Systematics of the Notonemouridae (Plecoptera) of southern Africa

Duncan Mark Stevens

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Systematic methods have advanced enormously in just a few decades, but Edward Newman's (1853) words are still frustratingly true today:

" … for the uninstructed mind acknowledges Nature's grouping, without the aid of science: an infant will distinguish a bird or a fish, without knowing the characters which separate both from a mammal; and however the man of science may blunder in defining those characters, however unsatisfactory to himself and to others may be his definitions, still a bird and a fish will ever be recognised as things distinct and separate from each other and from mammals. Nothing is more certain than that Nature has distinguished such groups: nothing is more probable than that man should fail in defining them."

Edward Newman 1853

(Proposed division of Neuroptera into two classes, *The Zoologist* 11, Appendix, Art XXIX: clxxxi-cciv)

A mating pair of *Aphanicerca* stoneflies

DECLARATION

The work presented in this thesis is my own original research, both in concept and execution, except as outlined below.

Chapters 1, 3 and 4 were entirely my own work. Four published taxonomic papers were condensed and amalgamated as Chapter 2 in order to provide an essential foundation for the remaining chapters. Those original publications were co-authored with my principal supervisor, Dr Mike Picker. My role in these Chapter 2 publications included conceptualization of the studies, planning the methodology, performing the laboratory practical work and statistical analyses, and preparation of the written part of the papers. Dr Picker's contribution to Chapter 2 comprised preparation of the insect drawings, guidance and critical review. The entire thesis benefited from valuable scientific and editorial input from both Dr Picker and co-supervisor Dr Jacqui Bishop.

This thesis has not been submitted for a degree at any other university.

Duncan Mark Stevens

DISCLAIMER

Chapter 3 presents the resolution of the *Aphanicerca capensis* Tillyard species complex. The species delimitations therein resulted in the recognition of 11 new species which were informally named at the conclusion of that chapter to facilitate analyses and discussion in the following chapter. In addition, two new species of *Aphanicercella* Tillyard were named in Chapter 4. None of these 13 new species has been described, and as such, this disclaimer serves notice according to Articles 8.1 to 8.3 of the International Code of Zoological Nomenclature (1999) that this work is not issued for public and permanent scientific record. Accordingly, these species names are disclaimed for nomenclatural purposes.

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ABSTRACT

The Plecoptera (stoneflies) is a minor, basal, aquatic order of the lower Neoptera, with about 3500 species worldwide, occurring on all continents except for Antarctica. Of the 16 extant families of Plecoptera, only two occur in southern Africa, namely the Perlidae and the Notonemouridae. The Gondwanan relictual Notonemouridae are represented by 90 species distributed among Australia, New Zealand, Madagascar and South America, with an additional 22 described species in five genera in southern Africa. Taxonomic revisions as the groundwork for this thesis increased the number of described South African species to 31 in six genera. The systematics section of this thesis added an additional 13 undescribed species. The South African Notonemouridae have received little taxonomic attention (the most recent taxonomic paper treating the southern African notonemourids was published in 1999, only the tenth since the first in 1931), in spite of their ecological dominance in many South African streams. They are also an ideal group for investigating processes of evolution and speciation within the geologically complex folded mountain systems of the Western Cape. However, phylogenetic and biogeographical questions can only be addressed given a sound taxonomic framework for the group involved. The Notonemouridae are used here as an exemplar relictual taxon of Gondwanan ancestry, to address patterns of endemism and speciation in the light of the geological and climatic history of southern Africa. Their cryptic diversity, low vagility and ecological montane requirements are shared by most of the other relictual taxa of southern Africa, so that drivers of speciation for the Notonemouridae might be applicable to a wider range of taxa, notably relictual taxa occupying the same habitat. The broad aims of this research, following the establishment of a firm taxonomic base for the southern African Notonemouridae, were to investigate species boundaries and to infer phylogenetic relationships in the *Aphanicerca capensis* Tillyard species complex using morphological, behavioural and molecular data (mtDNA), and to model historical biogeographic patterns and drivers of cladogenesis. Additional aims included an evaluation of the cytochrome oxidase subunit I gene as a molecular barcode in the Notonemouridae, a test of the monophyly of the genera, the construction of a phylogeny for the southern African Notonemouridae and the identification of clade synapomorphies.

The taxonomic background chapter unifies the results of four publications which were a necessary prerequisite to deeper examination of the systematics of the southern African Notonemouridae. A sound taxonomic platform is essential to phylogenetic accuracy. Morphological detail including some of the genitalic features required for the phylogenetic reconstructions later in the thesis are derived from this published work. These morphology-based revisions set the context for the molecular and morphological systematics and biogeography explored in the remaining chapters. They include the descriptions of a new genus, *Balinskycercella* Stevens & Picker, and the confirmation, using morphology and mate choice data, that *Aphanicercella barnardi* Tillyard comprises a species complex, resulting in the descriptions of five new species. Four new species in other genera were described, one each in *Desmonemoura* Tillyard and *Afronemoura* Illies, and two in *Aphanicerca*. Larval taxonomy produced characters for separating all genera and some species.

Numerous lines of evidence were examined within a practical application of the unified (general lineage) species concept for divergent *Aphanicerca capensis* Tillyard populations from the Cape Folded Mountains of South Africa. These lines of evidence included assessments of: allopatric fragmentation, genetic structuring, intrinsic reproductive isolation (four types – syntopic, sympatric, and complete and incomplete premating isolation during mate choice trials), morphological phenetic distinguishability, morphological diagnosability, monophyly and reciprocal monophyly. Two out of the ten lines of evidence provided parallel lines of support for all 12 morphogroups as independently evolving metapopulation lineages (i.e. species), namely morphological phenetic distinguishability and male morphological diagnosability. Sole reliance on any one of the other criteria failed to delimit all 12 morphogroups simultaneously as species. Morphology alone was sufficient to differentiate between these new species, but the additional lines of evidence afforded additional support for species delimitation. Analysis of morphometric characters provided support for the evolutionary relationships among these new species and drew attention to the characters that delimited members of this species complex. Of interest was the finding that the *Aphanicerca* COI gene tree and species tree (as hypothesized from

morphological relationships) were incongruent; moreover, the COI gene did not appear to be an efficient molecular "barcode" marker for this group. Because syntopic but distinct morphogroups shared haplotypes, it is clear that the sole use of genetic distance alone is inappropriate in species delimitation in the southern African Notonemouridae. There is evidence that whilst reproductive cohesion can break down in recently diverged species, species unity can be maintained in sympatry; rates of change in mate recognition systems may lag behind those of morphological and genetic divergence during vicariant speciation. In addition, there is also evidence for the distribution of spatially structured morphological lineages across the Cape Folded Mountains, evidence of mitochondrial introgression (possibly historical) or incomplete lineage sorting (or both) within the species complex, and evidence of a centre of origin of the species complex in the central region of the Langeberg in the Southern Folded Mountains.

The phylogenetic aims of the thesis were approached using forty of the forty four species (including the 13 undescribed species of the *A. capensis* complex) across the six genera of southern African notonemourids; they were included in a morphological and mtDNA molecular (39 species) analysis to test the monophyly of the genera and to estimate phylogenetic relationships. All morphological characters were newly conceived for separate parsimony and Bayesian analyses. Under the parsimony criterion, five weighting schemes (equal, *a priori*, successive approximations, implied and self) were employed. Partial COI sequences were used in maximum parsimony, maximum likelihood and Bayesian analyses, and in combined analyses with the morphology data in parsimony and Bayesian analyses. All five morphology parsimony weighting scheme and BI morphology cladograms were in agreement on the monophyly of the genera, the clade (*Aphanicercella*, *Balinskycercella*), and the clade (*Afronemoura*, *Aphanicerca*). The model-based analyses (Bayesian and maximum likelihood) of both the mtDNA partition and combined analyses are regarded as less reliable than the parsimony (morphological and molecular) analyses in light of recovery of nonmonophyly of two genera. Morphological and molecular parsimony cladograms were largely in agreement, and were congruent in generic relationships. The generic relationships under the parsimony criterion could be divided into those that were stable and those that were unstable. The *a priori* combined morphology and molecular consensus cladogram (*Aphanicercopsis* ((*Aphanicercella*, *Balinskycercella*), (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*)))) is favoured because at generic level it was fully resolved. Unambiguous character states that defined the stable and unstable clades are given for cladograms with equal and *a priori* weights.

Paraproct glands were described for the first time in Plecoptera, and possibly in Insecta. Unusual paired structures (probably spermathecae), not previously described in Plecoptera but with possible homology in *Capnioneura* (Capniidae), were described in female *Aphanicercopsis* excepting *A. outeniquae*. Some important and phylogenetically useful characters were found to be degree of fusion of ventral nerve cord abdominal ganglia, male paraproct glands (occurrence and form), and accessory glands of the male seminal vesicle.

Distribution maps have been provided for all species, and distributions described and discussed. Two main biogeographic areas were defined on species composition, namely the Eastern Highlands and the Cape Folded Mountains, with some overlap, and one additional minor zone, the Namaqua Highlands. The intersection zone of the Southern Folded Mountains and Western Folded Mountains was particularly rich in palaeogenic biota, with 24 of the 44 species. Local endemism, at mountain range scale, was common, with almost 41% of the species endemic to a single mountain range group. A hypothesis forwarded for the evolution of the southern African Notonemouridae proposes that the common ancestor of the six genera dispersed from a Cape Folded Mountains origin, to become widespread across the montane areas of the southern tip of the African continent after the separation from Gondwanaland, including the Cape Folded Mountains, Amatola and Drakensberg regions. Because allopatric speciation is believed to be far more prevalent than sympatric speciation, and because there are four genera present in the Cape Folded Mountains and usually multiple genera within one stream, it is likely that populations of this most recent common ancestor of these genera became separated by vicariant events (or surrogates such as topographical complexity) within the Cape Folded Mountains, allowing the genera to evolve. Species within these genera subsequently underwent cycles of range expansion and speciation in allopatry. Secondary contact would ultimately have occurred resulting in generic sympatry.

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Chapter 1
Introduction

Brief review of notonemourid biology

The Plecoptera is a minor, basal, aquatic order of the lower Neoptera, with about 3500 species worldwide (Fochetti & Tierno de Figueroa 2008), occurring on all continents except for Antarctica (Theischinger 1991). The larvae of almost all species are aquatic, with most being found in cool, perennial streams. Their larvae cling to submerged rocks, stones and the gravel on the stream bed, and to leaf packs and twigs. The larvae of Notonemouridae occur in cold, low order, fast-flowing streams with stony substrates and even in small moss-covered seepages on mountainsides, providing these are perennial or flow underground during the dry season. Adult stoneflies are found on rocks within and adjacent to streams and on riparian vegetation, but also on vegetation some distance from streams. The larvae generally are intolerant of thermal or organic pollution and so are useful indicators of water quality (Dallas & Day 1993).

The stonefly imago lives for only a few days to a few weeks. Females are generally larger than males, and males of many species of the suborder Arctoperlaria attract and court females by drumming the abdomen on the substrate to produce species-specific vibrational signals. Locally, this behaviour does occur at least in some *Aphanicerca*. During mating, the male mounts the female and curls the tip of his abdomen underneath that of the female, enabling the female subgenital plate to interlock with the male epiproct (supra-anal lobe), and/or other copulatory structures. The terminalia of male Plecoptera vary greatly among the different families and genera. Mating may last several hours during which sperm are transferred by, or aided by, one of a variety of structures such as the aedeagus (not in Notonemouridae), and the paraprocts. Eggs laid on the surface of the water, or below the surface become attached to the substrate by various anchoring structures (Stewart & Harper 1996). The (hemimetabolous) life cycle is most commonly univoltine, but many species are semivoltine, with the life cycle lasting from two to as long as six years (Frutiger & Imhof 1997). Diapause is known to occur in both the embryonic and larval stages under extreme environmental conditions (Stewart & Harper 1996). Eggs take three to four weeks to hatch, followed by a variable number of larval instars. The final instar larva, easily recognisable by its black wing pads, crawls out the water onto a dry stone or vegetation to emerge from its exuvium as the adult stonefly. These exuviae (shucks) remain attached to the substrate for a few weeks after the adult has emerged, and adult stoneflies are often found in close proximity to their shucks. Details of the life cycle of southern African plecopterans are unknown, and although the life cycle of the Notonemouridae is probably univoltine, the number of instars is yet to be established.

Feeding habits of the larvae are varied and include detritivory and herbivory (shredders, gatherers, scrapers), omnivory, and predation (e.g. in members of the second South African stonefly family, Perlidae (Picker 1985)). Feeding habits may even change with the developmental stage of the larva (Stewart & Harper 1996). The larvae of *Aphanicerca* Tillyard (Notonemouridae) from the Western Cape Province (South Africa) are shredders of allochthonous leaf detritus, a major source of carbon in streams. The microbial slime layer on decomposing leaf material is thought to provide a source of nitrogen (Reynolds *et al*. 1997). In the laboratory, adult notonemourids feed on proteinaceous foodstuffs, so they probably do feed in the wild.

Of the 16 extant families of Plecoptera, only two occur in southern Africa, namely the Perlidae, found throughout Africa, and the Notonemouridae which is restricted to southern Africa. The family Perlidae is represented locally by a species complex (Picker 1980) of an unknown number of species of *Neoperla*, while the family Notonemouridae is better known with 22 described species in five genera (these figures apply to the status quo prior to the publications and findings presented herein). Worldwide, there are about 121 species of Notonemouridae (Fochetti & Tierno de Figueroa 2008). The centre of adaptive radiation for African Notonemouridae is the south western parts of the Western Cape Province. Most of the southern African species are narrow endemics. It is therefore likely that further notonemourid species remain to be discovered in remote mountain streams of southern Africa. In addition, the application of molecular and behavioural techniques is likely to reveal additional (cryptic) species.

Review of the phylogenetic placement and morphology of the family Notonemouridae

The monophyly of the Plecoptera is supported by few apomorphic characters (Zwick 2000) namely, gonads forming loops, two superimposed seminal vesicles each forming a loop, the presence in the larva of strong oblique, intersegmental ventro-longitudinal muscles for laterally undulating swimming, the general absence of ovipositors (with few exceptions where they are secondarily derived), and possibly the presence of an accessory circulatory organ in some families. Terry & Whiting (2005) using a molecular approach found support for the monophyly of the order, although the taxon sampling was not that dense. The order falls within the Polyneoptera. The interordinal relationships of the Plecoptera are unresolved, although some authors (e.g. Hennig 1981) regard them as sister to the other Polyneoptera. A combined molecular and morphology-based cladogram separated Plecoptera with their sister group of Dermaptera and Zoraptera from the other Polyneoptera, and placed them as sister to Holometabola, thus showing the paraphyly of the Neoptera. However, the position of Plecoptera

(and other orders) varies according to character sets included, and they conclude that Plecoptera and its as yet unidentified sister group are distantly related.

Two suborders, Antarctoperlaria and Arctoperlaria (Zwick 1973) are recognized. Notonemouridae falls within the latter. Support for the Arctoperlaria is weak, although drumming (vibrational communication used in mate location) is a character that supports monophyly of the group (Zwick 2000). Zwick (2000) recognizes two major groupings within the Arctoperlaria, the Systellognatha and the Euholognatha, the latter containing the Notonemouridae. The three synapomorphies of the Euholognatha are an unpaired corpus allatum fused to the aorta, a soft chorion of the egg, and segmental nerves coursing under longitudinal abdominal muscles (Zwick 2000).

Within the Euholognatha, the superfamily Nemouroidea is characterized by synapomorphies which include: the tenth tergite is completely reduced and paraprocts lie directly behind sternite nine, laterally in contact with lateral parts of tergite 10 and the cercus bases or attached sclerites; male paraprocts comprise a medial lobe and an outer lobe with an internal muscle (Zwick 2000).

Although Ricker (1950) established the subfamily Notonemourinae within the Nemouridae Klapálek, Kimmins (1951) considered his own definition more inclusive, and defined it purely on wing venation as follows: “Fore wing with Cu2 long, generally extending well into the apical half of the wing; cross-vein in the pterostigmatic area of both wings either absent or present. Hind wing with the media forking at or before the radio-medial cross-vein”. Included in the new subfamily were the South African taxa (Kimmins 1951). Zwick (1973) elevated the Notonemourinae (Ricker 1950) to family rank (Notonemouridae), although he regarded the taxon as a paraphyletic grouping of early or pre-nemourid lines (Zwick 2000). Although the monophyly of the family has been questioned because of structural diversity (Zwick 2000, McLellan 2000), Terry & Whiting (2003), in a combined morphological (using familial characters from Zwick 2000) and molecular study, proposed a phylogeny where Notonemouridae is monophyletic and sister to a group comprising Nemouridae, Capniidae, Taeniopterygidae and Scopuridae. The Notonemouridae and Nemouridae were regarded by Zwick (2000) as sister groups, together forming a monophyletic group which he designated Nemouridae (*sensu lato*). The following synapomorphies characterize the Nemouridae (*sensu lato*); forked mesothoracic pleural arm; absence of penis and associated muscles; unpaired strongly muscular ejaculatory duct opening at gonopore at tip of sternite nine; abdominal nerve cord having fused posterior ganglia with no more than six free ganglia (Zwick 2000). Interestingly, Tillyard (1931) also recognized a grouping Nemouridae (*sensu lato*) and Nemouridae (*sensu strictu*), the former, equivalent to Zwick’s Nemouroidea, containing what

are now the Nemouridae, Notonemouridae, Taeniopterygidae and Leuctridae. Tillyard regarded what is now recognized as Notonemouridae as a link between the Nemouridae (*sensu strictu*) and the Leuctridae, similar to Zwick's concept of Nemouridae (*sensu lato*) as sister to Leuctridae and Capniidae. Tillyard (1931) placed the South African notonemourids in his possession into the family Nemouridae and the subfamily Nemourinae, on the basis of tarsal characters.

As yet, no synapomorphies are recognized for the Notonemouridae (Zwick 2000). As members of the Arctoperlaria, the family is thought to have its origins in the northern hemisphere. The Perlidae and the Notonemouridae are the only members of the suborder to survive in the southern hemisphere. The notonemourids have a Gondwanan distribution, being found in southern Africa, South America, Madagascar, New Zealand and Australia. McLellan (1991) revised the generic groups of the Notonemouridae which had been proposed by Zwick (1973, 1981). Zwick (1981) proposed two groups, viz. *Austrocercella* and *Notonemoura*, which were differentiated on the structure, position and development of male and female external genitalia. He regarded the southern African and Madagascan fauna as part of the *Austrocercella* group, but cautioned that the diverse African group may bear only superficial resemblance to the other members of the group. McLellan (1991) retained the names of the groups and added a third, the *Spaniocercoides* group to which he assigned the southern African and, with reservations, the Madagascan fauna. The defining characters of this group are: five hindwing anal veins with 2A free from 3A (plesiomorphic); Male – subgenital plate long, paraprocts entire (apomorphic); paraprocts free from subgenital plate (plesiomorphic), gonopore posterior on sternite 9 (plesiomorphic), epiproct usually a simple hook (apomorphic), tergite 9 without lateral processes; Female – gonopore usually at rear of sternite 8 (plesiomorphic), subgenital plate formed by extensions of posterior sternites, if gonopore central on sternite 8 or in front of it, there may be either a lobe or a plate anterior to it, or an anterior and a posterior lobe (apomorphic) (polarity of character states defined by McLellan 1991). Later, McLellan (2000) felt that only the *Notonemoura* group was monophyletic and that the southern African and Madagascan genera should perhaps be assigned to a separate group or higher taxon. Using mainly ovipositor structure, Zwick (2000) provided an alternative view on generic groupings without attaching names to them, which differed both from his original and McLellan's groups. With exceptions, this grouping largely clusters geographically. He points out that taxa without ovipositors are not catered for and that the presence of an ovipositor cannot establish notonemourid monophyly. Typical nemourid larval coxal structure was not found in notonemourids, providing more evidence for monophyly of the Nemouridae, but not of the Notonemouridae (Zwick 2006). The phylogeny proposed by Terry & Whiting (2003) shows that the southern African genera form a monophyletic group, providing support for McLellan's view

on a separate generic or higher grouping for these taxa (however Madagascan genera were not included in the Terry & Whiting (2003) study).

The concept of generic groups for the Notonemouridae was developed because of the wide morphological diversity and the doubted monophyly of the family. There is no consensus on the definition or composition of these groups which serve to provide a starting point for further research culminating in a formal taxonomic classification. This study provides a more detailed morphological analysis of the southern African notonemourids than was previously available, in the hope that it will contribute toward solving the problem of uncertain notonemourid phylogenetic relations, specifically to help clarify the utility and status of the generic groups. The aim of this study is not to resolve that issue, but is a first step toward that goal which will require study of foreign material to realize.

The taxonomic history of the southern African Notonemouridae

Southern African notonemourid stoneflies were the subject of only six publications until 1980, the first in 1931. A further four papers were published by D. M Stevens and M.D. Picker between 1995 and 1999 which are unified as Chapter 2 of this thesis in order to provide essential background to the systematics chapters.

The first taxonomic treatment of stoneflies from southern Africa was published in 1931 by Tillyard, on specimens collected mostly by K.H. Barnard, the then assistant director of the South African Museum (Tillyard 1931). As mentioned above, Tillyard assigned these notonemourids to Nemouridae: Nemourinae. He recognized two new genera, *Aphanicerca* (type species *A. capensis* and *Desmonemoura* (type species *D. pulchellum*) based largely on wing venation, characters useful for distinguishing higher taxonomic groupings, but less useful at the generic level. *Aphanicerca* was assigned three species, namely *A. capensis* Tillyard, *A. denticulata* Tillyard and *A. barnardi* Tillyard, differentiated from each other by wing and genitalic characters. Using wing venation as the primary distinguishing characters of the genera, resulted in two of the three species being erroneously assigned to *Aphanicerca*. Tillyard, however, evidently did appreciate the wide variation in genitalic structure when erecting two subgenera within *Aphanicerca*, namely *Aphanicerca* and *Aphanicercella*. The subgenus *Aphanicerca* contained the type species *A. capensis* (type locality Table Mountain, Cape Peninsula), based on the presence of dorsal processes "on segment seven" (actually they are on tergite nine), and "without a dorsal grooved appendage below and between cerci". This is a reference to the epiproct which he recognized in the other two species which he placed in the subgenus *Aphanicercella*. However, the epiproct does in fact exist in *Aphanicerca*. Tillyard (1931) actually illustrates this epiproct (Fig. 4d, p.120), but it is labelled paraproct. He indicated that

there are two of these structures lying close together, but in fact there is only one in the dorsal midline. This error probably arose from examination of the insect distorted by flattening on a glass microscope slide.

The second subgenus, *Aphanicercella*, was assigned the species *A. denticulata* and *A. barnardi*. Barnard (1934) transferred *Aphanicerca denticulata* to a new genus *Aphanicercopsis*, and elevated the subgenus *Aphanicercella* to genus rank, with *Aphanicercella barnardi* as the type species. When describing the female of *Aphanicerca barnardi,* Tillyard (1931) erroneously figured the female of *Desmonemoura pulchellum* (Fig. 9, p. 125). The female described by him as *Desmonemoura pulchellum* allotype presumably belongs to one of the other genera. Tillyard did remarkably well considering he had just one species from each genus, and that most of the specimens he had to work from were dried and pinned (Barnard 1934).

The second major contribution to the stoneflies of southern Africa was authored by Barnard (1934), as part of a series on the fauna of the Cape Mountain ranges, with additional distributional data provided subsequently (Barnard 1936). Barnard correctly placed emphasis on male, and to a lesser extent female, genitalia for generic and specific characterization and phylogenetic inference, providing some accurate and useful characters. Barnard did not elaborate on larval identification or phylogenetically relevant features, but stated that larvae of the genera are "practically indistinguishable" (Barnard 1934). He further noted micropterism in a female *Aphanicercella* from Robinson Pass in the Outeniqua Mountains, and apterism in two *Aphanicercopsis* females from the Palmiet River near Kleinmond, and in a male *Aphanicercopsis hawaquae* from Jonkershoek, Stellenbosch.

Barnard (1934) dissolved the subgenus category and redescribed *Aphanicerca capensis* Tillyard, and additionally described another five new *Aphanicerca* species, namely *A. uncinata* Barnard, *A. lyrata* Barnard, *A. bicornis* Barnard, *A. bovina* Barnard, and *A. tereta* Barnard. Of importance was the recognition by Barnard of different allopatric "varieties" of *A. capensis* males based on the shape of the dorsal process of tergite 9, from Wellington, Montagu Pass and Tulbagh, as well as females with variably-shaped subgenital plates from various localities. He stated that the slight variations in male and female genitalia did not justify assigning varietal names to them. The suggestion that *A. capensis* might comprise a species complex (Picker & Stevens 1999; Chapter 2 of this thesis) is investigated in more detail in Chapter 3 of this thesis.

Barnard (1934) erected the new genus *Aphanicercopsis* for *Aphanicercopsis denticulata* (Tillyard) and for three new species *A. tabularis* Barnard, *A. outeniquae* Barnard, and *A. hawaquae* Barnard. Tillyard's subgenus of *Aphanicerca*, *Aphanicercella* Tillyard, was elevated

to genus status by Barnard (1934), and contained the type species, *Aphanicercella barnardi*, and five new species *A. scutata* Barnard, *A. cassida* Barnard, *A. bifurcata* Barnard, *A. quadrata* Barnard, and *A. nigra* Barnard. As with *A. capensis*, *A. barnardi* was recognized by Barnard as a variable species with "transitional forms" that did not justify unique names. This morphological variation within *A. barnardi* is addressed in Stevens & Picker (1997) (Chapter 2 of this thesis).

Lastly, Barnard (1934) redescribed *Desmonemoura* Tillyard, and corrected Tillyard's error by describing the female of *D. pulchellum* which Tillyard had previously described as the *A. barnardi* female.

Balinsky (1956) described five new species of stonefly from the eastern, summer rainfall region of South Africa, which he classified together with the rest of the South African taxa as belonging to the family Leuctridae. He was of the opinion that similarity in wing venation placed the notonemourids in the Leuctridae, in spite of the fact, as he pointed out, that the paraproct is more similar to that of the Nemouridae (Balinsky 1956). He described a new species from Grahamstown and the Hogsback (Amatolae Mountains) in the Eastern Cape Province, which he assigned to *Aphanicercopsis* Barnard. The new species, *Aphanicercopsis amatolae*, was characterized by a pair of sharp spines on the posterior margin of the ninth tergite. Although he had collected one male of similar morphology but with a single median spine, he did not describe it as a new species until he had collected more specimens from the same area. These were described as *Aphanicercopsis spinulata* Balinsky (Balinsky 1967). As pointed out by Illies (1980), Balinsky (1956) acknowledged that *A. amatolae* "differs very considerably from the four species of the genus listed by Barnard". With regard to the female, Balinsky stated that the subgenital plate was quite typical for *Aphanicercopsis* and thus based his generic allocation on this character. Balinsky (1956) states that "The classification of my species as an *Aphanicercopsis* would appear to be based mainly on negative characters if only the ♂♂ are taken into consideration". Yet, he describes the species accurately, providing unique characters such as the pair of sharp spines on the posterior margin of the ninth tergite, broadly and uniformly convex posterior margin of ninth tergite, and 10th tergite comprising two broad heavily chitinised plates. Balinsky seems to have viewed the absence of appendages on tergite nine (e.g. in *Aphanicerca* and *Desmonemoura*), and the absence of a clasper-like structure on the tenth pleurite (as in *Aphanicercella*), as characters, instead of focusing on what actually was present. Fitzhugh (2006) points out that coding a character as "absent" can only be interpreted as "a shorthand term for what actually is observed". So, the absence of processes on tergite nine is better described (coded for in a cladistic sense) as "posterior edge broadly and uniformly convex, bearing a pair of sharp spines near the midline…", which is a direct quotation from Balinsky's (1956) description. Had Balinsky focused on his own description and not on what his

new species did not have in relation to existing genera, he may have erected a new genus himself. Instead, this was done later by Illies (1980) who established the genus *Afronemoura* to accommodate Balinsky's *Aphanicercopsis amatolae* and *A. spinulata* (Balinsky 1967). Illies also described the larva of *Afronemoura*, which has a unique feature not found in the other genera, namely a tuft of bristles about one third of the way up the antennae. He also pointed out the non-overlapping distributions of *Aphanicercopsis* and *Afronemoura* (Illies 1980). The second part of Balinsky's 1956 paper is the description of three new species allocated to *Aphanicercella* Tillyard. As he noted for the *Afronemoura* Illies species, these new species form a morphologically distinct unit (Balinsky 1956). A new genus, *Balinskycercella* Stevens & Picker (Stevens & Picker 1995) (Chapter 2 of this thesis), was erected to accommodate this distinctive clade, which was recognised as the sister group of *Aphanicercella*.

Species concepts

A vast literature on species concepts exists, a summary and analysis of which is beyond the scope of this project. Any species concept will have one or more shortcomings (Hull 1997), and any operational method will sometimes fail to delimit species correctly (Crowe 1999, Sites & Marshall 2004). Because there are many species concepts (e.g. 22 recognized by Mayden 1997), and no universal consensus on any of them, attempts to delimit a species by whatever means or criteria may fail by the criteria of a rival species concept. If an organism is well delimited in terms of one species concept and not of another, can it justifiably be accorded formal species status and described? Because species are described daily using one or other criterion, the practical answer to that question is yes, regardless of what the philosophical answer may be. It is therefore a subjective or qualitative judgment that results in that species description, regardless of the degree of objectivity of the delimitation method employed. The term "subjective" is used here not in the sense of arbitrary, but in the sense of "interpretative". In the words of Joel Cracraft (2000), "[systematists] bring to bear on the interpretation of that variation their prejudices, experiences, the data available, and the theoretical framework of the species concept within which they operate – hardly arbitrary or subjective." Over three decades ago Doyen & Slobodchikoff (1974) outlined an operational method of species delimitation using morphometric (phenetic), reproductive and ecological parameters. They presented a graphical method which appeared objective, but nevertheless stated that "it is still up to individual taxonomists to decide how close two populations have to be in the three-dimensional space in order to be conspecific". This subjectivity as part of rigorous methodology persists today, where even choosing a species concept for delimitation will affect the species that are delimited (de Queiroz 2007). Subjectivity will always be a part of systematics and taxonomy (Mallet 1995), but nevertheless steps to minimize it are required for repeatability and reduction of controversy. Objective and repeatable methods are desirable (Sites & Marshall 2004). For conservation of

biodiversity purposes, any well supported lineage deserves to be conserved, regardless of the operational approach used, and that has led to the development of the concepts Evolutionary Significant Units (ESU's) and Management Units (MU's) (Moritz 1994) for conservation purposes. One problem with these concepts is that they fall into the same trap as the species concept – they also need to be defined using certain criteria, and how is agreement reached on those criteria? It can be difficult to decide whether to accord species or ESU status to members of a species complex.

Because of the stochastic nature of gradual biological change that leads to speciation, species boundaries will be nebulous, and therefore a single operational criterion to delimit species is unlikely to be sufficient for more than clear cut cases. Because species concepts and their operational criteria (when they have them) are constructs of the human mind and not of nature, the likelihood of one definition or method fitting all biological systems and pleasing all human minds is remote. What is far more plausible is that all species delimitation methods and species concepts provide some information that approximates truth. Finding this information in multiple methods may allow the investigator to make more accurate inferences of species status than would be possible when relying on a single approach, and to be more confident about the subjective part of those decisions.

A philosophy tying together all species concepts is the idea that species comprise segments of separately evolving metapopulation lineages (Wiens 2007, de Queiroz 2007). This unified concept of species (de Queiroz 2007), also known as the general lineage concept (de Queiroz 1998, 1999), recognizes the primary, and only necessary, property of a species as being a segment of a population-level lineage. Secondary properties (criteria) are those that are contingent and not necessary as defining properties for a lineage to be considered a species (de Queiroz 1999). Many have advocated the use of multiple lines of evidence (i.e. these secondary properties or criteria) in species delimitation (e.g. Ross 1974, Sites & Marshall 2004, Coyne & Orr 2004, Knowles & Carstens 2007, Petersen *et al.* 2007). The definition of the unified species concept is that species are segments of separately evolving metapopulation lineages, while the operational approach to delimiting species in terms of this definition is to use any number of secondary criteria to infer that a metapopulation is a separately evolving lineage. In this way, the problems of failure of species delimitations by criteria of rival concepts is done away with, and the only remaining controversy will be regarding methodology (de Queiroz 2007) and sufficiency of evidence for membership of a separately evolving lineage. The unified species concept (general lineage concept) regards the species category as nonrelational. However, the secondary properties or criteria are relational (de Queiroz 1999) in that a group of organisms can be a segment of a separately evolving metapopulation lineage (i.e. a species) based on, for

example, the morphological criterion relative to one lineage, and on the reciprocally monophyletic criterion relative to another. Ideally, it would be useful to cite one or more criteria that distinguish a certain species from all other known species. Factors confounding this might include the practical inability to undertake such a comparison, cryptic species (morphological similarity with genetic or other divergence), incomplete lineage sorting (retained ancestral polymorphisms), saturation, relationships affected by choice of outgroups, hybridization and mitochondrial introgression, incipient speciation, and small amounts of gene flow between populations that maintain morphological or ecological distinctiveness. It is useful therefore to compare relationally, using various lines of evidence, closely related species such as members of a species complex, which are generally recognized and defined by morphological characters (of which synapomorphies will be useful phylogenetically). Morphological characters may take the form of morphometrically analyzed data, meristic, discrete, and continuous characters, of which the last two especially will utilize a certain amount of subjectivity in their formulation and analytical approach. It is this morphological character analysis that must always remain the mainstay of taxonomy, and is essential in describing the biodiversity of our planet. Here I use various lines of evidence in the broad categories of morphology, mate choice biology and mitochondrial DNA, to investigate species boundaries in the *Aphanicerca capensis* species complex.

Phylogeny and biogeography

Knowledge of species distributions and phylogenetic relationships within the notonemourid stoneflies of southern Africa are needed to provide a hypothesis for the evolution of other members of the well-represented palaeogenic fauna (basal taxa currently occupying relictual habitats) of the region (Stuckenberg 1962). Ecologically, the Notonemouridae share a number of features characteristic of other members of the relictual invertebrate fauna of southern Africa; cryptic speciation, low vagility and restriction to temperate montane refugia. This makes them an ideal model for examining possible drivers of speciation. Up to the present though, scant attention has been paid to the biogeography of southern African stoneflies. Balinsky (1962) and Stuckenberg (1962) emphasized the family's Gondwanan origins and distributional similarities with other local relictual montane faunal invertebrates, particularly within the Cape Folded Mountains (CFM) and the Eastern Highlands (EH). It is thought that the relictual fauna of southern Africa are currently restricted to small temperate refugia as a result of their once wider distributions being contracted following gradual climate warming and aridification that occurred as Africa moved northwards after the fragmentation of Gondwanaland (Day 2005). These organisms have survived in temperate refugia (mountain streams, caves, forest) present in the complex geological formations of the CFM, a region rich in both fauna and flora (Taylor 1978). Stuckenberg (1962) partly attributed the general species richness of the CFM to the antiquity of

the landscape, the varied topography, and the climate. Price *et al*. (2007) discussed the controversy regarding climatic conditions present in the Cape Floristic Region (CFR) during the Pliocene and Pleistocene, namely stability versus rapid, dramatic change. One view is that the CFR may have been spared the climatic cycles that caused extinctions of flora in northern temperate areas during this period (Barraclough 2006). Pleistocene glaciation was largely a northern phenomenon, from which southern Africa was largely spared (Barraclough 2006). However, the glaciation that did occur in southern Africa is thought to have been more extreme in the south-eastern Cape region than in the south-western Western Cape Province, leading to more extinctions in the former (Cowling *et al*. 1996); indeed, the Cape Folded Mountains were evidently not high enough to have been glaciated (Deacon 1983). It is likely that a combination of rapid speciation and low extinction rates led to the overall species richness of the flora in this region (Cowling *et al*. 1996). Overall, there is no consensus on a causal relationship between any one main environmental variable and the high levels of speciation of the palaeogenic (relictual) invertebrates in the CFM, and the answer probably lies in a multiplicity of factors (Day 2005), including those which resulted in the remarkable diversification of the flora. Aspects of these biogeographical events are further explored in Chapter 4.

Broad aims of this thesis

Collecting of southern African Notonemouridae had been carried out sporadically and superficially, moreover little of the limited material in existing collections was found to be sufficiently well-preserved for taxonomic purposes. Far greater sampling was required across the whole country for thorough taxonomic revision, and the work presented in this thesis is based on 14 years of collecting effort. Live and fresh material was also required for mate choice trials and mitochondrial DNA sequencing respectively, both essential in resolving species complexes. This initial alpha taxonomic work resulted in the publication of four papers (co-authored with my principal supervisor, M. D. Picker). These publications are included here in modified form, as **Chapter 2**, in order to provide essential updated taxonomy and morphological detail for accurate phylogenetic reconstruction, and are referred to in the later chapters. A new genus, *Balinskycercella* Stevens & Picker, was described. *Aphanicercella barnardi* Tillyard was confirmed to be a species complex using morphology and mate choice data, resulting in the descriptions of five new species. Four new species in other genera were described, one each in *Desmonemoura* Tillyard and *Afronemoura* Illies, and two in *Aphanicerca*. The female of *Aphanicerca bovina* Barnard was described for the first time. Larval taxonomy produced characters for separating all genera and some species, and a key to genera. Separate dichotomous generic keys to adult males and females, and to males of all species were produced.

Chapter 3, the resolution of the *Aphanicerca capensis* species complex, is presented as one unified theme for purposes of continuity of the thesis and to avoid repetition, but for publication purposes is probably better divided into smaller units. In this chapter I examine evidence for cryptic speciation. Barnard (1934) had noted the presence of 'varieties' of *A. capensis*, but considered them too minor to warrant species status. The primary aim of this study is to determine species boundaries between 12 *Aphanicerca capensis* morphogroups using multiple lines of evidence according to the unified (general lineage) species concept (de Queiroz 1998, 1999, 2007). I use mate choice behaviour, morphometric data and mitochondrial DNA to this end. These lines of evidence include assessments of: allopatric fragmentation, genetic structuring, intrinsic reproductive isolation (four types – syntopic, sympatric, and complete and incomplete premating isolation during mate choice trials), morphological phenetic distinguishability (using morphometrics), morphological diagnosability, monophyly and reciprocal monophyly.

Chapter 4 addresses the phylogeny and biogeography of the group in light of the historical landscape scenario briefly introduced above. In this chapter I present a morphological and mitochondrial DNA molecular phylogeny of the southern African Notonemouridae. This is the first morphological cladistic analysis of the southern African Notonemouridae. I develop a morphological character matrix for analysis under maximum parsimony and Bayesian inference approaches. Various weighting schemes (equal, implied, self, successive approximations and *a priori*) are employed and contrasted under parsimony. I also present the first molecular analysis using mitochondrial DNA that includes all six genera and almost all species. I use equal weighting parsimony, Bayesian and maximum likelihood approaches for the mitochondrial DNA partition analysis, and Bayesian and parsimony (equal and *a priori* weighted) for the combined partition analyses. The mitochondrial DNA cytochrome oxidase I (COI) gene is used in this study as it is widely used in phylogenetics and is therefore useful for comparative purposes (Caterino *et al*. 2000) and, as it is the gene chosen by Hebert *et al*. (2003) as the DNA barcode marker, in order to test its utility in the southern African Notonemouridae as a DNA barcode. I use these results in a discussion of the current and historical biogeography of the group.

Specific aims

1. To provide a review of the taxonomy of the southern African Notonemouridae using extensive material to provide a sound framework for subsequent systematic, phylogenetic and ecological studies.
2. To examine 'varieties' (divergent populations) of *Aphanicerca capensis* following the unified species concept (de Queiroz 2007) to delineate recent cladogenic events.

3. To examine the relationship between mate recognition systems and morphological and genetic divergence in the *A. capensis* species complex.
4. To determine possible processes that shaped regional speciation within the *A. capensis* species complex using the constructed phylogenies.
5. To evaluate the utility of the COI gene as a molecular "barcode".
6. To formulate hypotheses of the historical biogeography of the southern African Notonemouridae using species distributions and the phylogeny.
7. To examine the phylogenetic relationships among the species and genera using morphological and molecular cladistic methods, and to compare the morphological and molecular trees for congruence.
8. To identify the morphological synapomorphies that define the genera and clades. These characters may prove useful in future studies that attempt to resolve intercontinental relationships between genera of the Notonemouridae.

Chapter 2
Taxonomic revision of southern African Notonemouridae (Plecoptera), with description of *Balinskycercella* gen. n.

This chapter presents the unified results of my four publications (Stevens & Picker 1995, 1999; Picker & Stevens 1997, 1999) which were a necessary prerequisite to deeper examination of the systematics of the southern African Notonemouridae. A sound taxonomic platform is essential to phylogenetic accuracy. Morphological detail required for the phylogenetic reconstructions later in the thesis is derived from this published work. Later chapters refer to figures in this chapter. The morphology-based revisions in this chapter set the context for the molecular and morphological systematics and biogeography explored in the remaining chapters. The work done for this chapter was my own, except for the figures which were prepared by my principal supervisor, MDP (hence alternating of senior authorship) who also provided guidance throughout.

A new genus, *Balinskycercella*, is described for three species of stoneflies from the Lesotho-Drakensberg Highlands, resulting in the new combinations *B. gudu* (Balinsky), *B. tugelae* (Balinsky) and *B. fontium* (Balinsky). The genus *Aphanicercella* Tillyard is revised based on conventional characters of the genitalia. A number of *A. barnardi* Tillyard forms that differ from one another and the other known species of *Aphanicercella* are distinguished. These were morphologically discrete, and there was no evidence of intermediates. An evaluation of the biological species status of the forms was carried out using mate choice experiments. The results showed clear positive assortative mating within forms, indicative of reproductive isolation between them. Further indications that these forms should be accorded species status derived from the absence of morphological intermediates in the field. The forms are consequently considered to be valid species and are formally described, with all five new species evidently being closely related. They differed in minor, but consistent features of the male and female genitalia, and had smaller geographical ranges than the other, more morphologically diverse species. They are grouped together with *A. barnardi* in the *A. barnardi* species complex. The genera *Desmonemoura* Tillyard, *Aphanicerca* Tillyard, *Afronemoura* Illies, *Aphanicercopsis* Barnard and *Balinskycercella* gen. n. are also revised. *Desmonemoura brevis* sp. n., *Aphanicerca gnua* sp. n., *Aphanicerca chanae* sp. n. and *Afronemoura stuckenbergi* sp. n. are also described, with all new species increasing the total number of described southern African notonemourid stoneflies from 22 to 31. The female of *Aphanicerca bovina* Barnard is described for the first time. Larvae of the genera *Aphanicerca*, *Desmonemoura*, *Aphanicercopsis*, *Afronemoura*, *Aphanicercella* and *Balinskycercella* gen. n. are described, some for the first time. Setal and other characters facilitated simple and accurate generic and often species identification. Diagnostic abdominal setal patterns of 13 of the widespread species are illustrated. Although the examination of black-wingpad larvae with adult genitalia visible through the cuticle remains the most reliable means of species identification, the rarity of this stage of the life cycle necessitates examination of the more abundant earlier instars. In addition to a generic larval key, keys are provided to males and females to genus level, and to males of all described species.

Keywords: Notonemouridae**,** taxonomy, *Balinskycercella*, stoneflies, South Africa, Lesotho, species complex, mate choice, larva

INTRODUCTION

The genus *Aphanicercella* Tillyard is restricted to South Africa, where most species are concentrated in the south-western Western Cape Province. The type species, *Aphanicercella barnardi* Tillyard was placed in the subgenus *Aphanicercella* (of the genus *Aphanicerca*) by Tillyard (1931), which was later treated as a genus by Barnard (1934). The genus *Aphanicercella* as defined in this revision comprises *A. barnardi*, which is considered to be a species complex, as well as *A. scutata* Barnard, *A. bifurcata* Barnard, *A. quadrata* Barnard, *A. nigra* Barnard and *A. cassida* Barnard (Tillyard 1931; Barnard 1934). Although Barnard (1934) observed four forms within *A. barnardi*, he did not assign species status to these because of the insignificant morphological differences, and because they were connected by what he perceived to be transitional forms. These, and additional, morphologically defined forms were tested for biological species status (*sensu* Mayr 1942, 1970) using mate choice experiments in this study. Similar methodology has previously been used to reveal the presence of sibling species within Plecoptera (Picker 1980), and other groups, for example, in closely related species of Orthoptera (Dagley *et al*. 1994). Morphological character evidence relied mainly on the male paraprocts and epiproct, and the female subgenital plate.

Balinsky (1956) described a morphologically compact group of notonemourid stoneflies; the three species which he designated *Aphanicercella gudu* Balinsky, *Aphanicercella tugelae* Balinsky and *Aphanicercella fontium* Balinsky, are endemic to the Lesotho and KwaZulu-Natal Drakensberg and the Maluti Mountains of Lesotho. The unique set of morphological characters of the group are described and contrasted with those of the rest of the genus, with a view to erecting a new genus (Stevens & Picker 1995). The taxonomy of the other genera, *Afronemoura* Illies, *Aphanicerca* Tillyard, *Aphanicercopsis* Barnard and *Desmonemoura* Tillyard is also revised.

The adults of the southern African Notonemouridae and their distributions have been reasonably well described (Tillyard 1931; Barnard 1934, 1936; Balinsky 1956, 1967; Illies 1980), but the larvae are poorly known. The descriptions of Tillyard (1931) and Barnard (1934) of the larvae of *Aphanicerca* and reference to larvae of other genera are too vague to facilitate the distinction of genera, something the latter admitted when stating that "Nymphs of all four Nemourine genera are practically indistinguishable". Illies (1980) described the larva of *Afronemoura* using a unique feature that ensures reliable identification of this genus. While it is understandable that the first taxonomic treatments should have focused on adults, larval identification merits attention, as it is this stage of the life cycle that is most frequently encountered in ecological surveys. Adults are present for a brief period compared to the aquatic larvae, and are frequently scarcer and more difficult to collect. Moreover, limnological studies

invariably sample a larval stage. There is consequently a need to provide ecologists with a means of identifying larvae, at least to the generic level. In the south-western region of the Western Cape Province, the distribution centre of southern African Notonemouridae (Stevens & Picker 2003), these insects may attain considerable biomass and ecological importance in the mountain streams in which they occur (Davies *et al.* 1993). Moreover, they have considerable potential as bioindicators, being restricted to pristine mountain streams (Dallas & Day 1993). For these reasons, larval identification was emphasised in this chapter.

The aim of the papers comprising this chapter was to re-evaluate the taxonomy of the southern African Notonemouridae, and in so doing to describe new taxa, identify new distribution records, develop a larval identification system and provide identification keys. The study of notonemourid larvae may provide larval character states useful in resolving the question of the monophyly of the group (Zwick 1990), will assist limnologists in larval and adult identifications and will facilitate the production of a phylogeny and biogeographical hypotheses of the group (see Chapter 4). This chapter serves to lay the taxonomic groundwork for the main section of the thesis, Chapters 3 and 4.

MATERIALS AND METHODS

Morphology

All examined material from private collections and museums had been stored in ethanol, usually 70%. Specimens were examined under a Wild stereo-microscope using varying magnifications for descriptions. Drawings were done freehand. Notonemourid taxonomy has been based primarily on external genitalia of the male (Barnard 1934; Illies 1975; McLellan 1991). Morphological terminology follows Illies (1975) and McLellan (1991). Characters of the male useful for the separation of genera and species are: processes on tergite 9, numerous features on segment 10 including pleural processes, dorsal plates, hooks, paraprocts, and the epiproct (segment 11). Females are distinguished by the shape and sclerotization of the subgenital plate (sternite 7 or 8), sclerotization patterns on the sternites, shape and size of the cerci, and the shape of the subanal plates. In this study new terminology has been introduced or existing terminology (of Barnard (1934)) modified to facilitate species distinction. Homology of these characters within the Nemouroidea (Nelson 1984) is not dealt with here. These terms are especially relevant to *Aphanicercella* in which the described structures are all present, but not always so in the other genera. The basal supporting processes of the "titillators" (i.e. paraprocts) (Barnard 1934) are redefined as structures originating on the transverse rods lateral to the median arch of the paraprocts (Fig. 2.18). The transverse rods (the curved, chitinised struts of Barnard (1934)) are transverse struts medially producing the basal supporting processes and primary supporting struts of the paraprocts. Thinner secondary supporting struts may also be

present. Paraprocts are paired structures that play a role in copulation and sperm transfer (Barnard 1934). The arch processes are extensions of the paraproct median arch, which is situated between the bases of the paraprocts. The spinous process is a slender process arising from the arch process in some species. Claspers (Barnard 1934) are the digitate extensions of pleurite 10 (Fig. 2.18). The "supra-anal lobe" (Barnard 1934) is the epiproct. Females are not as readily distinguished as are males. The most diagnostic character is the shape and degree of sclerotization of the subgenital plate. The only other useful character is the extent and pattern of pigment patches on the sternites (Fig. 2.19). However, age of specimen and fading in alcohol both influence the intensity of these markings. In all species sternites 1 and 2 are similarly and entirely pigmented (except *A. bifurcata*).

Larval taxonomy (Picker & Stevens 1997)

All notonemourid larvae are covered in fine setae, named clothing hairs by Hynes (1941), which have not been used as distinguishing characters in this paper. In addition, because they are not taxonomically useful, the cluster of setae on segment 11 in most species is not depicted in the Figures. Only the more robust longer and thicker setae are used as definitive characters ('bristles' when short and tapered, and 'hairs' when finer and longer (Hynes 1941)). They are particularly important when situated on the posterior margin of abdominal segments, as well as on the antennae, wingpads, and dorsal and ventral parts of abdominal segments. The term setae thus refers to hairs longer and thicker than the general body covering. Setation patterns have been used in the description of New Zealand notonemourid larvae (McLellan 1991), and are generally useful in the taxonomy of larval Plecoptera (Hynes 1941).

Similarly, in the African Notonemouridae, comparative larval setal patterns and other characters are useful in discriminating between genera (pers. obs). The setal patterns of earlier instars correlated well with those of mature larvae. Characters used in the separation of genera are assumed to be genus-specific as they were consistent for all of the species examined within each genus. It was not possible to obtain mature larvae of certain species, some of which are known only from a single adult. Moreover, unequivocal association of larva and adult can only be made using mature larvae of the black-wingpad stage, which reveal adult genitalia under the larval cuticle. For these reasons I described only one (the most common) species for each genus. In addition, for *Aphanicerca* two of the six species, *Aphanicercella* three of the six species and for *Aphanicercopsis* two of the four species are compared and discussed. The remaining species are mostly known from limited material and are unlikely to be encountered.

All measurements and descriptions of larvae were taken from mature (black-wingpad) larvae. The extent of fusion of the tergite and sternite of the abdominal segments is a useful

character, where there is a trend for the anterior-most segments to have the tergite and sternite separated by a pleurite, but more posteriorly the tergite and sternite may fuse to form a ring (Hynes 1941; Suter & Bishop 1990). Reported fractional values represent cases of incomplete fusion of sternite and tergite. Colour patterns are variable and, as such, are not widely used (Hynes 1941). The mandibular formula expresses the number of incisors of the right:left mandible.

Mate choice experiments (Stevens & Picker 1999)

Mate choice experiments were carried out using field-collected adults of varying age. Some of these insects had possibly already mated, but this apparently did not affect the behaviour of males, and the large number of successful pairings of within-population trials suggests that it was not necessary to use teneral individuals. The new species names are used here, prior to the results of the experiments and the species descriptions, for convenience sake. The following trials were conducted using members of the *A. barnardi* species complex: *A. clavata* sp. n. (Table Mountain, Cape Peninsula) with *A. bullata* sp. n. (Cederberg Mountains), and *A. clavata* sp. n. with *A. flabellata* sp. n. (Jonkershoek, northern Hottentots Holland Mountains). Two-species trials using *A. clavata* sp. n. and *A. scutata* served as a control. Field-collected individuals were isolated until the mate choice trials commenced (about three hours after capture). One male of each of two forms or species, and one female of one of the forms (or alternatively, females of two forms and a male of one of the forms) were placed together in a closed Petri dish containing a small circle of moist filter paper. All mate-choice trials were closely observed for the entire duration of the experiment (usually about six hours), and any attempted mating was recorded. A mating was scored as successful if mounting and copulation persisted for longer than four minutes. Successful mating lasted at least four minutes after which most pairs were forcibly separated. Pairs that were not separated often remained *in copulo* for five hours or longer. As the various species and forms are almost identical macroscopically, species were marked by cutting off the tips of the wings. Unsuccessful attempts at mating were easily identified by characteristic behaviour patterns of both males and females.

Source of material and abbreviations

Material examined was derived from the following sources: Albany Museum, Grahamstown (AMGC); Private collection of D.M. Stevens & M.D. Picker, University of Cape Town; Max-Planck Institute for Limnology, Schlitz, Germany (MPIL); South African Museum, Cape Town (SAMC); Transvaal Museum, Pretoria (TMSA); Private collection of L. Minter, Pietersburg.

RESULTS AND DISCUSSION

Aphanicercella barnardi species complex (Stevens & Picker 1999)

In the mate choice experiments, males appeared to be indiscriminate in the initial selection of a mate, often attempting to mount females of both available species. However, once genitalic contact had been made, males rejected females of another species. Females could reject males by moving away, or by curling the abdomen dorsally or laterally and by raising the wings over the body. The rejected male would occasionally successfully mount the female but would not be able to copulate. In these cases the pairs remained together for less than four minutes. In successful mating, the pair would not separate if disturbed, whereas in unsuccessful mating where the male had mounted a female but had not copulated, disturbance would result in immediate separation.

In controls where males of *A. scutata* and *A. clavata* were paired with females of both species, nine intraspecific matings occurred from 12 mountings ($P < 0.005$, χ^2 test, $n = 9$) (Table 2.1). Three interspecific mountings did not lead to mating. Where males of *A. flabellata* sp. n. and *A. clavata* were paired with females of these taxa, 34 positive assortative matings resulted from 48 mountings ($P < 0.001$, χ^2 test). Eleven intertaxon mountings resulted in one probable mating. In the trials where *A. bullata* and *A. clavata* males were given a choice of females of these taxa, nine positive assortative matings resulted from 10 mountings ($P < 0.005$, χ^2 test). There were neither inappropriate matings nor mountings (Table 2.1).

Table 2.1. Results of three mate choice experiments: *Aphanicercella clavata* sp. n. paired with *A. scutata; A. flabellata* sp. n. with *A. clavata* sp. n.; and *A. bullata* sp. n. with *A. clavata* sp. n.

Pairings	Mean duration and range (min)	Number of successful matings	Number of trials
A. clavata sp. n. x *A. clavata* sp. n.	11.8 (5-34)	5	
A. scutata sp. n. x *A. scutata* sp. n.	22.0 (5-39)	4	9
A. clavata sp. n. x *A. scutata* sp. n.	0	0	
A. flabellata sp. n. x *A. flabellata* sp. n.	103.5 (4-300)	12	
A. clavata sp. n. x *A. clavata* sp. n.	69.6 (2-195)	22	35
A. flabellata sp. n. x *A. clavata* sp. n.	60	1	
A. bullata sp. n. x *A. bullata* sp. n.	67.3 (30-120)	6	
A. clavata sp. n. x *A. clavata sp. n.*	19.7 (4-40)	3	9
A. bullata sp. n. x *A. clavata* sp. n.	0	0	

Both males and, to a lesser extent, females of the species of *Aphanicercella* were readily distinguished morphologically. The six species comprising the *A. barnardi* species complex were also clearly distinguishable, although the similarity of structures such as the epiproct suggested a close phylogenetic relationship for members of this species complex. Very little intraspecific variation and no intermediate forms were observed, implying a lack of interbreeding between the forms. These observations were further supported by the positive

assortative matings observed for those members of the *A. barnardi* species complex tested in mate choice trials. Although it was only feasible to test a limited number of forms against one another, the conclusive results suggested that the consistent morphological differences separating all the forms are a morphological reflection of their valid biological species status (Mayr 1970). Mate-choice trials are very informative in cases where alpha-taxonomy is able to resolve forms on consistent but minor differences, but a decision concerning the biological species status of such taxa is uncertain. In some cases speciation is not accompanied by concomitant morphological changes, at least not initially. In such instances, the general interpretation is that speciation has been recent. Dagley *et al*. (1994) found that in *Chorthippus parallelus* (Zetterstedt) grasshoppers, positive assortative mating based on changes in acoustic components of the courtship preceded major morphological changes, possibly initiated by geographical isolation during the Pleistocene. However, they also recorded that in cases where genitalia function in a 'lock and key' manner (Shapiro & Porter 1989), morphological changes in genitalia would be expected to match parallel divergence in mating behaviour and genitalia.

The sensory mode used for species recognition in *Aphanicercella* is not known. Drumming, a widespread communication medium used in plecopteran courtship (Stewart & Maketon 1990), was not observed in *Aphanicercella*, although Nelson (1984) recorded its occurrence in Notonemouridae, and it does occur in *Aphanicerca* (pers. obs.). The various species within the *A. barnardi* complex can be regarded as sibling species since they can only be distinguished morphologically by minor, yet consistent differences in genitalia. Their geographical distributions were also found to be far more localized than the other species of *Aphanicercella* (Appendix 7.7), supporting the contention that they had speciated relatively recently, and had not expanded their ranges to any degree. Aspects of their biogeography are covered in Chapter 4.

Possibly the best indicator of lack of significant gene flow between the various species of the *A. barnardi* complex was the absence of intermediates in the field. However, most species of *Aphanicercella* occur allopatrically, and collections of notonemourids typically comprised sympatric communities of single species representatives from a few genera. Only rarely are congeneric species collected at the same locality. The other genera of Notonemouridae in South Africa may also include sibling species complexes: varieties have been noted by Barnard (1934) in *Aphanicerca capensis*, and are investigated in Chapter 3.

The combination of significant variation in the genitalia of both males and females of the varieties of *A. barnardi*, and the positive assortative mating observed during experimental trials, confirmed the existence of multiple species within the previously recognised single species. As

a result, the type specimen of *A. barnardi* retains the designation, and the varieties are described below as *A. bullata* sp. n., *A. clavata* sp. n., *A. flabellata* sp. n., *A. securata* sp. n. and *A. spatulata* sp. n. These descriptions have been published in Stevens & Picker (1999).

Revision of the remaining genera (Picker & Stevens 1999)

Sufficient morphological differentiation was found between *Aphanicercella gudu, A. tugelae* and *A. fontium* and the rest of the genus *Aphanicercella* to justify erection of a new genus, *Balinskycercella*, to accommodate these three species (Stevens & Picker 1995). No new species of *Aphanicercopsis* (four species described by Barnard (1934)) and *Balinskycercella* gen. n. (three species described by Balinsky (1956)) have been found. A second species of *Desmonemoura* is described here from the Oudtshoorn area, whereas the distribution of *D. pulchellum* is centred in the south-western Western Cape Province. Two additional and localised species of *Aphanicerca* and a third species of *Afronemoura* are also described. The previously unknown female of *Aphanicerca bovina* Barnard is described (Picker & Stevens 1999).

Comparative larval morphology (Picker & Stevens 1997)

Taxonomically useful differences in pleurite number (Table 2.2) and dorsal and ventral abdominal setation patterns between genera and between some species were found (Table 2.3; Figs 2.1-2.3) (Picker & Stevens 1997), in spite of species identification of larval Plecoptera being generally difficult (Hynes 1941). McLellan (1991) described many New Zealand species that appear far more distinct from one another than the southern African genera. It is possible to distinguish the six southern African genera of Notonemouridae using many of the characters used by McLellan. Species identifications, based on setae, could only be made for certain species, including the most common species in the Western Cape Province. Ideally, large collections, preferably containing black-wingpad nymphs, should be used for identifications. The latter, if sufficiently mature, will have adult genitalia visible through the larval cuticle, and can easily be identified on adult features (Barnard 1934; Balinsky 1956, 1967; Illies 1980). Correlations with any adults that may be present are also very useful. Distributional data may also be useful indicators of species identifications, as most genera and species have limited distributions.

Table 2.2. Number of segments with pleurites (pleurites are present on the first and a variable number of consecutive segments). In all cases standard deviation = 0.

Species	Mean no. of pleurites
Afronemoura amatolae	5.0 (*n* = 1)
Aphanicerca bicornis	5.5 (*n* = 1)
Aphanicerca capensis	5.0 (*n* = 7)
Aphanicerca lyrata	5.0 (*n* = 1)
Aphanicercella bifurcata	6.0 (*n* = 14)
Aphanicercella cassida	6.0 (*n* = 11)
Aphanicercella clavata sp. n.	6.0 (*n* = 19)
Aphanicercella scutata	6.5 (*n* = 1)
Aphanicercopsis denticulata	4.0 (*n* = 2)
Aphanicercopsis outeniquae	3.0 (*n* = 4)
Aphanicercopsis tabularis	3.5 (*n* = 5)
Balinskycercella tugelae	7.5 (*n* = 1)
Desmonemoura pulchellum	5.0 (*n* = 3)

The genera of the southern African Notonemouridae were readily separable, both as adults and larvae, although species identification of larvae could be difficult. In addition to the generic larval key, keys are provided to males and females to genus level, and to males of all described species. A species key to females is impracticable due to the close similarity of species within most genera. This is because it was found that very few characters (namely, shape of the subgenital and subanal plates, shape of ovipositor when present and sclerotization patterns) could be used to distinguish females of different species. Species differences between females could be very subtle, and confident determinations were best done in associations with males.

Biogeography

The biogeography is treated in more detail in Chapter 4. The distribution of the southern African Notonemouridae agrees closely with that of other palaeogenic invertebrates, following the boundaries of the Cape Folded Mountains (*sensu* Kleynhans *et al*. 2005) and the Great Escarpment. This substantiates many of Stuckenberg's (1962) observations for distribution trends of the palaeogenic invertebrates. There is, for example, a distinction between the notonemourid fauna of the Cape Folded Mountains compared to that of the Great Escarpment. More specifically, the former is both more speciose, and supports a greater number of genera. The KwaZulu-Natal Drakensberg and Maluti Mountains of Lesotho support the endemic genus *Balinskycercella*. The Grahamstown area is regarded by Stuckenberg as a transition zone between the Cape and the Eastern Highland centres. Its distinction is based on climatic and geological isolation from the major palaeogenic habitats (Stuckenberg 1962). The occurrence of a single species of notonemourid in the Grahamstown area and the sudden transition to new genera east of Grahamstown supports this distinction.

Table 2.3. Average number of setae per abdominal segment. Range in brackets. *Aphanicercopsis* species: *A. tabularis*, *A. denticulata*, *A. outeniquae*; *Balinskycercella tugelae*; *Desmonemoura pulchellum*; *Aphanicerca* species: *A. capensis*, *A. bicornis*, *A. lyrata*; *Aphanicercella* species: *A. clavata* sp. n., *A. bifurcata*, *A. cassida*, *A. scutata*; *Afronemoura amatolae*.

Dorsal abdominal hairs						
Abdominal segment	*A. tabularis* *n* = 5	*A. denticulata* *n* = 2	*A. outeniquae* *n* = 4	*B. tugelae* *n* = 1	*A. amatolae* *n* = 1	*D. pulchellum* *n* = 3
1	3.6 (1-6)	3.5 (3-4)	2.8 (1-4)	8	2	1.0 (0-3)
2	5.8 (5-7)	3	1.8 (0-4)	8	3	1.3 (0-4)
3	8.0 (6-10)	6.5 (6-7)	5.5 (4-8)	8	4	6.7 (3-12)
4	9.6 (8-12)	7.0 (6-8)	8.0 (6-10)	16	4	7.7 (5-12)
5	10.8 (10-12)	11.0 (8-14)	9.3 (5-12)	24	5	9.3 (4-16)
6	11.2 (10-12)	14.0 (12-16)	7.5 (6-10)	26	11	12.0 (4-20)
7	14.4 (8-26)	24.0 (22-26)	7.0 (4-8)	40	10	13.0 (3-20)
8	12.0 (8-16)	17.0 (6-26)	9.0 (6-12)	60	10	16.7 (12-22)
9	12.4 (10-14)	16.0 (8-24)	12.0 (8-14)	60	4	16.0 (10-22)
10	17.6 (8-50)	5.5 (3-8)	7.0 (4-8)	70	8	8.0 (6-10)

Dorsal abdominal hairs							
Abdominal segment	*A. capensis* *n* = 7	*A. bicornis* *n* = 1	*A. lyrata* *n* = 1	*A. clavata* sp. n. *n* = 19	*A. bifurcata* *n* = 16	*A. cassida* *n* = 12	*A. scutata* *n* = 2
1	0	0	0	2.5 (2-5)	0	5.3 (2-8)	1.0 (0-2)
2	0	0	0	2.4 (2-4)	0	3.9 (2-6)	2
3	0	0	0	2.3 (2-4)	0	4.3 (2-7)	2
4	0	0	0	2.1 (1-4)	0	4.5 (2-6)	2
5	0	0	0	2.0 (1-3)	0	5.7 (4-8)	1.5 (1-2)
6	0	0	0	2	0	6.9 (5-10)	2
7	0	0	0	2.0 (1-3)	0	10.4 (8-18)	2
8	0.1 (0-1)	0	0	3.1 (2-6)	1.3 (0-2)	14.5 (12-18)	3.5 (3-4)
9	1.0 (0-2)	0	0	5.1 (4-9)	4.7 (3-8)	19.1 (14-22)	6.5 (6-7)
10	2.9 (0-8)	3	0	5.7 (3-9)	6.0 (4-8)	20.5 (16-24)	9.0 (6-12)

Ventral abdominal hairs						
Abdominal segment	*A. tabularis* *n* = 5	*A. denticulata* *n* = 2	*A. outeniquae* *n* = 4	*B. tugelae* *n* = 1	*A. amatolae* *n* = 1	*D. pulchellum* *n* = 3
1	0.8 (0-4)	0	0	0	6	0
2	3.2 (2-6)	0	0	12	4	0
3	4.8 (3-10)	4.0 (0-8)	0	10	4	0
4	6.8 (4-14)	8	0	8	4	0
5	9.2 (6-12)	10.0 (8-12)	0.5 (0-2)	8	2	3.3 (2-4)
6	11.2 (6-16)	15.0 (14-16)	2.0 (0-8)	20	4	4.0 (2-6)
7	18.0 (12-22)	23.0 (20-26)	4.0 (0-10)	20	4	2.7 (2-4)
8	34.8 (24-44)	26.0 (22-30)	12.5 (10-16)	30	6	6
9	44.0 (26-60)	24.0 (20-28)	16.5 (14-20)	40	6	12 (6-20)
10	5.2 (2-6)	3.0 (2-4)	0	10	2	2

Ventral abdominal hairs							
Abdominal segment	*A. capensis* *n* = 7	*A. bicornis* *n* = 1	*A. lyrata* *n* = 1	*A. clavata* sp. n. *n* = 19	*A. bifurcata* *n* = 16	*A. cassida* *n* = 12	*A. scutata* *n* = 2
1	0	0	0	0	0	0.1 (0-2)	0
2	0	0	0	2.0 (1-4)	0	1.5 (0-4)	2.0 (0-4)
3	0	0	0	1.9 (0-4)	0	3.1 (0-4)	3.0 (2-4)
4	0	0	0	1.9 (0-4)	0	3.1 (2-4)	2
5	0	0	0	1.8 (0-2)	0	3.9 (2-6)	3.0 (2-4)
6	0	0	0	1.9 (1-3)	0	4.3 (2-6)	3.0 (2-4)
7	0.3 (0-2)	0	0	1.9 (1-2)	0.1 (0-1)	6.5 (4-10)	2
8	0.9 (0-2)	0	0	2.2 (1-4)	2.0 (0-4)	9.5 (6-20)	2.5 (2-3)
9	3.7 (2-8)	4	2	3.8 (1-6)	6.3 (4-8)	16.3 (8-22)	6
10	4.6 (0-10)	5	0	0.6 (0-4)	0	2.2 (2-4)	2

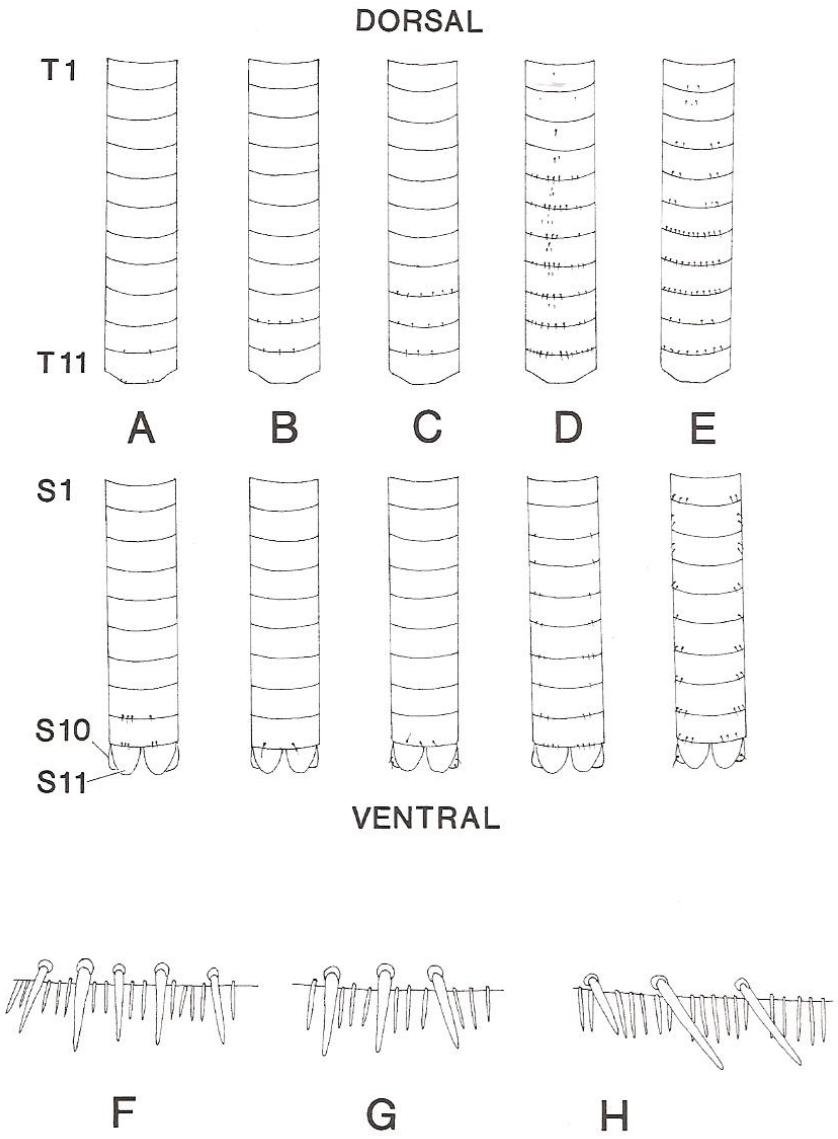


Fig. 2.1. *Aphanicerca*, *Desmonemoura* and *Afronemoura* schematic abdominal setal patterns (**A - E**), and magnifications (x 1000) of portions of tergite 8 (**F**, **G** & **H**). **A**, *Aphanicerca bicornis* (Langrivier, Swartboskloof, Stellenbosch, northern Hottentots Holland Mountains); **B** & **F**, *Aphanicerca lyrata* (Molenaars River, Du Toitskloof, northern Hottentots Holland Mountains); **C**, *Aphanicerca capensis* (Platteklip Stream, Table Mountain, Cape Peninsula); **D** & **G**, *Desmonemoura pulchellum* (Molenaars River, Du Toitskloof, northern Hottentots Holland Mountains); **E** & **H**, *Afronemoura amatolae* (Graskop, Mpumalanga Drakensberg). Abbreviations: S = sternite; T = tergite.

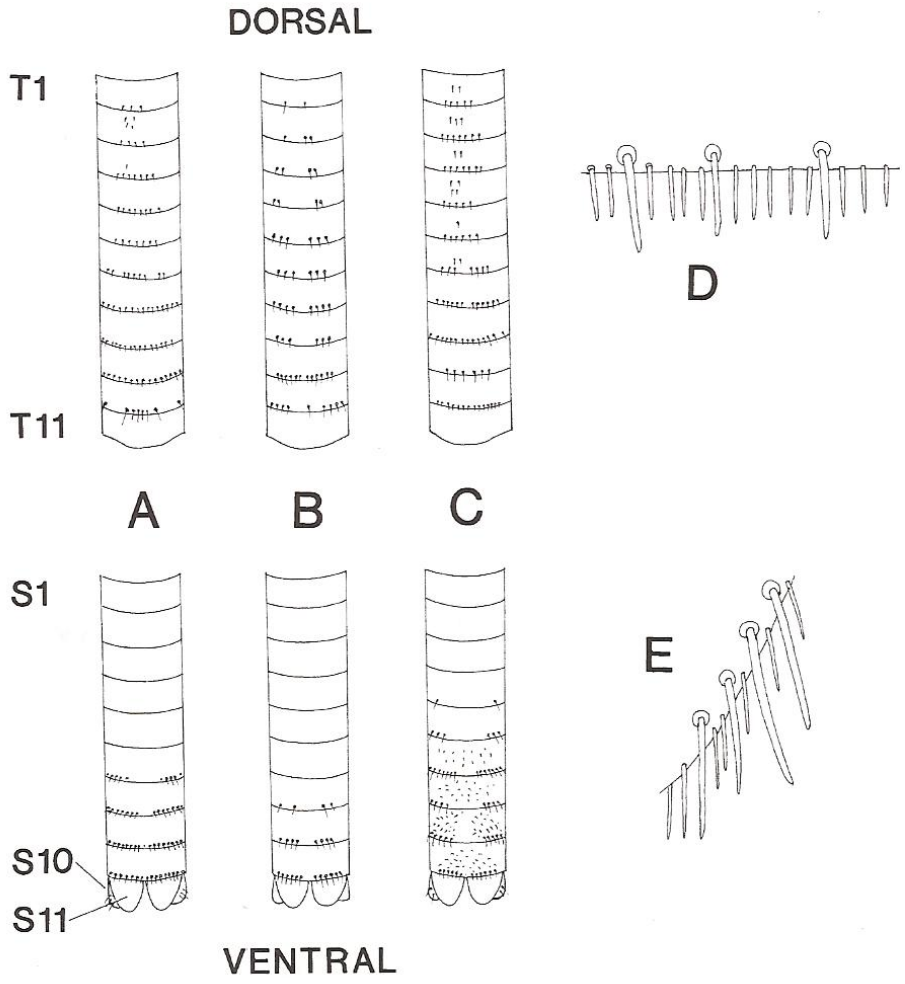


Fig. 2.2. *Aphanicercopsis* schematic abdominal setal patterns (**A** - **C**), and magnifications (x 1000) of portions of tergite 8 (**D**), and subanal plate (**E**). **A**, *Aphanicercopsis denticulata* (Pilkington Bridge, Bain's Kloof, northern Hottentots Holland Mountains); **B**, *Aphanicercopsis outeniquae* (Prince Alfred's Pass, Knysna, Outeniqua Mountains); **C**, **D** & **E**, *Aphanicercopsis tabularis* (Twelve Apostles, Cape Peninsula). Abbreviations: S = sternite; T = tergite.

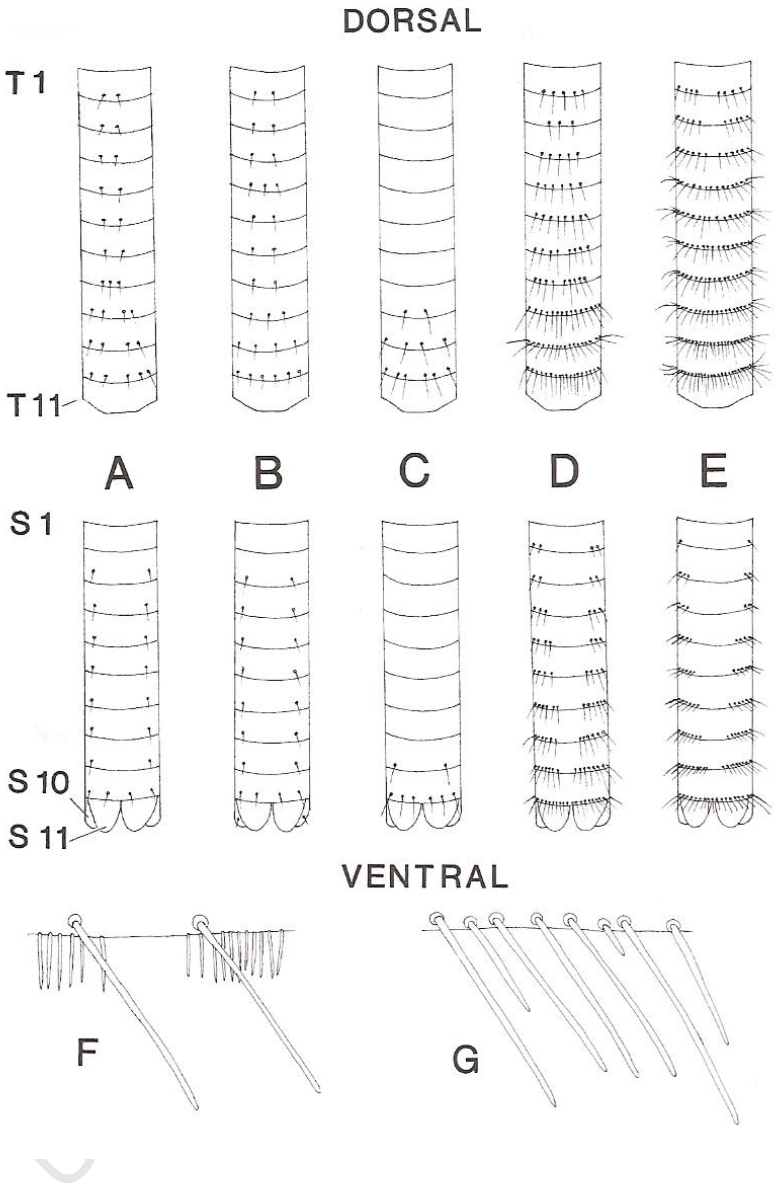


Fig. 2.3. *Aphanicercella* and *Balinskycercella* schematic abdominal setal patterns (**A** - **E**), and magnifications (x 1000) of portions of tergite 8 (**F** & **G**). **A**, *Aphanicercella barnardi* (Platteklip Stream, Table Mountain, Cape Peninsula); **B** & **F**, *Aphanicercella scutata* (Witte River, Bain's Kloof, northern Hottentots Holland Mountains); **C**, *Aphanicercella bifurcata* (Hoekwil, George district, Outeniqua Mountains); **D**, *Aphanicercella cassida* (Loerie, Eastern Cape); **E** & **G**, *Balinskycercella tugelae* (Mangaung River, Lesotho). Abbreviations: S = sternite; T = tergite.

TAXONOMY (Stevens & Picker 1995, 1999; Picker & Stevens 1997, 1999)

This section describes the new genus *Balinskycercella* gen. n., followed by the taxonomic revision of *Afronemoura*, *Aphanicerca*, *Aphanicercella*, *Aphanicercopsis*, and *Desmonemoura*.

Genus ***Balinskycercella* gen. n.**

Type species: *Aphanicercella gudu* Balinsky, 1956: 294.

Diagnosis

The new genus is most similar to *Aphanicercella* as regards male and female genitalia. Detailed descriptions of the three known species are given in Balinsky (1956). The species of *Balinskycercella* are larger then *Aphanicercella*, have an unpigmented patch in the middle of the forewing, and have lightly pigmented femorotibial junctions (black in *Aphanicercella*). In the male, anterior apex of the median plate of tergite 10 bears a recurved hook, not present in *Aphanicercella*. Claspers formed by pleurite 10 shorter and more robust than in *Aphanicercella*. The epiproct is more rounded basally and more sclerotized than in *Aphanicercella*. The female genitalia of the two genera are very similar.

Description

General colouration dark-brown. Abdomen lighter-brown, but terminal segments 9 and 10 dark-brown. *Pronotum* broader than long, with weak longitudinal suture. *Head* dark-brown, clypeus and scape dull-orange. Compound eyes violet-brown; ocelli cream-coloured. Femora light-tan, darkening distally. *Wings* smoky-brown (greyer in hind wings) with darker shading on veins and in costal and basal regions. Unpigmented line dividing radiomedial cell longitudinally. Intercubital crossveins 7-13 in number. Clear patch present in middle of forewing, at junction of MA_1 and MA_2 with RS.

Male genitalia. Sternite 9 extended posteriorly, ending in a short rounded tip. Basal process of sternite 9 short to moderately long with terminal bulb. Tergite 9 excised posteriorly and anteriorly (but only posteriorly in *B. fontium*). Tergite 10 comprises two lateral sclerotized plates narrowing posteriorly, between which lies a median triangular sclerotized plate. Base of median plate concave, and anterior tip narrow and produced dorsally to form a single or bifid, highly sclerotized recurved hook (Fig. 2.26E). Two small sclerites posterior to median plate support the epiproct. Lateral plates lacking scabrous knobs. Pleurites of segment 10 produced posteriorly, narrowing gradually to form stout claspers with subacute tips. Epiproct robust, with rounded base. Lateral margins broadly chitinized. Paraprocts broad, long, and curved upwards, with medial chitinized strip. Cerci of variable length, membranous medially.

Female genitalia. Subanal plates variable in shape, but rounded and frequently narrowed posteriorly. Subgenital plate weakly sclerotized. Sclerotized, rounded vaginal plate may be present (Fig. 2.27E). Anterior margin of sternite 9 with median, weakly chitinized crescent or square. Cerci of variable length, membranous medially. Tergite 10 with rounded tip posteriorly.

Larva. The larva of *B. tugelae* is described below (Fig. 2.4). The larvae of the other two species are unknown.

Distribution. Headwaters in the Mont-aux-Sources district of the north-eastern escarpment of the Lesotho-Drakensberg Highlands, and Maluti Mountains of western Lesotho.

Etymology. Named after B.I. Balinsky, in recognition of his contribution to the taxonomy of the southern African Notonemouridae, and in combination with the closely related genus, *Aphanicercella*.

Balinskycercella gudu (Balinsky) **comb. n.**

Aphanicercella gudu Balinsky, 1956: 294.

Type material examined. 4♂, 12♀, paratypes: 'Gudu R. Mt. aux Sources / Natal. B. Balinsky 31.i.1954' (SAMC C003173/3217).

Additional material examined. LESOTHO: 3♂, 8♀, Tributary at Qiloane Falls, Makheleng River, Maluti Mountains, 29.24S 27.55E, 7.i.95, D.M. Stevens Private Collection.

Balinskycercella tugelae (Balinsky) **comb. n.**

Aphanicercella tugelae Balinsky, 1956: 297.

Description of larva. Most parts of body covered in long setae (Figs 2.4, 2.3E,G).

Size (mm). Medium-sized to large larvae; male body length 6.4.

Head. Mottled reddish-brown; three small ocelli; compound eyes black; whorl of fine short setae on anterior margin of antennal segments.

Thorax. *Prothorax* mottled reddish-brown, with pale median stripe; head width (male 1.0 mm) similar to pronotum (0.9 mm) and mesonotum; pronotum extending laterally beyond margins of prothorax; margins of prothorax bearing fringe of long setae. *Mesothorax and metathorax* pale-brown, with long setae along anterior and posterior margins and medial to area of wingpad attachment.

Wingpads. Dorsal surface with numerous long setae.

Legs. Pale-yellow-brown, with long setae laterally; setae sparse on lateral surface of tibia.

Abdomen. Mottled reddish-brown, with conspicuous dorsal whorls of long setae (complete on segments 8-10) (Fig. 2.3E,G); pleurites usually evident on first 8 segments (Table 2.2).

Cerci. Whorl of short bristles on posterior margin of segments.

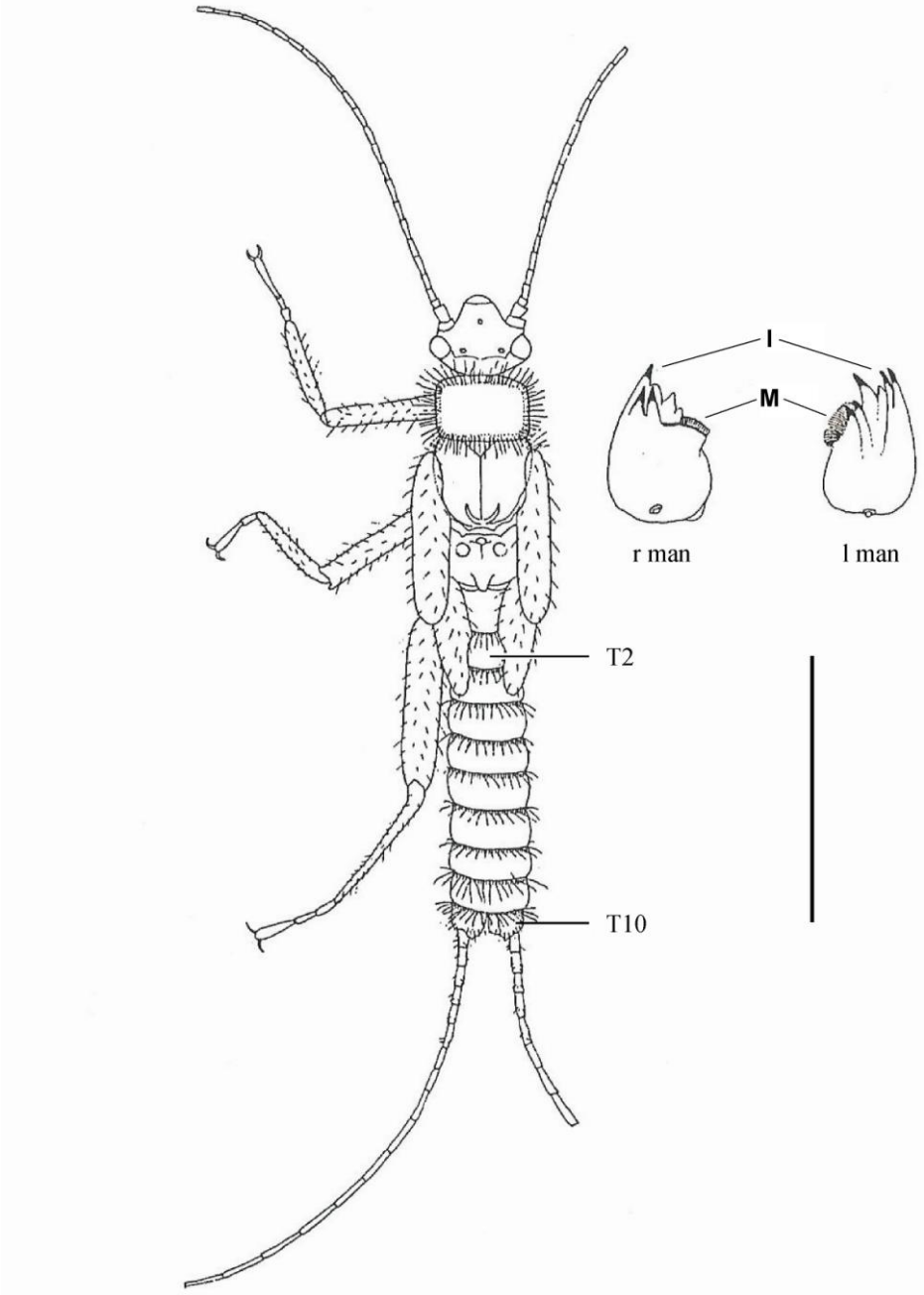


Fig. 2.4. Dorsal view of final instar (black-wingpad) larva of *Balinskycercella tugelae* (Mangaung River, Lesotho). Abbreviations: I = incisor; l man = left mandible; M = molar; r man = right mandible; T2 = tergite 2; T10 = tergite 10. Scale bar = 2 mm.

Type material examined. 3♂, 7♀, paratypes: 'Tugela R. Mt. aux Sources / Natal / 25.i.1954.' (coll. B. I. Balinsky) (SAMC C003172/3216).

Additional material examined. SOUTH AFRICA: *KwaZulu-Natal*, 2♂, 14♀, Gudu Falls, Mont-aux-Sources, Royal Natal National Park, 28.41S 28.44E, 28.xii.58, B.I. Balinsky (TMSA, no. 16); 4♂, 6♀, Gorge, Mont-aux-Sources, Royal Natal National Park, 28.41S 28.44E, 31.xii.58, B.I. Balinsky (TMSA); 1♂, 6♀, streams near Crystal Falls, Champagne Castle, 29.05S 29.20E, 6.i.58, B.I. Balinsky (TMSA). LESOTHO: 1♂, 11♀, Oxbow, 28.46S 28.36E, 21.i.90, L. Minter (LRMC); Larva: LESOTHO: 1♂, Mangaung River, 29.33S 29.13E, 25.xi.1988, L.R. Minter.

Balinskycercella fontium (Balinsky) **comb. n.**

Aphanicercella fontium Balinsky, 1956: 299.

Type material examined. Holotype ♂, allotype ♀, 'Tugela R. Mt. aux Sources, Natal / 25.i.1954' (TMSA).

Genus ***Afronemoura*** Illies

Afronemoura Illies, 1980: 211.

Type species: *Aphanicercopsis amatolae* Balinsky, 1956: 290, by subsequent designation of Illies (1980).

Afronemoura stuckenbergi **sp. n.**, Figs 2.6-2.8

Diagnosis

The male is most similar to *A. spinulata* from which it differs by having two spines on the bilaterally concave posterior margin of tergite 9 (bilaterally concave margin supporting a single spine on a very slight median convexity in *A. spinulata*), and by the shape of the dorsal plates (anterior margin more angular in *A. spinulata*). In *A. amatolae* the posterior margin of tergite 9 is convex. The female differs from the other two species in that the lateral margins of the subgenital plate bear a rounded lateral projection halfway along its length.

Description

Male. *Size*. Body length 6.0 mm, *n* = 1.

Male genitalia. (Figs 2.6, 2.7). Posterior margin of tergite 10 bilaterally concave and produced into a median projection comprising two spinous projections joined together by a sclerotized band. Dorsal plates of tergite 10 subtriangular with convex lateral margin, concave medial margin and rounded transverse anterior margin. Posteroventral angle sclerotized and

serrated with the most lateral denticle more prominent. Epiproct thin and fusiform. Primary supporting strut of the paraprocts situated laterally and is flat and broad, with concave anterior margin and convex posterior margin, and terminating acutely; continuous with transverse rod which is incomplete centrally. Median arch flat and broad and produced into a slender, apically acute arch process. Sternite 9 subtriangular and elongated.

Female. *Size*. Body length = 7.2 mm, *n* = 1.

Female genitalia. (Fig. 2.8). Subgenital plate tapers to a parallel-sided terminal projection of which the distal half is bifid; extends almost as far as the apex of the subanal plates; lateral margins bear a rounded projection. Subanal plates triangular, with the dorsal margin abruptly concave halfway along its length.

Larva. Unknown.

Etymology. Named in honour of Dr Brian Stuckenberg (former Director of Natal Museum) for his contribution to southern African entomology.

Type material examined. Holotype ♂, 1 ♀ paratype, SOUTH AFRICA: *Mpumalanga*, 'Mariepskop / E. Transvaal / 4 October 1956 / B. Stuckenberg', 24.32S 30.53E, (MPIL).

Afronemoura amatolae (Balinsky)

Aphanicercopsis amatolae Balinsky, 1956: 290.

Afronemoura amatolae (Balinsky): Illies, 1980: 211.

Description of larva. Appearing smooth, but with stout setae on tergites 8-10 (Figs 2.1E,H, 2.5).

Size (mm). Large larvae; male body length 6.8.

Head. Pale-brown, with irregular reddish-dark-brown markings anterior to transverse ecdysial suture only; three small black ocelli; large black-violet compound eyes; whorls of setae on distal margin of antennal segments; diagnostic group of enlarged setae situated approximately one third of the way up antennae, first evident as gradual enlargement of setae for 7 segments, followed by a dense tuft of enlarged setae on the next 3-4 segments.

Thorax. *Prothorax* brown; similar in width (male 1.0 mm) to head capsule (male 0.9 mm), but wider than mesonotum; extending laterally beyond margins of prothorax; margin lined with short, stout hairs. *Mesothorax and metathorax* brown; short setae on anterior margin, and a cluster of longer setae at anterolateral margins.

Wingpads. Appearing smooth, but with enlarged setae proximally.

Legs. Yellow-brown, with short hairs laterally, and glabrous stripe on femur.

Abdomen. Pale-brown with irregular darker patterning; enlarged stout spines on posterior margins of tergites 8-10 (Fig. 2.1E,H); pleurites evident on first five segments (Table 2.2).

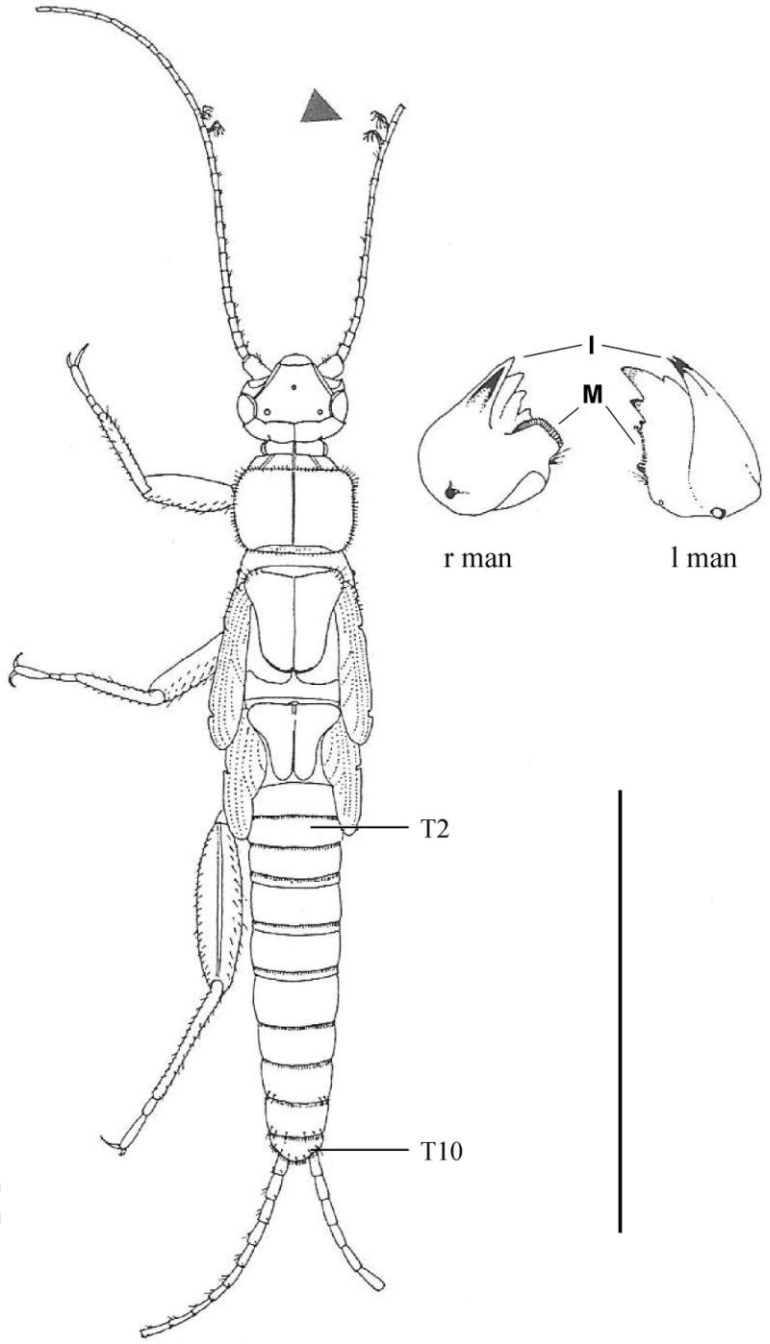


Fig. 2.5. Dorsal view of final instar (black-wingpad) larva of *Afronemoura amatolae* (Graskop, Mpumalanga). Arrow indicates diagnostic medial tuft of antennal hairs. Abbreviations: I = incisor; l man = left mandible; M = molar; r man = right mandible; T2 = tergite 2; T10 = tergite 10. Scale bar = 2 mm.

Cerci. Whorl of short stout bristles on distal margins of segments.

Remarks. This species has a disjunct distribution, occurring in the Amatola Mountains in the south and the Mpumalanga Drakensberg in the north. It has not been recorded from suitable mountainous habitat between these zones. It is sympatric with *A. spinulata* in the Amatola Mountains and Grahamstown areas.

Material examined. SOUTH AFRICA: *Mpumalanga*, 1♂, Mt. Sheba, 24.57S 30.44E, 6.xi.1985, B. I. Balinsky (TMSA); 1♂, Graskop, 24.55S 30.50E, 10.iv.1976, M.D. Picker (SAMC). *Eastern Cape Province*, 1♂, 3♀, Hogsback, way to Waterfall, seepage area, 32.34S 26.58E, 29.xi.1979, J. Illies (MPIL); 8♂, same data but Hogsback, Madonna & Child Falls; 1♂, 9♀, same data but Hogsback, Waterfall; 3♂, 7♀, Tyume Cascades, Hogsback, 32.39S 26.53E, 25.xi.1964, J. Illies (MPIL).

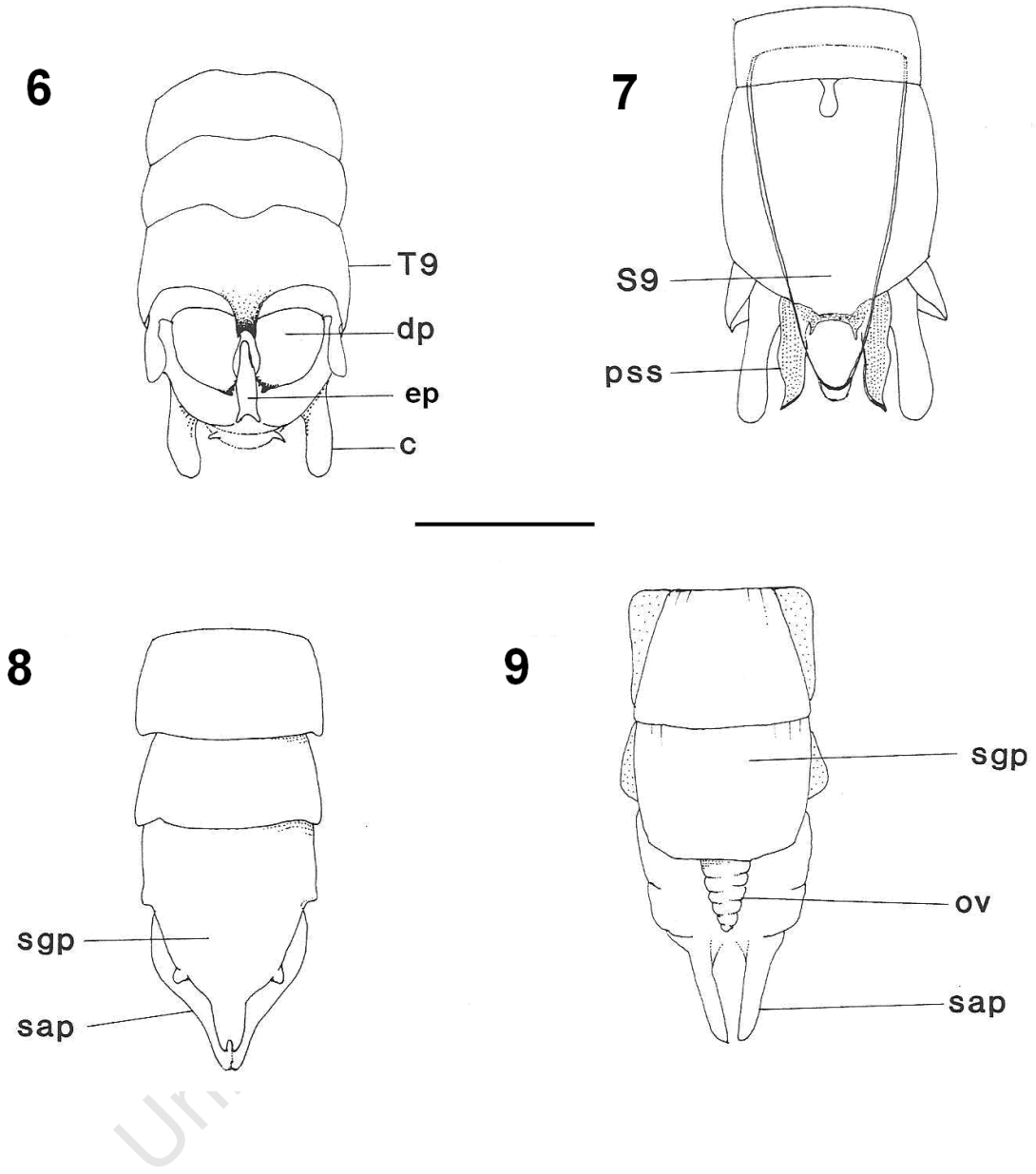
Afronemoura spinulata (Balinsky)

Aphanicercopsis spinulata Balinsky, 1967: 148.

Afronemoura spinulata (Balinsky): Illies, 1980: 211.

Remarks. *A. spinulata* has a widespread distribution from Grahamstown, Hogsback and the Amatola Mountains in the south, to the KwaZulu-Natal midlands, apparently restricted to forest streams. The species has not been recorded from mountain streams of the nearby KwaZulu-Natal Drakensberg. Specimens from Ngome Forest are unusual in that the males have a particularly large spine on tergite 9 and females show a polymorphism for subgenital plate (ovipositor) length, which is either short or very long.

Material examined. SOUTH AFRICA: *KwaZulu-Natal*, 4♂, 1♀, Mkomazi River, Himeville district, 29.36S 29.38E, 11.xii.1979, J. Illies (MPIL); 2♂, 1♀, Nottingham Road, 50 km from Underberg, 11.xii.1979, J. Illies (MPIL); 8♂, 25♀, Town Bush, Pietermaritzburg, 29.34S 30.18E, v.1974, R. Miller (MPIL); 3♂, 35♀, Ngome Forest, 27.51S 31.25E, 31.x-4.xi.1970, H. & M. Townes (MPIL); 17♂, 35♀, Karkloof, 30.25S 30.17E, 23.xi-5.xii.1970, H. & M. Townes (MPIL); 1♂, 1♀, Ngele Forest near Mackton Cottage, 30.31S 29.44E, 1.iii.1990, collector unknown (AMGC). *Eastern Cape Province*, 1♂, 6♀, Hogsback, 32.35S 26.56E, 6.ix.1986, S. van Noort (SAMC).



Figs 2.6-2.9. **6-8**, *Afronemoura stuckenbergi* sp. n.. **6**, male genitalia, dorsal view; **7**, male genitalia, ventral view; **8**, female genitalia, ventral view. **9**, *Aphanicerca bovina*, female genitalia, ventral view. Abbreviations: c = cercus; dp = dorsal plates of tergite 9; ep = epiproct; ov = ovipositor; pss = primary supporting strut; S9 = sternite 9; sap = subanal plate; sgp = subgenital plate; T9 = tergite 9. Scale bar = 1 mm.

Genus ***Aphanicerca*** Tillyard

Aphanicerca Tillyard, 1931: 117.

Type species: *Aphanicerca capensis* Tillyard, 1931: 119 by original designation.

Aphanicerca bicornis Barnard

Aphanicerca bicornis Barnard, 1934: 530.

Remarks. Although not nearly as common as *A. capensis*, this is the second-most widely distributed species of *Aphanicerca*. Intermediate forms, possibly representing hybrids between *A. bovina* and *A. bicornis* have been collected at Jonkershoek (see under additional material examined), where both putative parental species occur. The dorsal process of tergite 9 resembles that of *A. bovina*, but the epiproct resembles that of *A. bicornis*. The sclerotized knobs on the dorsal plates of tergite 10 have features of both species, being situated posteriorly but lacking the spine of *A. bovina*. The shape of the subgenital plate of the female is also intermediate between that of the two species. However, it is also possible that this 'form' represents a distinct species.

Type material examined. No holotype designated. Lectotype ♂, SOUTH AFRICA: *Western Cape Province*, 1♂, E side of Franschhoek Pass, 33.55S 19.05E, 7.v.1933, H.G. Wood (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 9♂, 12♀, Langrivier, Jonkershoek, 33.57S 18.56E, 5.vii.1996; 2♂, 3♀, same data but 5.viii.1995; 2♀, Eerste River, Tweede Tol, Bain's Kloof, 33.36S 19.08E, 4.vii.1994, M. D. Picker (SAMC); 18♂, 7♀, Franschhoek Pass, 33.55S 19.05E, 23.v.1993; 15♂, 7♀, same data but 23.v.1995; 14♂, 14♀, Jan Joubertsgat Bridge, Franschhoek Pass, 33.56S 19.10E, 8.v.1994; 1♂, 1♀, Molenaars River, 33.44S 19.08E, 16.vi.1994; 6♂, 2♀, High Noon, N Villiersdorp, 34.58S 19.11E, 8.v.1994; 2♂, E side of Franschhoek Pass, 33.55S 19.05E, 7.v.1933, H.G. Wood (SAMC); 1♂, Fouche's Hoek, Mostertshoek Mountains, 33.27S 19.17E, 17.iv.1933, K.H. Barnard (SAMC); all M.D. Picker & D.M. Stevens (SAMC) unless otherwise stated.

Intermediate form, SOUTH AFRICA: *Western Cape Province*, 2♂, 3♀, Cape Nature Conservation Offices, Jonkershoek, 33.57S 18.56E, 15.viii.1995; 2♂, Langrivier, Stellenbosch, 33.57S 18.56E, 5.vii.1996; both M.D. Picker & D.M. Stevens (SAMC).

Aphanicerca bovina Barnard, Fig. 2.9

Aphanicerca bovina Barnard, 1934: 531.

Description

Female. *Size*. Body length 6.2 ± 0.4 mm, *n* = 12 (allotype ♀ = 6.1 mm).

Female genitalia. (Fig. 2.9). All sternites completely sclerotized save laterally. Subgenital plate short and broad with slightly convex posterior margin and connected to ovipositor above by a thin, median, dorsally-directed sclerotized band. Elongated ovipositor lies posterodorsal to the subgenital plate, has sclerotized ventral surface and crimped lateral margins and is triangular with its apex reaching about halfway to the apex of the subanal plates. Subanal plates triangular in lateral view, with concave dorsal margin.

Remarks. The original description of *A. bovina* (Barnard 1934) was based solely on male specimens as the female was unknown. This rare species has only once been collected from the type locality in Franschhoek. It has subsequently only been found in Jonkershoek near Stellenbosch. The males most closely resemble *A. bicornis* which has a weak convexity of the internal face of the epiproct (there is no convexity of the epiproct of *A. bovina*). Additionally, the internal denticles of the epiproct of *A. bicornis* do not extend as far apically as those of *A. bovina*. In *A. bicornis* the rounded sclerotized knobs of the dorsal plates of tergite 10 are centrally placed, while in *A. bovina* they are acute and located posteriorly. *Aphanicerca bicornis* females differ from this species in only having sternites 1, 2, 7 and 8 (subgenital plate) completely sclerotized, and in the elongate subgenital plate covering the ovipositor completely. Intermediate forms which may represent hybrids between *A. bovina* and *A. bicornis* are discussed under *A. bicornis*.

Type material examined. No holotype designated. Lectotype ♂, SOUTH AFRICA: *Western Cape Province*, 1♂, Franschhoek Pass, 33.55S 19.05E, 1.x.1932, H. G. Wood (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, Langrivier, Jonkershoek, Stellenbosch, 33.57S 18.56E, 18.vi.1985, M. D. Picker (SAMC); 9♂, 12♀, Swartboskloof, Stellenbosch, 33.57S 18.56E, 5.vii.1996, 18.viii.1996, D. M. Stevens & M. D. Picker (SAMC).

Aphanicerca capensis Tillyard

Aphanicerca capensis Tillyard, 1931: 119.

Description of larva. Appearing smooth, but covered evenly in very fine hairs (Figs 2.10, 2.1C).

Size (mm). Large larvae; body length male 9.6 (9.0 - 10.2); female body length 12.0 (11.4 - 12.8).

Head. Pale-brown, with three distinct ocelli; compound eyes black to violet; segments on proximal two thirds of antennae with whorl of hairs on distal margin; proximal third of antennae including scape with medial hairs longer than lateral.

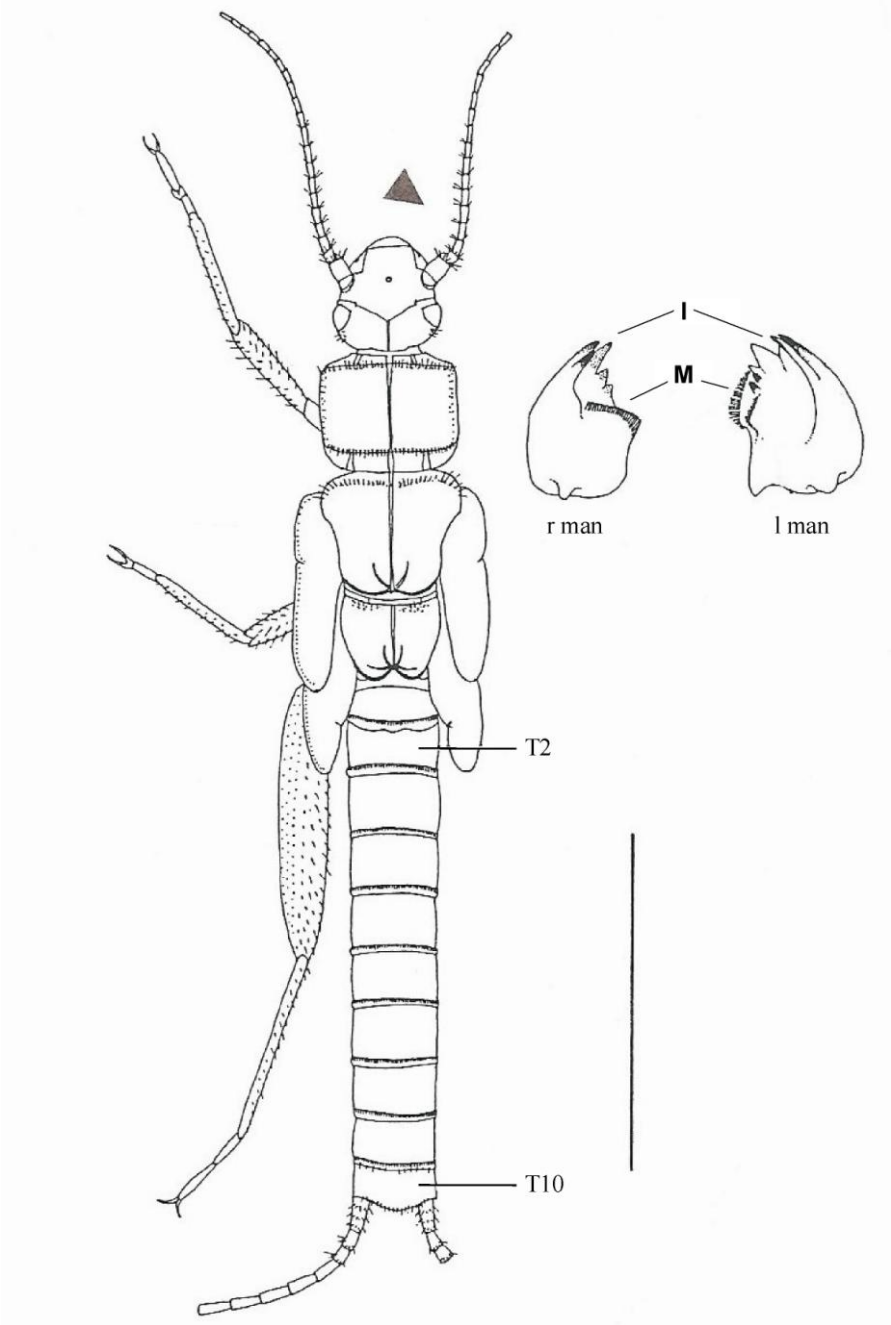


Fig. 2.10. Dorsal view of final instar (black-wingpad) larvae of *Aphanicerca capensis* (Platteklip Stream, Table Mountain). Arrow indicates longer medial hairs on proximal antenna. Abbreviations: I = incisor; l man = left mandible; M = molar; r man = right mandible; T2 = tergite 2; T10 = tergite 10. Scale bar = 2 mm.

Thorax. *Prothorax* pale-brown; pronotum width (male and female 1.9 mm) equal to head (male and female 1.8 mm) and mesonotum width; having marginal short bristles; pronotum extended laterally beyond margins of prothorax. *Mesothorax and metathorax* pale-brown, with enlarged setae on lateral margins.

Wingpads. Black, but with pale-yellow region, appearing smooth.

Legs. Pale-yellow-brown; evenly covered in fine bristles, but with a few large stouter setae on dorsal aspect of femur and tibia, and ventral aspect of the tibia; glabrous stripe on lateral aspect of femur and tibia.

Abdomen. Pale-brown; appearing smooth; fine comb of setae on posterior margins of segments (Fig.2.1C); pleurites evident on first five segments (Table 2.2).

Cerci. Whorl of short bristles on posterior margin of segments; lateral bristles twice as long as medial bristles.

Larval Material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, 1♀, Vlermuis waterfall, Donkerkloof, Montagu, 33.45S 20.07E, 20.xi.93, D.M. Stevens; 1♂, Garcia Pass, Riversdale, 33.54S 21.13E, 8.iii.1996, D.M. Stevens; 2♀, Langrivier, Swartboskloof, Stellenbosch, 33.54S 18.55E, 18.viii.1996, D.M. Stevens & M.D. Picker Private Collection.

Remarks. *Aphanicerca capensis* is the most common southern African notonemourid, with a wide geographical distribution within the Western Cape Province and into the western Eastern Cape. Barnard (1934) recorded some morphological variation between populations, and referred these morphological variants to four 'forms', distinguished on the shape of the epiproct and dorsal processes of the male, and subgenital plate of the female. This study has also revealed morphological variation between certain populations. Morphologically, these forms are worthy of further examination, and mate choice experiments that revealed a species complex within *Aphanicercella barnardi* could be usefully applied here. The larvae of the three most common species, *A. bicornis* Barnard, *A. lyrata* Barnard and *A. capensis* (Fig. 2.1A-C), are relatively hairless, and although distinguished by the mean number of setae per segment, an overlap in the ranges negates the use of this character (Table 2.3). Nevertheless, *A. capensis* has higher setal counts on average, and is by far the largest of the three species (body length of *A. lyrata* (male) = 7.2 mm; body length of *A. bicornis* (female) = 6.9 mm). Setae under high magnification are similar in all species examined (Fig. 2.1F). Adult genitalia present in black-wingpad larvae are useful aids to species identifications. *Aphanicerca tereta* Barnard, *A. uncinata* Barnard and *A. bovina* Barnard are rare species unlikely to be encountered.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*. '*Aphanicerca capensis* Till. Table Mt. 25.1.29. K. H. B.'. Slide, wings only (SAMC). Paratype ♂ (pinned), Table Mtn, Cape Town, 33.57S 18.25E, date unknown, K.H. Barnard (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, 2♀, Gardens, Table Mountain, Cape Town, 33.57S 18.22E, 16.vi.1993; 2♂, 1♀, Boyes Drive, Cape

Peninsula, 34.07S 18.27E, 25.iv.1993; 3♂, Tafelberg Road, Table Mountain, Cape Town, 33.57S 18.25E, 15.v.1993; 1♂, 1♀, Slangolie Ravine, Twelve Apostles, Cape Peninsula, 33.58S 18.24E, 21.vi.1993; 1♀, Silvermine, Cape Peninsula, 34.05S 18.23E, 10.vi.1993; 1♂, 1♀, Liesbeeck River, Kirstenbosch, Cape Peninsula, 33.59S 18.25E, 21.ix.1993; 3♀, Skeleton Gorge, Table Mountain, Cape Town, 33.59S 18.25E, 25.x.1993; 8♂, 5♀, Langrivier, Stellenbosch, 33.57S 18.56E, 25.x.1993, K. Snaddon (SAMC); 4♂, 4♀, same data but 25.ix.1993; 29♂, 17♀, Jonkershoek, Stellenbosch, 33.57S 18.56E, 15.viii.1995; 7♂, 6♀, Pilkington Bridge, Bain's Kloof, 33.37S 19.06E, 10.vii.1994; 8♂, 2♀, same data but cement bridge 1 km N Pilkington Bridge; 2♂, 2♀, same data but 1.6 km N Pilkington Bridge; 7♂, 8♀, Gawie se Water, Bain's Kloof, 33.37S 19.06E, 10.vii.1994; 2♀, Eerste Tol, Witte River, Bain's Kloof, 33.36S 19.08E, 29.vi.1994, M.D. Picker (SAMC); 3♂, 2♀, Wellington, base of Bain's Kloof Pass, 33.39S 19.05E, 10.vii.1994; 1♀, Wolwekloof, Michell's Pass, 33.25S 19.15E, 12.vi.1994; 2♀, Krom River, E Huguenot Tunnel, Witteberg, 33.44S 19.05E, 27.xii.1997, D.M. Stevens (SAMC); 1♀, Molenaars River, Du Toitskloof, 33.44S 19.08E, 24.v.1994; 1♀, same data but 5.xii.1993; 1♀, same data but 26.vii.1981, M.D. Picker (SAMC); 1♂, Du Toits River Bridge, Franschhoek Pass, 33.55S 19.05E, 23.v.1993; 1♀, same data but 3 km N Du Toits River bridge, Franschhoek Pass; 1♂, 1♀, Harold Porter Nature Reserve, Betty's Bay, 34.20S 19.02E, 31.v.1993; 5♂, 5♀, Clarence Drive, between Gordon's Bay and Rooiels, 34.12S 18.46E, 31.v.1993; 1♂, Voelklip Nature Reserve, Hermanus, 34.25S 19.16E, 10.iv.1997, T. Branch (SAMC); 2♀, Berg River, Franschhoek district, 33.52S 18.59E, 21.viii.1997, H. Dallas (SAMC); 4♂, 2♀, Pniel, 33.54S 18.57E, 12.vi.1997; 1♀, Nuweberg, Palmiet River, 34.07S 19.08E, 11.vii.1992; 19♂, 10♀, Kleinboontjies River, between Ceres and Wolseley, 33.22S 19.13E, 19.vi.1993; 5♂, 5♀; Seweweekspoort, Riversdale district, 33.25S 21.24E, 1.vii.1995; 13♂, 10♀, Garcia Pass, 33.58S 21.13E, 1.vii.1995; 1♀, Kristalskloof, Riversdale district, 33.58S 21.13E, 1.vii.1995; 35♂, 22♀, Oubos, Riviersonderend Mountains, 34.06S 19.49E, 13.ii.1999; 4♂, 5♀, Marloth Nature Reserve, Swellendam, 33.58S 20.25E, 4.iv.1998, D.M. Stevens (SAMC); 1♀, 12 km W Swartberg Pass, 33.21S 21.58E, 4.xii.1994; 6♂, 2♀, Prince Alfred's Pass, near Avontuur, 33.46S 23.10E, 3.xii.1994; 1♀, 3 km N Bergplaas, George district, 33.52S 22.41E, 3.xii.1994; 1♂, 2♀, 3 km N Hoekwil, George district, 33.59S 22.37E, 3.xii.1994; 1♂, 1♀, Gouna Forest, Knysna district, 33.58S 23.03E, 2.iii.1996, D. M. Stevens (SAMC); 3♂, 1♀, Ysternek Nature Reserve, Knysna-Uniondale road, 33.57S 23.06E, 4.xii.1994; all M.D. Picker & D.M. Stevens (SAMC) unless otherwise stated. *Eastern Cape Province*, 1♂, Vark River, Tsitsikamma Mountains, 33.57S 23.40E, 3.xii.1979, J. Illies (MPIL).

***Aphanicerca chanae* sp. n.**, Figs 2.14-2.16

Diagnosis

The gradual sigmoid curve, length, stoutness and distal medial spinules of the dorsal process lobes of tergite 9 distinguish the male of this species. The female is most similar to *A. lyrata*, but the apex of the subgenital plate is bifid (single in *A. lyrata*), the dorsal surface of the subanal plate is sigmoid in lateral view (concave in *A. lyrata*) and the subanal plates are shorter.

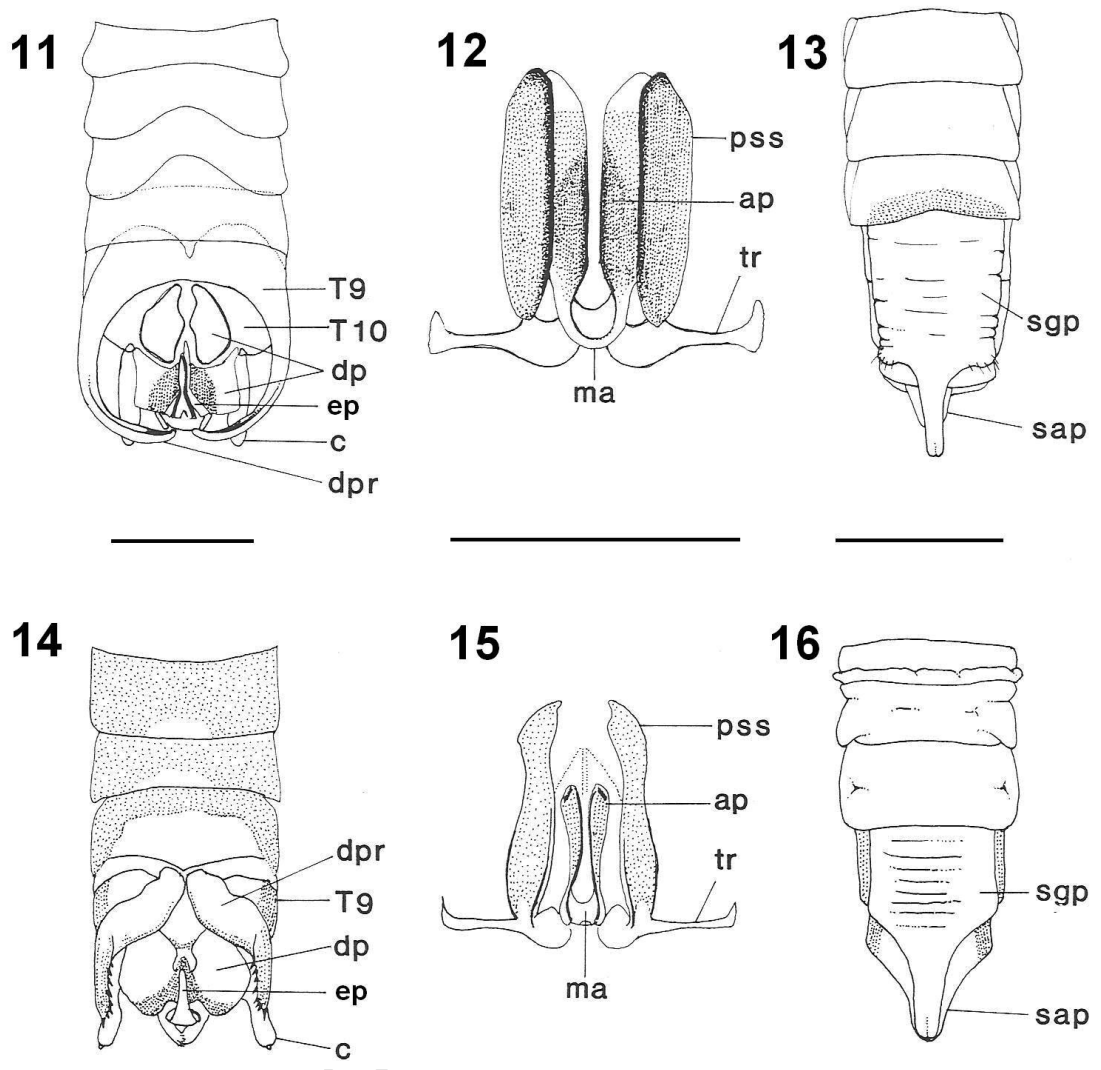
Description

Male. *Size*. Body length 5.4 ± 0.7 mm, $n = 9$ (holotype = 7.0 mm). Males are significantly smaller than females (*t*-test, $P < 0.05$).

Male genitalia. (Figs 2.14, 2.15). Dorsal process of tergite 9 bilobate, lobes basally stout and separated and divergent for half their length before bending medially and attenuating to become subparallel; the medial and lateral margins being gently sigmoidal; distal quarter-third denticulate on medial margin with small apical spine, dorsal process extends approximately to posterior margin of dorsal plates of tergite 10. Tergite 9 not sclerotized except for the following areas: a large area laterally, a strut joining the base of the process lobes to the lateral margins, a narrow strip across the base of the process lobes, the entire denticulate part of the lobes, and a lateral and medial stripe the length of the lobes. The other tergites are wholly sclerotized except for a pale rectangular to semicircular area on posterior margin of tergite 8 which decreases in size towards tergite 6. Dorsal plates of tergite 10 swollen, narrower anteriorly and broadly rounded posteriorly. Epiproct broad proximally and narrowing uniformly to denticulate area half way along its length, becoming linguiform to the acute apex. In lateral view denticulate area slightly convex. Primary supporting struts of the paraprocts very broad and apically truncate, and basally continuous with the transverse rod. Median arch produced into arch processes which are broad, shorter than the primary supporting struts, bear minute spinules apically, and are joined to the primary supporting struts by the membranous part of the paraprocts. Transverse rod incomplete centrally, each half being broad and rounded medially, narrowing abruptly before the junction of the primary supporting strut and terminating laterally in an acutely tipped perpendicular strut.

Female. *Size*. Body length 5.7 ± 0.3 mm, $n = 5$.

Female genitalia. (Fig. 2.16). All sternites completely sclerotized. Anterior half of sternite 8 (subgenital plate) parallel-sided, but narrowing gradually and concavely in posterior half to subacute bifid apex, extending posteriorly to same level as the apex of the subanal plates. Dorsal surface of subanal plates gradually sigmoid in lateral view.



Figs 2.11-2.16. *Aphanicerca* species. **11-13**, *Aphanicerca gnua*; **11**, male genitalia, dorsal view; **12**, paraprocts; **13**, female genitalia, ventral view; **14-16**, *Aphanicerca chanae*; **14**, male genitalia, dorsal view; **15**, paraprocts; **16**, female genitalia, ventral view. Abbreviations: ap = arch process; c = cercus; dp = dorsal plates of tergite 9; dpr = dorsal process of tergite 9; ep = epiproct; ma = median arch; pss = primary supporting strut; sap = subanal plate; sgp = subgenital plate; T9 & T10 = tergites 9 & 10; tr = transverse rod. Scale bars: **11** & **14** = 1 mm, **12** & **15** = 0.7 mm, **13** & **16** = 1 mm.

Larva. Larval taxonomic characters such as abdominal setation patterns concur with those given for the genus. The species may be distinguished by the fringe of long setae on the anterodorsal margin of the scape, and the long setae on the lateral aspect of the frons. There are no stout setae on the tergites or sternites (save sternite 11).

Etymology. Named in memory of the late Dr Chana Willemse, a close friend and stonefly collecting companion of DMS.

Type material examined. Holotype ♂, *SOUTH AFRICA:* Western Cape Province, 'Grootvadersbosch River, under road bridge, Grootvadersbosch Nature Reserve, Boosmansbos Wilderness Area, Langeberge, 33.52S 20.23E, 18.ii, 9.iii.1996, D.M. Stevens' (SAMC). Paratypes, 8♂, 4♀, same data as holotype.

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 10♂, 6♀, 1 mature larva, Marloth Nature Reserve, Swellendam, 33.58S 20.25E, 4.iv.1998, D.M. Stevens (SAMC).

***Aphanicerca gnua* sp. n.**, Figs 2.11-2.13

Diagnosis

The pronounced curvature and length of the lobes of the dorsal process of tergite 9 distinguish the males. The females are easily identified by the shape of the subgenital plate, which has slightly swollen posterior angles leading to transverse margins that give rise to a narrow posterior projection.

Description

Male. *Size*. Body length 5.7 ± 0.6 mm, $n = 5$ (holotype = 5.8 mm). Males are significantly smaller than females (*t*-test, $P < 0.005$).

Male genitalia. (Figs 2.11, 2.12). Dorsal process of tergite 9 bilobate, each long and robust corniform lobe directed posteroventrally proximally and curving strongly to become dorsally directed, bearing a row of very short spinules on a dorsolateral ridge on distal third, with a terminal spine. Anterior half of plates of tergite 10 swollen, each half narrow and rounded anteriorly, with a straight medial margin which may bear a medial bulge, and with a teardrop-shaped posterolateral margin. Posterior part of the plates flattened with truncate posterior margins and heavily sclerotized medial surfaces, between which lies the epiproct. Epiproct broad basally with sclerotized lateral margins and membranous central area, narrowing evenly and gradually to the anterior half. The lateral margins are slightly convex, the apex subacute. In lateral view, anterior margins bulge anteriorly in the middle denticulate third. Cerci long and cylindrical. Primary supporting struts of the paraprocts broad and apically rounded; medial secondary supporting struts broad, shorter than the primary supporting struts, continuous with a median arch and joined to the primary supporting struts by the membranous part of the paraprocts. Transverse rod (to which primary supporting strut is attached basally) is incomplete centrally; each half comprising an oval-shaped section medially, narrowing abruptly to a thin strut which gradually broadens laterally to an axe-shaped apex.

Female. *Size*. Body length 6.2 ± 1.0 mm, $n = 2$.

Female genitalia. (Fig. 2.13). All sternites completely sclerotized. Anterior two-thirds of sternite 8 (subgenital plate) rectangular with slightly swollen posterior angles; posterior margin runs transversely from posterior angle for almost a quarter the width of the sternite before being produced into a subtriangular, apically bifid plate which extends beyond the apex of the subanal plates; rectangular part of subgenital plate with dark rectangular patch posterolaterally. Subanal plates subtriangular with a gradual sigmoid curve in lateral view.

Larva. Unknown.

Etymology. Named for the resemblance of the dorsal process of tergite 10 to the horns of the wildebeest or gnu.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, '2.5km along Boontjiesrivier road off R46, Klein Boontjiesrivier, nr Wolseley, 33.23S 19.13E, 12.vi.1994, M.D. Picker & D.M. Stevens' (SAMC). Paratypes, 3♂, 1♀, same data as holotype; 1♂, same data as holotype but 10.vii.94.

Aphanicerca lyrata Barnard

Aphanicerca lyrata Barnard, 1934: 529.

Remarks. This species is only known from Franschhoek and the Jonkershoek valley (Stellenbosch) occurring sympatrically with *A. bicornis* and *A. bovina* at the latter locality. The shape of the dorsal process of tergite 9 is diagnostic.

Type material examined. No holotype designated. Lectotype ♂, SOUTH AFRICA: *Western Cape Province*, 1♂, Jonkershoek, Stellenbosch, 33.57S 18.56E, v.1924, H. G. Wood (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 2♀, Jonkershoek, Stellenbosch, 33.57S 18.56E, 10.vi.1993, D.M. Stevens (SAMC); 1♀, same data but 6.iii.1993, D. M. Stevens & M. D. Picker (SAMC); 2♂, 2♀, Swartboskloof, Jonkershoek, Stellenbosch, 33.57S 18.56E, 27.iv.1986, M. D. Picker (SAMC); 2♂, 2♀, Langrivier, Jonkershoek, Stellenbosch, 33.57S 18.56E, 22.v.1996, T. Behrens (SAMC); 1♂, 1♀, Swiss Farm Excelsior, Franschhoek, 33.55S 19.07E, 8.v.1994, D.M. Stevens & M. D. Picker (SAMC); 2♂, 3♀, Jonkershoek, Stellenbosch, 33.57S 18.56E, v.1924, H. G. Wood (SAMC).

Aphanicerca tereta Barnard

Aphanicerca tereta Barnard, 1934: 531.

Remarks. This rare species has not been collected since Barnard's first collection of four males. It resembles *A. bovina*, but has 3-4 large and sharp denticles on the inner margin of the dorsal processes of tergite 9.

Type material examined. No holotype designated. Lectotype ♂ (pinned), SOUTH AFRICA: *Western Cape Province*, Riviersonderend Mountains, xi.1928, K. H. Barnard (SAMC).

Aphanicerca uncinata Barnard

Aphanicerca uncinata Barnard, 1934: 528.

Remarks. This is another of Barnard's rare species, known only from the type locality of Landdroskop in the Hottentots Holland Mountains between 1916 and 1933, until I rediscovered at Betty's Bay in 2000. The recurved and truncate tips of the dorsal processes of tergite 9 are distinctive. The males from the type collection could not be found.

Material examined. No holotype designated. SOUTH AFRICA: *Western Cape Province*, 3♀, E side of Hottentots Holland Mountains, i.1933, K.H. Barnard & H.G. Wood (SAMC); ♂♂, ♀♀, Leopard's Kloof, Harold Porter Nature Reserve, Betty's Bay, 22.viii.2000, D.M. Stevens Private Collection; 5♂, 5♀, same locality, 16.v.2004, D.M. Stevens Private Collection; 2♂, 3♀, same locality, 16.vi.2007, D.M. Stevens Private Collection.

Genus ***Aphanicercella*** Tillyard

Aphanicercella Tillyard, 1931: 124.

Type species: *Aphanicerca* subgenus *Aphanicercella barnardi* Tillyard, 1931: 122 by original designation

***Aphanicercella barnardi* species complex**

Aphanicercella barnardi Tillyard, Figs 2.18B, 2.19B

Aphanicerca subgenus *Aphanicercella barnardi* Tillyard, 1931: 122.

Aphanicercella barnardi Tillyard: Barnard 1934: 537.

Redescription

Male (Fig. 2.18B). Body length: 4.82 ± 0.70 mm, n = 5. Epiproct triangular with rounded apex bearing minute ventral projection. Lateral dorsal plates of tergite 10 with small knob present on each plate. Median dorsal plate of tergite 10 crescentic, with apically round or truncate anterior extension. Pleurite 10 with entire clasper heavily sclerotized; medial margin of apex produced into very short terminal spine; clasper half to three-quarters the length of the dorsomedial margin of the pleurite; base of clasper swollen. Transverse rod with rounded apex, but internal sclerotized strut terminating acutely. Basal supporting process broad and long, but slightly shorter than the arch process; fused proximally to arch process. Median arch produced

into spatulate arch process and a short and thin medial, caudally-directed spinous process. Paraprocts having primary supporting strut much thicker and longer than medial secondary supporting strut. Primary strut originating from median arch, joined to the basal supporting process by a sclerotized strip. Medial secondary supporting strut attached proximally to primary supporting strut by a transverse sclerotized bar.

Female (Fig. 2.19B). Body length: 6.80 ± 1.10 mm, *n* = 13. Subgenital plate entire, sclerotization not uniform, imparting diagnostic pattern: lateral margins dark-brown with indented paler transverse band on distal half. Sternites 3-6 and 8 with an unbroken/broken bar of pigment on posterior margin. Sternite 8 with pigmented lateral margins. Sternite 9 pigmented except for median unpigmented rectangle on anterior margin.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Fairy Glen, Worcester, 33.33S 19.27E, 4.vi.1929, K.H. Barnard (SAMC). Paratypes, 3♂, 8♀, 79 km N Ceres, 19.15S 22.47E, 13.vi.1993, D.M. Stevens & M.D. Picker (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 2♂, 1♀, 27 km E Clanwilliam, Cederberg, 32.06S 19.11E, 10.ix.1994; 2♂, 4♀, same data but 9.ix.1997; 10♂, 6♀, Fairy Glen, Worcester, 33.33S 19.27E, 4.vi.1929, K.H. Barnard (SAMC); all D.M. Stevens & M.D. Picker (SAMC) unless otherwise stated.

***Aphanicercella bullata* sp. n.**, Figs 2.18E, 2.19E

Description

Male (Fig. 2.18E). Body length: 5.57 ± 0.51 mm, *n* = 9. Epiproct triangular, with rounded apex bearing minute acute ventral projection. Each lateral dorsal plate of tergite 10 bears a small knob. Median dorsal plate of tergite 10 crescentic, with apically rounded anterior extension. Pleurite 10 clasper heavily sclerotized distally, swollen basally, about half the length of dorsomedial margin of pleurite; medial margin of apex produced into short terminal spine. Transverse rod with rounded apex, but internal sclerotized strut terminates acutely. Basal supporting process short, rounded terminally, forming and overlying thickened base of primary supporting strut of paraprocts. Median arch bears a thick and apically rounded arch process with a thin, acuminate terminal extension. Primary supporting strut of paraprocts gives off a shorter supporting strut proximolaterally, from which it diverges. Medial secondary supporting strut very slender, closely apposed to primary strut, diverging distally; membranous part contains two other outer supporting struts.

Female (Fig. 2.19E). Body length: 6.94 ± 0.52 mm, *n* = 14. Subgenital plate with slightly convex posterior margin, sclerotization almost complete with concave anterior margin. Sternites 3-6 with unbroken bar of pigment of variable size on posterior margin. Sternite 8 with lightly

pigmented lateral margins. Sternite 9 pigmented except for median unpigmented rectangle on the anterior margin.

Etymology. Latin (*bulla*) for the knob-like basal supporting processes of the paraprocts.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, 27 km N Riversdale, Garcia Pass, 33.55S 21.12E, vii.1995, D.M. Stevens & M.D. Picker (SAMC). Paratypes: 4♂, 5♀, same data as holotype (SAMC).

Additional material examined. SOUTH AFRICA: 2♂, 8♀, Herrie Drif, Meiringspoort, 33.26S 22.34E, 2.vii.1995; 4♂, 6♀, Longmore Forest, Loerie, 33.55S 23.35E, 3.vii.1995; 1♂, Kristalskloof, 29 km N Riversdale, 33.57S 21.12E, 1.vii.1995; 2♂, 1♀, Garcia Pass, 24 km N Riversdale, 33.57S 21.12E, 1.vii.1995; 2♂, 1♀, 3 km N Bergplaas, 33.52S 23.40E, 3.xii.1994; all D.M. Stevens & M.D. Picker (SAMC).

***Aphanicercella clavata* sp. n.**, Figs 2.18D, 2.19D

Description

Male (Fig. 2.18D). Small species, body length: 4.36 ± 0.18 mm, *n* = 10. Epiproct triangular, with well-rounded, bead-like, cream-coloured apex; becoming concave towards apex. Lateral dorsal plates of tergite 10 with knob present medially on each plate, occasionally obscured by the epiproct; plates thickened and concave distally. Median dorsal plate of tergite 10 crescentic, with apically-rounded anterior extension. Pleurite 10 having apex only slightly more sclerotized than rest of clasper; medial margin of apex produced into very short heavily sclerotized terminal spine; base of clasper swollen; clasper and dorsomedial margin of pleurite equal in length. Transverse rod with acute apex. Apex of basal supporting process swollen medially. Median arch terminating in an elongate and very slender spinous arch process. Paraprocts with primary supporting strut much thicker than medial secondary supporting strut, the former continuous with the base of the basal supporting process. Medial secondary supporting strut thin and apposed to primary supporting strut proximally, but diverging distally.

Female (Fig. 2.19D). Body length: 5.80 ± 0.60 mm, *n* = 10. Subgenital plate diagnostic and very robust, having narrow incised notch on posterior margin, bounded by lobate lateral margins, uniformly and intensely sclerotized. Sternites 3-6 and 8 unpigmented, sternite 9 entirely pigmented.

Description of larva. Usually well-covered in enlarged setae (Figs 2.17, 2.3A).

Size (mm). Very small larvae; body length male 4.6; female 5.4 (5.0 - 5.8).

Head. Brown; three indistinct small dark ocelli; black compound eyes; antennal segments with whorl of fine short setae on distal margins.

Thorax. *Prothorax* brown with irregular darker markings; pronotum of similar width (male 0.6 mm, female 1.0 mm) to head (male 0.8 mm, female 0.9 mm) and mesonotum; slightly wider

than long; margins with numerous long setae and shorter bristles; pronotum extended laterally beyond margins of prothorax. *Mesothorax and metathorax* brown with irregular darker markings; long and short setae forming a row on anterior margin; often with a pair of long setae posteriorly and medially.

Wingpads. Appearing smooth, but with well-developed cluster of long setae proximally.

Legs. Yellow-tan with numerous short to long setae laterally on tibia, with thin lateral glabrous stripe; joints often darkly pigmented.

Abdomen. Brown, with a pair of medial, long, stout setae on posterior margin of tergites, frequently with additional stout setae between tergites; similar arrangement of paired setae ventrally, but setae more widely spaced; setae more numerous on segments 9 and 10, forming a complete whorl on segment 9 (Fig. 2.3A); pleurites evident on first six segments (Table 2.2).

Cerci. Whorl of short stout bristles on distal margins of segments.

Etymology. Latin (*clava*) for the club-shaped basal supporting process of the paraprocts.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Cecilia State Forest, Cape Peninsula, 34.04S 18.23E, 25.vi.1993, D.M. Stevens & M.D. Picker (SAMC). Paratypes, 43 ♂, 20♀, same data as holotype.

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 31♂, 16♀, Gardens, Table Mountain, Cape Town, 33.57S 18.25E, 16.vi.1993, D.M. Stevens & M.D. Picker (SAMC); larvae: 1♂, 2♀, Platteklip Stream, Cape Peninsula, 33.57S 18.25E, 16.vi.1993, D.M. Stevens & M.D. Picker Private Collection.

Aphanicercella flabellata **sp. n.**, Figs 2.18F, 2.19F

Description

Male (Fig. 2.18F). Body length: 4.80 ± 0.32 mm, *n* = 19. Epiproct triangular, bearing a dorsal median ridge distally, terminating in a rounded apex bearing minute, acute, ventral projection. Each lateral dorsal plate of tergite 10 bears a small knob. Median dorsal plate of tergite 10 crescentic with rounded anterior extension. Clasper of pleurite 10 pale apically with a sclerotized rim; bearing a very short, central, terminal spine; three-quarters the length of dorsomedial margin of the pleurite. Transverse rod with acute apex. Basal supporting process absent or vestigial. Median arch bears an apically rounded arch process, and a slightly longer medial spinous process. Primary supporting strut of paraprocts thicker and longer than medial secondary supporting strut, to which it is joined by a transverse sclerotized band.

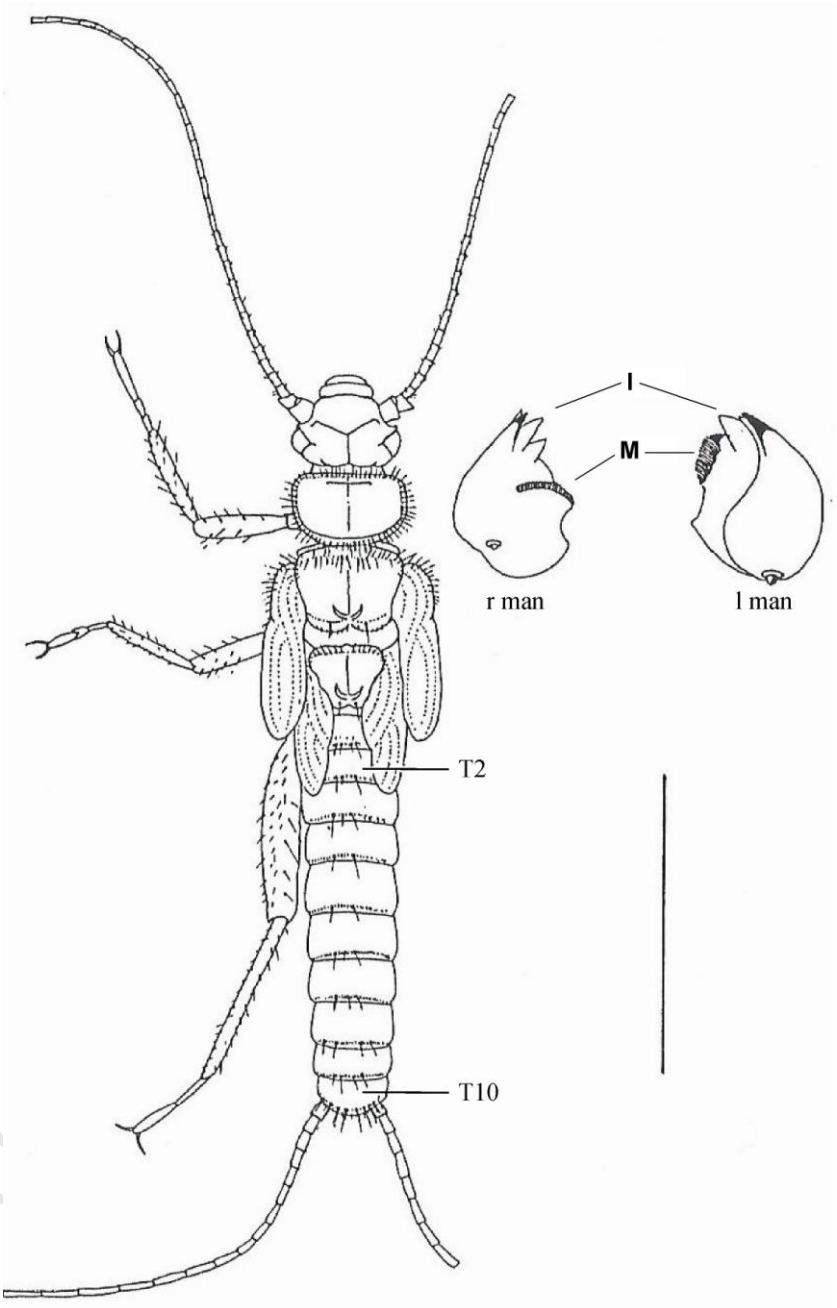


Fig. 2.17. Dorsal view of final instar (black-wingpad) larva of *Aphanicercella clavata* sp. n. (Platteklip Stream, Table Mountain). Abbreviations: I = incisor; l man = left mandible; M = molar; r man = right mandible; T2 = tergite 2; T10 = tergite 10. Scale bar = 2 mm.

Female (Fig. 2.19F). Body length: 5.80 ± 0.44 mm, *n* = 13. Subgenital plate with deeply concave posterior margin, sclerotization light, uniform and extensive. Sternites 3-6 with an unbroken bar of pigment on posterior margin. Sternite 8 unpigmented, sternite 9 pigmented except for a median unpigmented triangle on anterior margin.

Etymology. Latin (*flabellum*) for the fan-shaped arch process of the paraprocts.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Wellington, 33.45S 19.00E, 10.vii.1994, D.M. Stevens & M.D. Picker (SAMC). Paratypes: 6♂, 5♀, same data as holotype (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 75♂, 35♀, Kleinboontjies River, 2.5 km N Wolseley, 33.25S 19.12E, 19.vi.1993; 16♂, 9♀, Langrivier, Stellenbosch, 33.55S 19.00E, 22.v.1996, Behrens (SAMC); 4♂, 5♀, Gawie se Water, Bain's Kloof, 33.35S 19.07E, 10.vii.1994; 1♂, Franschhoek, 33.52S 19.06E, 8.v.1994; 15♂, 26♀, Swartboskloof, Stellenbosch, 33.54S 18.55E, 25.ix.1993; 3♂, 4♀, Jonkershoek, Stellenbosch, 33.54S 18.55E, 4.vii.1985, M.D. Picker (SAMC); 6♂, 2♀, Pniel, 33.52S 18.55E, 12.vi.1997; 4♂, 1♀, Du Toitskloof, 33.44S 19.07E, 15.vi.1981, M.D. Picker (SAMC); 1♂, 1♀, Wolwekloof, 8 km E Wolseley, 33.26S 19.14E, 12.vi.1994; 6♂, 5♀, 4 km E Wellington, 33.45S 19.05E, 10.vii.1994; 1♂, Karmel campsite, 4 km E Franschhoek, 33.52S 19.09E, 8.v.1994; all D.M. Stevens & M.D. Picker (SAMC) unless otherwise stated.

***Aphanicercella securata* sp. n.**, Figs 2.18C, 2.19C

Description

Male (Fig. 2.18C). Large species, body length: 5.90 ± 0.27 mm, *n* = 10. Epiproct triangular, with rounded apex bearing minute ventral projection; medial strut gradually broadening distally. Lateral dorsal plates of tergite 10 with small knob present on each plate. Median dorsal plate of tergite 10 crescentic, with short, rounded anterior median extension. Pleurite 10 with apex of clasper usually pale centrally with heavily sclerotized rim, but entire apex may be heavily sclerotized; very short central terminal point; clasper about two-thirds length of dorsomedial margin of pleurite. Transverse rod with acute apices. Basal supporting process parallel-sided, apically rounded, and about one-third the length of the arch processes. Median arch bears a large arch process which is rounded and laterally expanded apically, with a shorter, thin, medial, caudally-directed spine; arch process extends almost as far as the convergence of the primary and medial secondary supporting struts of paraprocts. Paraprocts with primary supporting strut thicker and longer than medial secondary supporting strut; supporting struts joined by a transverse sclerotized band.

Female (Fig. 2.19C). Body length: 6.90 ± 0.85 mm, *n* = 10. Subgenital plate with slightly concave posterior margin, sclerotization uniform and extensive, except for narrow anterior and

posterior unpigmented bands. Sternites 3-6 with an unbroken bar of pigment on posterior margin. Sternite 8 unpigmented, sternite 9 pigmented.

Etymology. Latin (*securis*) for the axe-shaped arch process of the paraprocts.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, 7 km N Villiersdorp, 33.56S 19.20E, 8.v.1994, D.M. Stevens & M.D. Picker (SAMC). Paratypes, 13 ♂, 4♀, same data as holotype.

***Aphanicercella spatulata* sp. n.**, Figs 2.18A, 2.19A

Description

Male (Fig. 2.18A). Body length: 5.20 ± 0.36 mm, *n* = 10. Epiproct triangular; lateral margins straight or slightly convex; apex bluntly rounded or subtruncate with minute ventral acute projection. Each lateral dorsal plate of tergite 10 subdivided into two, domed anterior part bearing a small knob, and a subtriangular posterior part. The median dorsal plate of tergite 10 crescentic with reduced anterior extension. The apex of the clasper of pleurite 10 pale with heavily sclerotized rim; terminal spine short and arising centrally, clasper about three-quarters the length of dorsomedial margin of pleurite. Transverse rod having a rounded apex, but with internal sclerotized strut terminating acutely. Basal supporting process short and rounded terminally, forming the thickened base of primary supporting strut of paraprocts. Median arch bearing a large spatulate arch process and a small and very thin dorsally-directed spinous process. Primary supporting strut of paraprocts much thicker than medial secondary supporting strut and continuous with the basal supporting process; medial secondary supporting strut thin, connected proximally to the primary supporting strut by an oblique sclerotized band.

Female (Fig. 2.19A). Body length: 5.90 ± 0.62 mm, *n* = 6. Subgenital plate with slightly concave posterior margin, sclerotization uniform and extensive. Sternites 3-5 with unbroken bar of pigment on posterior margin. Sternite 6 with dome-shaped pigmented band posteriorly. Sternite 8 unpigmented, sternite 9 entirely pigmented except for median unpigmented rectangle on irregular anterior margin.

Etymology. Named for the spatulate arch process of the paraprocts.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Bain's Kloof, 33.35S 19.07E, 12.vi.1994, D.M. Stevens & M.D. Picker (SAMC). Paratypes, 13♂, 5♀, same data as holotype (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 3♂, 14♀, Harold Porter Nature Reserve, Betty's Bay, 34.23S 18.53E, 31.v.1993, D.M. Stevens & M.D. Picker (SAMC); 1♂, Bain's Kloof, 33.35S 19.07E, 10.vii.1994, D.M. Stevens & M.D. Picker (SAMC); 1♂, Berg River, Groot Drakenstein Mountains, 33.55S 19.05E, 13.vi.1951, collector unknown

(AMGC); 1♂, 1♀, Driefontein Bridge, Greater Berg River, 33.52S 19.05E, 22.v.1962, collector unknown (AMGC); 1♂, Palmiet River, 34.12S 19.00E, 20.ix.1952, Harrison (MPIL).

The remainder of the Aphanicercella species

Aphanicercella bifurcata Barnard, Figs 2.18I, 2.19I

Aphanicercella bifurcata Barnard, 1934: 542.

Redescription

Male (Fig. 2.18I). Body length: 4.80 ± 0.37 mm, *n* = 12. Epiproct incised apically; anterior margin entirely concave; length and width of incision less than one-quarter the length of lateral margin of epiproct. Lateral dorsal plates of tergite 10 bears small knob; plates fused anteriorly. Median dorsal plate of tergite 10 with helmet-shaped anterior projection capped by transverse bar with swollen apices. Apex of clasper of pleurite 10 not sclerotized; medial margin of apex produced into short terminal spine; clasper about two-thirds the length of dorsomedial margin of pleurite. Transverse rod with acute apex. Basal supporting process apparently absent, although lateral margin of paraprocts has weak basal thickening. Median arch with slender arch process fused to medial surface of paraprocts. Primary supporting strut of paraprocts thicker and longer than medial secondary supporting strut, diverging distally.

Female (Fig. 2.19I). Body length: 6.00 ± 0.70 mm, *n* = 10. Subgenital plate entire; dome-shaped pigmentation on posterior margin, lateral margins pigmented. Sternites 1-6 and 8 unpigmented, sternite 9 entirely pigmented.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Oudebosch, Riviersonderend Mountains, 33.57S 21.06E, xi.1928, K.H. Barnard (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 9♂, 5♀, Grootvadersbosch Nature Reserve, 34.02S 20.46E, 18.ii.1996; 5♂, 1♀, Stinkhoutkloof forest trail, Bloukrans forest, Knysna District, 33.57S 23.05E, 4.iii.1996; 1♂, Jubilee Creek, Knysna, 34.00S 23.08E, 3.iii.1996; 1♂, 2♀, Touw River waterfall, Giant Kingfisher trail, Wilderness, 33.58S 22.35E, 7.iii.1996; 1♂, 5♀, Karatara River, Seven Passes Road, Barrington District, 33.52S 22.52E, 6.iii.1996; 2♂, 2♀, Kaaimans River, 5 km NE George, 33.25S 22.33E, 6.iii.1996; 3♂, 3♀, Brak River, Kom Se Pad, Knysna, 33.57S 23.05E, 6.iii.1996; 6♂, 3♀, Ysternek Nature Reserve, 7.5 km N Knysna, 33.57S 23.04E, 3.xii.1994, D.M. Stevens & M.D. Picker (SAMC); 31♂, 40♀, Big Tree, 6 km N Wilderness, 33.58S 32.34E, 3.xii.1994, D.M. Stevens & M.D. Picker (SAMC); all D.M. Stevens (SAMC) unless otherwise stated.

Aphanicercella cassida Barnard, Figs 2.18G, 2.19G

Aphanicercella cassida Barnard, 1934: 541.

Redescription

Male (Fig. 2.18G). Body length: 5.13 ± 0.34 mm, *n* = 16. Lateral margin of epiproct straight for two-thirds of its length and curving in medially, becoming convex then concave distally, terminating in small sharp point which bears a minute acute ventral projection. Lateral margin distal to medial strut obscured in normal dorsal view. Each lateral dorsomedial plate of tergite 10 bears a small knob. Median dorsal plate of tergite 10 crescentic with rounded anterior extension. Clasper of pleurite 10 heavily sclerotized apically; very short central terminal spine; half to three-quarters the length of the dorsal margin of pleurite. Transverse rod with flat, broadly rounded apices. Basal supporting process forms the heavily sclerotized and thickened base of primary supporting strut of paraprocts. Arch process short, thin, pale and spinous. Primary supporting strut of paraprocts thicker and longer than medial secondary supporting strut, diverging distally.

Female (Fig. 2.19G). Body length: 6.14 ± 0.89 mm, *n* = 14. Subgenital plate with deeply concave posterior margin, sclerotization forming a trilobate pattern. Sternites 3-6 and 8 unpigmented, sternite 9 entirely pigmented.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Kaaimans Gat, Wilderness, 33.57S 22.37E, 16.iv.1933, H.G. Wood (SAMC). Paratypes, 3♂, 8♀, Longmore Forest , Loerie, 33.55S 23.35E, 3.vii.1995, D.M. Stevens & M.D. Picker (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, Meiringspoort, 10 km N De Rust, 33.27S 22.32E, 2.vii.1995; 4♂, 1♀, Seweweekspoort, 59 km N Riversdale, 33.27S 21.25E, 1.vii. 1995. *Eastern Cape Province*, 1♂, 11♀, Groendal Nature Reserve, 10 km NW Uitenhage, 33.40S 25.18E, 3.vii.1995; 3♂, Palmiet River, Grahamstown, 33.16S 26.33E, 3.vii.1995; 2♂, 6♀, Poortjie, Baviaanskloof, 33.45S 24.28E, 2.vii.1995; 6♂, 4♀, Enkeldoorn, Baviaanskloof, 33.45S 24.26E, 2.vii.1995; 32♂, 29♀, Loerie, 33.55S, 23.35E, 3.vii.1995. *KwaZulu-Natal*, 1♂, 3♀, Balgowan, 29.25S 30.05E, 21.vii.1965, B. Stuckenberg (MPIL); 2♂, 2♀, Cathedral Peak, Natal Drakensberg, 29.02S 29.13E, 24.iv.1957, B. Stuckenberg (MPIL); all D.M. Stevens & M.D. Picker (SAMC) unless otherwise stated.

Aphanicercella nigra Barnard, Figs 2.18K, 2.19K

Aphanicercella nigra Barnard, 1934: 544.

Redescription

Male (Fig. 2.18K). Large species. Body length: 6.50 mm, *n* = 1. Apex of epiproct heavily incised; medial margins of the two arms convex; width of incision equal to the length of the

lateral margin of epiproct. Lateral dorsal plates of tergite 10 fused anteriorly; elbowed distally; knob absent. Median dorsal plate of tergite 10 crescentic. Apex of clasper of pleurite 10 heavily sclerotized with central acute spine; clasper longer than dorsal margin of pleurite; pleurite papillate. Apices of transverse rod rounded, but internal sclerotized rod terminating acutely. Basal supporting process absent, although lateral margin of paraprocts has sclerotized basal thickening. Median arch with slender arch process fused to medial surface of paraprocts. Medial secondary supporting strut of paraprocts very thin, lying adjacent to thicker primary supporting strut proximally, but diverging distally.

Female (Fig. 2.19K). Body length: 6.50 ± 0.06 mm, *n* = 3. Subgenital plate sclerotized on margins only, with slightly concave posterior margin. Sternites 3-6 with a broken/unbroken bar of pigment on posterior margin. Sternite 8 with pigmented lateral margins, sternite 9 entirely pigmented.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Franschhoek Pass, 33.52S 19.04E, 1.x.1933, K.H. Barnard (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, 2♀, Hex River, Uitsig, 17 km N Citrusdal, 22.33S 19.03E, 9.ix.1997. *Eastern Cape Province*, 1♂, 3♀, Groendal Nature Reserve, 10 km NW Uitenhage, 33.40S 25.18E, 3.vii.1995; both D.M. Stevens & M.D. Picker (SAMC).

Aphanicercella quadrata Barnard, Figs 2.18J, 2.19J

Aphanicercella quadrata Barnard, 1934: 543.

Redescription

Male (Fig. 2.18J). Large species. Body length: 6.17 ± 0.34 mm, *n* = 10. Apex of epiproct heavily incised; anterior margin entirely concave and heavily sclerotized as a broad band; length and width of incision about one third and about half the length of the lateral margin respectively. Papillate lateral dorsal plates of tergite 10 not fused; knob present. Median dorsal plate of tergite 10 crescentic with tip of elongate anterior extension swollen. Apex of clasper of pleurite 10 heavily sclerotized: medial margin of apex produced into short terminal spine; clasper about half the length of dorsomedial margin of pleurite; pleurite, but not clasper papillate. Apices of transverse rod truncate, but internal sclerotized rod terminates acutely. Basal supporting process absent, although lateral margin of paraprocts has heavily sclerotized basal thickening. Median arch produced into spinous arch process, extending distally as far as the origin of medial secondary supporting strut of paraprocts. Primary supporting strut of paraprocts with base expanded laterally, and distal two thirds heavily sclerotized. Medial secondary supporting strut very thin, lying adjacent to thicker primary supporting strut proximally but diverging distally; a thin supporting strut follows the outer margin of the paraprocts.

Female (Fig. 2.19J). Body length: 8.50 ± 0.84 mm, *n* = 8. Subgenital plate entire, sclerotization bell-shaped. Sternites 3-5 with an unbroken bar of pigment on posterior margin. Sternite 6 almost entirely pigmented with dome-shaped pattern. Sternite 8 with pigmented lateral margins. Sternite 9 pigmented except for median unpigmented rectangle on anterior margin.

Remarks. Barnard (1934) incorrectly assigned the female of *A. scutata* to this species.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Clanwilliam District, Ceder Mountains, 32.08S 18.58E, ix.1923 K.H. Barnard (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 9♂, 5♀, Pakhuispad, 20 km E Clanwilliam, 32.06S 19.11E, 10.ix.1994; 1♂, 3♀, Cederberg, Wolfberg, 32.23S 19.04E, ix.1996; both D.M. Stevens & M.D. Picker (SAMC).

Aphanicercella scutata Barnard, Figs 2.18H, 2.19H

Aphanicercella scutata Barnard, 1934: 540**.**

Redescription

Male (Fig. 2.18H). Body length: 5.20 ± 0.42 mm, *n* = 10. Epiproct subtriangular with truncate lightly sclerotized apex; sclerotized lateral margins end subterminally and are connected by very thin V-shaped sclerotized strip. Lateral dorsal plates of tergite 10 flat laterally, elevated and cuneiform medially to form a ridge bearing a small knob centrally on its medial margin; incision caudomedial to the knob. Median dorsal plate of tergite 10 crescentic, with anterior extension capped by a thin transverse bar joining the two lateral plates. Pleurite 10 with apex of clasper usually pale centrally with a heavily sclerotized rim, but may be entirely sclerotized; medial margin of apex produced into terminal spine; clasper equal in length to medial margin of pleurite. Transverse rod with acute apices. Basal supporting process forms short lateral thickenings of base of paraprocts. Median arch produced into an apically acute arch process and a shorter, much thinner medial spine. Medial secondary supporting strut of paraprocts very thin and lies adjacent to thicker primary strut.

Female (Fig. 2.19H). Body length: 6.70 ± 1.30 mm, *n* = 10. Subgenital plate diagnostic and very robust, having broad and deep concave incision on posterior margin; sclerotization intense and uniform. Sternites 3-6 with a broken/unbroken bar of pigment on posterior margin. Sternite 8 unpigmented, sternite 9 entirely pigmented.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, Witte River, Wellington Mountains, 33.35S 19.07E, ix.1933, H.G. Wood (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 20♂, 21♀, Witte River, Eerste Tol, Bain's Kloof, 33.37S 19.07E, 17.vi.1994; 10♂, 6♀, Gawie se Water, Bain's Kloof, 33.35S 19.07E, 10.vii.1994; 3♀, Tweede Tol, Steenboks Nature Park, Bain's Kloof,

33.35S 19.04E, 10.vii.1994; 2♂, Palmiet River, Nuweberg, 34.05S 19.03E, 11.viii.1992, M.D. Picker (SAMC); 1♂, Pniel, 33.52S 18.55E, 12.vi.1997; 2♀, Gydo Pass, 15 km N Ceres, 33.14S 19.17E, 13.vi.1993; 1♂, 1♀, Berg River, 33.55S 19.05E, 21.viii.1997; 1♀, Harold Porter Nature Reserve, Betty's Bay, 34.23S 18.53E, 31.v.1993; 1♂, 2♀, Jonkershoek, Stellenbosch, 33.54S 18.55E, 15.vi.1993; 7♂, 5♀, Franschhoek Pass, 33.52S 19.04E, 23.v.1993; 6♂, 8♀, Clarence Drive, 6 km N Rooiels, 34.17S 18.48E, 31.v.1993; 3♀, Stellenbosch, Swartboskloof, 33.54S 18.55E, 5.vii.1996; 2♂, 4♀, Pilkington Bridge, Bain's Kloof, 33.37S 19.07E, 10.vii.1994; 5♂, 4 ♀, Du Toitskloof, Molenaars River, 33.44S 19.07E, 6.vii.1994; 2♀, 26 km E Caledon, 34.14S 19.45E, 1.vii.1995; all D.M. Stevens & M.D. Picker (SAMC) unless otherwise stated.

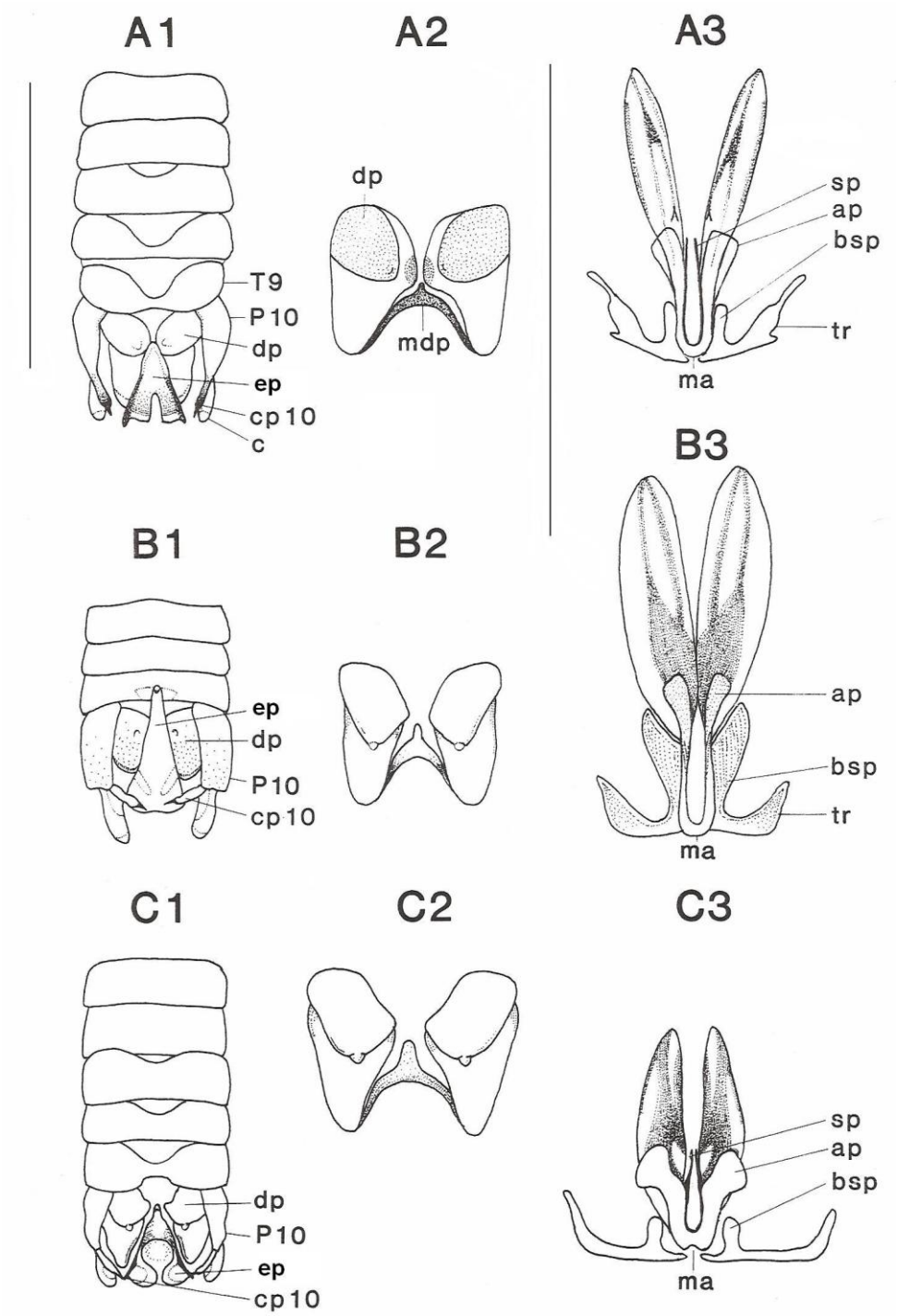


Fig. 2.18. Male genitalia of all described species of *Aphanicercella*. For all species illustrated: **1**, dorsal view of abdomen and male external genitalia; **2**, dorsal plates of tergite 10; **3**, paraprocts and associated structures. **A**, *A. spatulata*; **B**, *A. barnardi*; **C**, *A. securata*; **D**, *A. clavata*; **E**, *A. bullata*; **F**, *A. flabellata*; **G**, *A. cassida*; **H**, *A. scutata*; **I**, *A. bifurcata*; **J**, *A. quadrata*; **K**, *A. nigra*. External genitalia: c = cercus; cp10 = claspers of pleurite 10; dp = dorsal plates; ep = epiproct; P10 = pleurites 10; T9, T10 = tergites 9 and 10. Dorsal plates: dp = dorsal plates; mdp = median dorsal plate. Paraprocts: ap = arch process; bsp = basal supporting process; ma = median arch; sp = spinous process; tr = transverse rod. Scale bars = 1 mm.

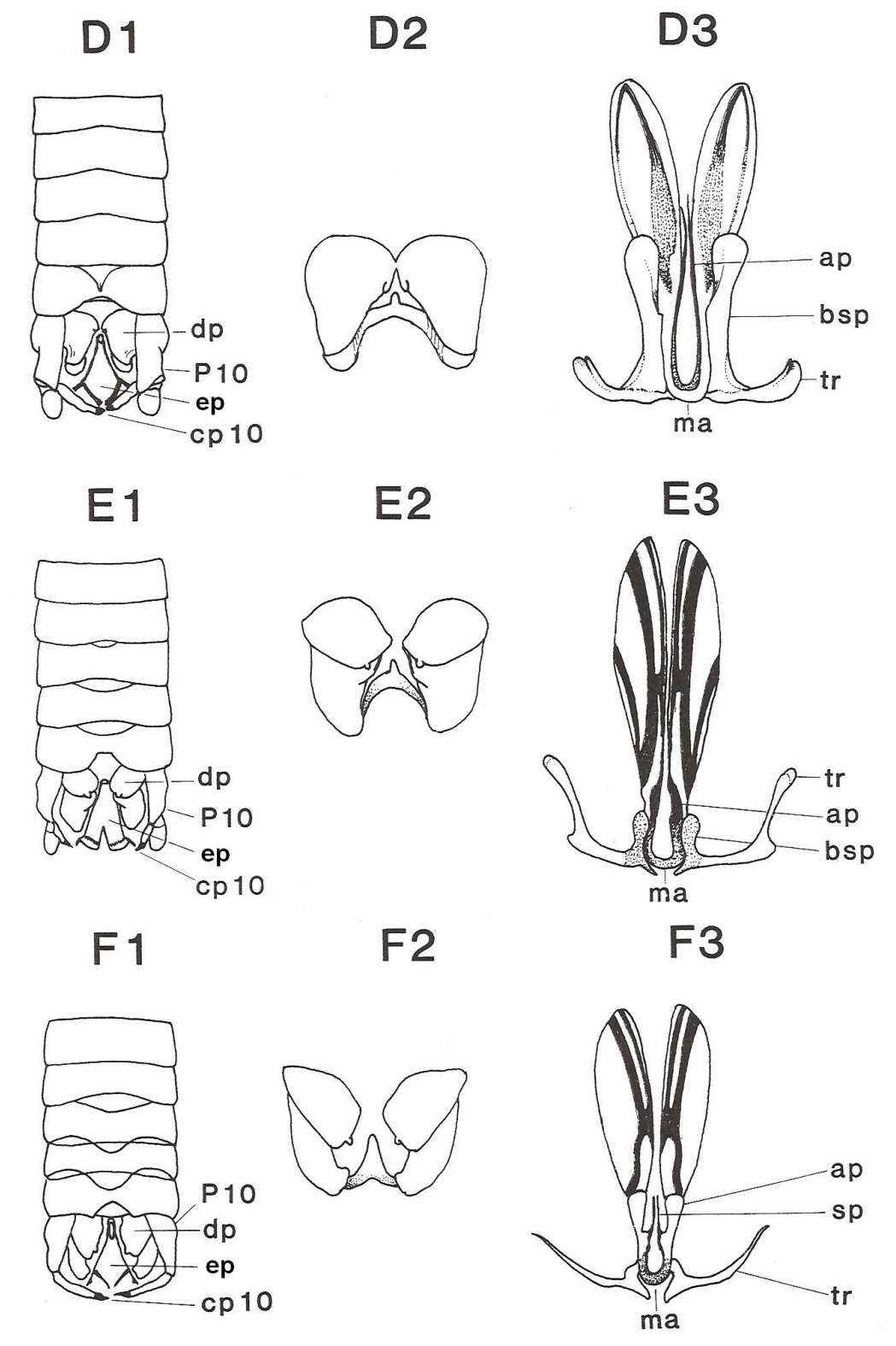


Fig. 2.18. Continued.

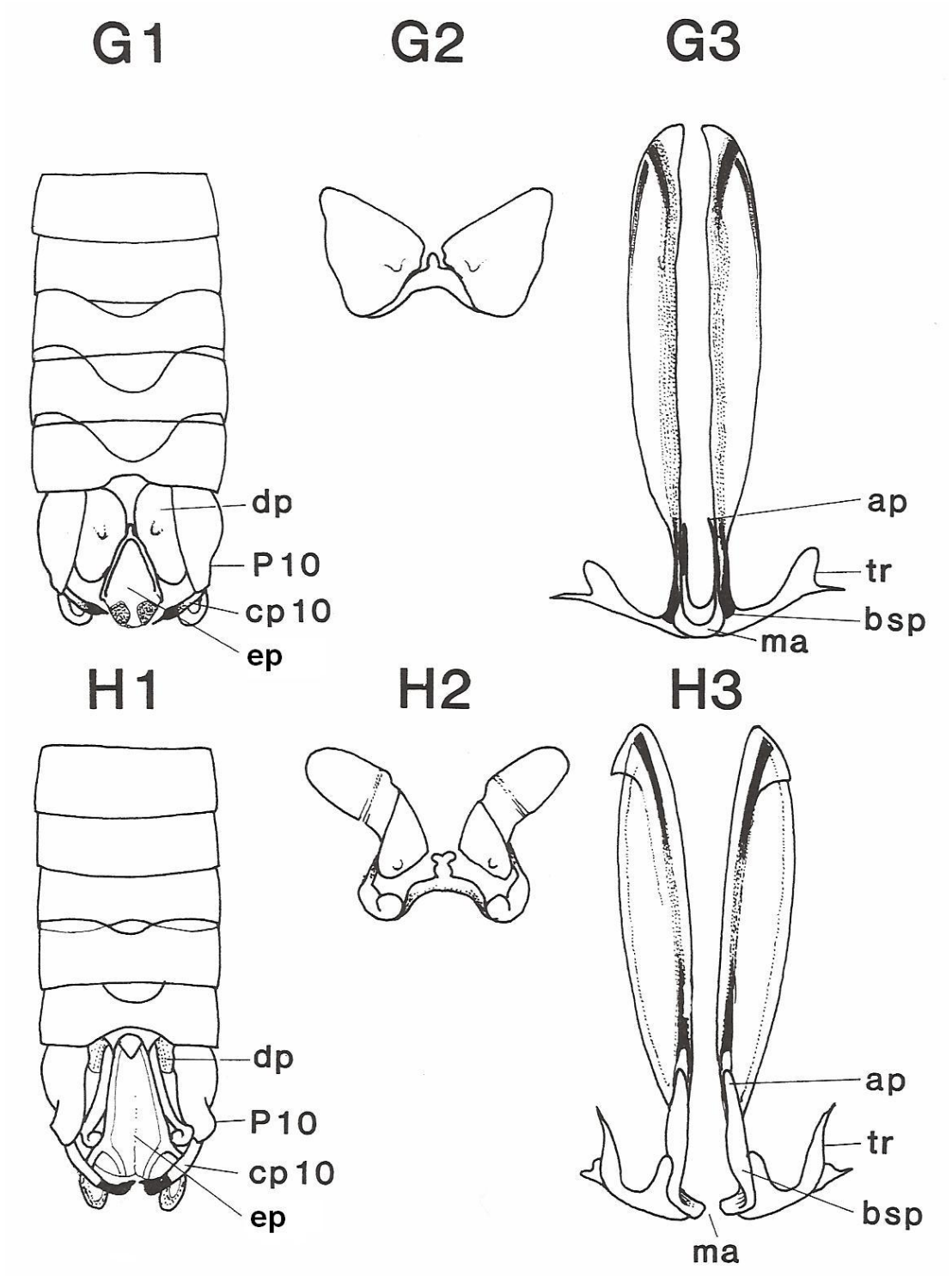


Fig. 2.18. Continued.

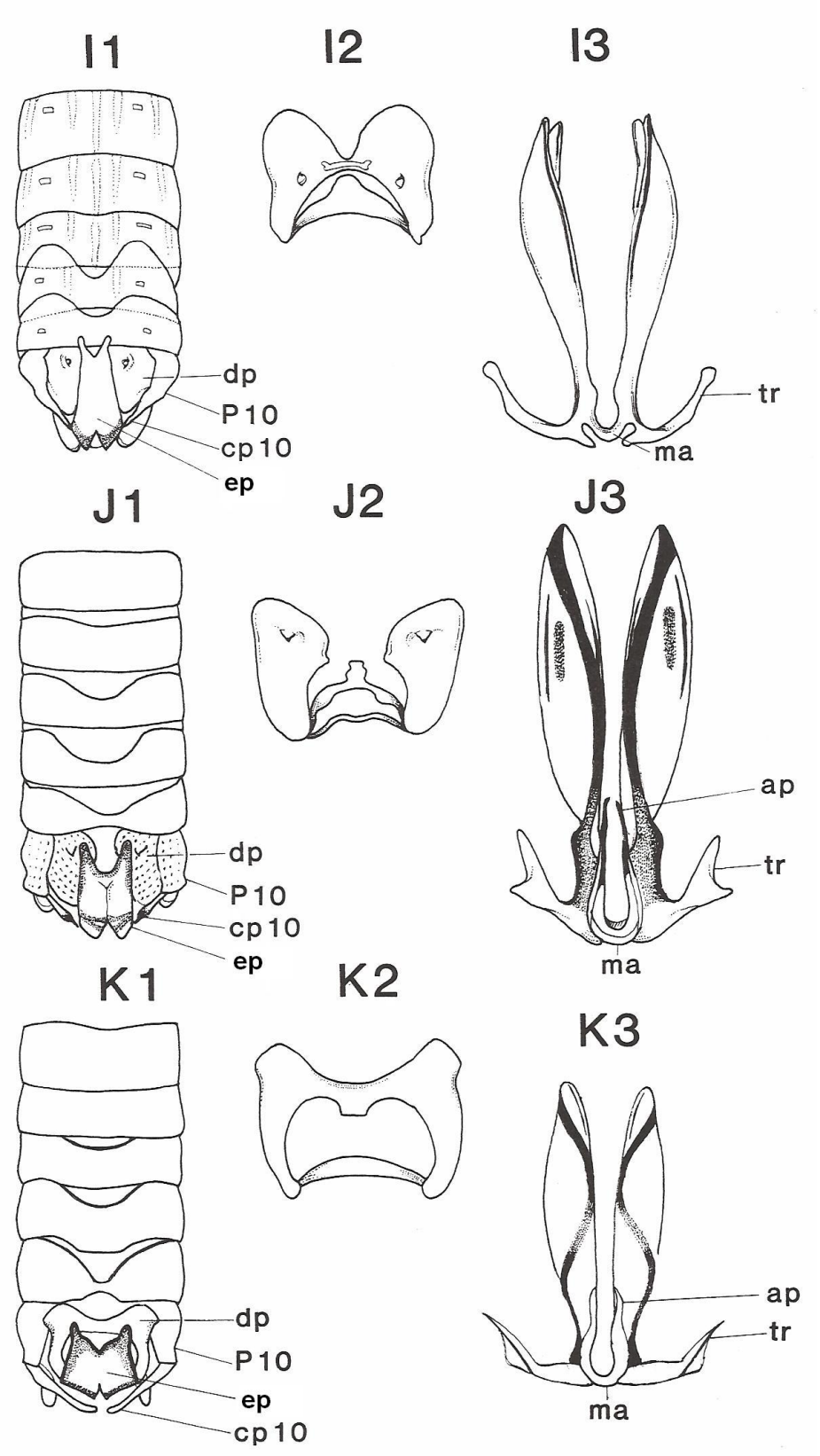


Fig. 2.18. Continued.

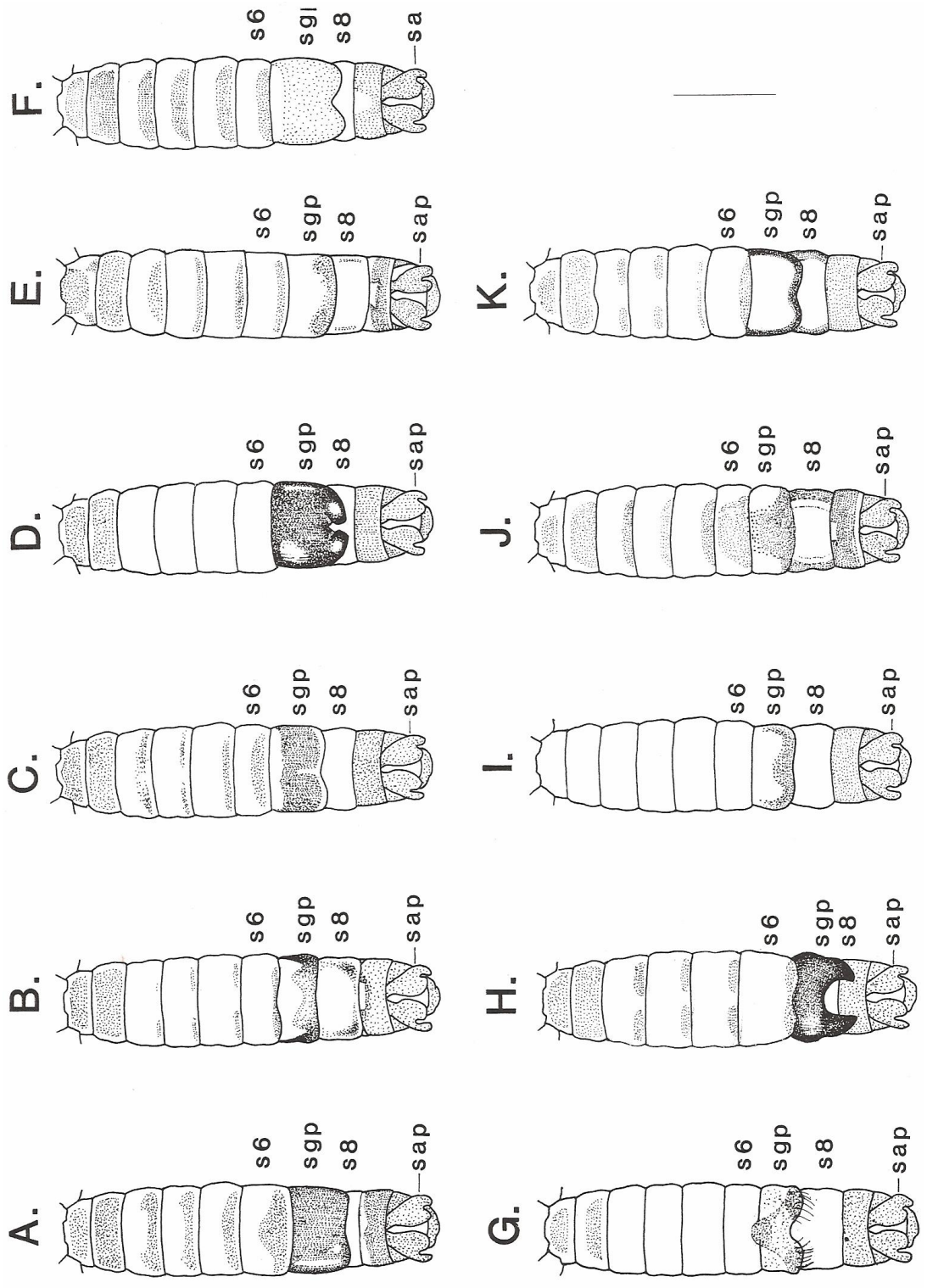


Fig. 2.19. Female genitalia of all known species of *Aphanicercella*. **A**, *A. spatulata*; **B**, *A. barnardi*; **C**, *A. securata*; **D**, *A. clavata*; **E**, *A. bullata*; **F**, *A. flabellata*; **G**, *A. cassida*; **H**, *A. scutata*; **I**, *A. bifurcata*; **J**, *A. quadrata*; **K**, *A. nigra*. s6, s8, sternite 6 and 8; sap, sub-anal plate; sgp, subgenital plate. Scale bar = 1 mm.

Genus ***Aphanicercopsis*** Barnard

Aphanicercopsis Barnard, 1934: 532.

Type species: *Aphanicerca denticulata* Tillyard, 1931: 119, by subsequent designation of Barnard (1934).

Aphanicercopsis denticulata Tillyard

Aphanicerca denticulata Tillyard, 1931: 121.

Aphanicercopsis denticulata (Tillyard): Barnard, 1934: 534.

Remarks. This species has a distribution running along a north-south axis, from Tulbagh through Bain's Kloof and Franschhoek Pass to Kleinmond. It is distinguished by the absence of a basal expansion of the epiproct (present in *A. tabularis* and *A. outeniquae*), and can be separated from *A. hawaquae* by the shape and denticulation of the epiproct.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, '*Aphanicerca denticulata* Till. Winterhoek Mts. viii.'29. K.H.B.' (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 6♂, 14♀, Pilkington Bridge, Bain's Kloof, 33.37S 19.06E, 10.vii.1994, D. M. Stevens & M. D. Picker (SAMC); 1♂, same data but Eerste Tol, Bain's Kloof; 9♂, 11♀, 26.4 km E Caledon, 34.11S 19.43E, 1.vii.1995, D. M. Stevens & M. D. Picker (SAMC); 1♂, 1♀, Witte River, Wellington Mountains, x.1933, H.G. Wood (SAMC); 1♂, Nonna Kloof, Keeromsberg, Worcester, 33.34S 19.36E, ix.1930, K.H. Barnard (SAMC).

Aphanicercopsis hawaquae Barnard

Aphanicercopsis hawaquae Barnard, 1934: 536.

Remarks. This species has a wide distribution from Jonkershoek in the west to Meiringspoort in the east, and is easily recognized by the broad base and distal narrowing of the epiproct.

Type material examined. No holotype designated. Lectotype ♂, SOUTH AFRICA: *Western Cape Province*, Franschhoek Pass, E side, 1.x.1932, H. G. Wood (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, Eerste Tol, Bain's Kloof, 33.36S 19.08E, 10.vii.1994; 2♂, Steenboks Nature Park, Bain's Kloof, 33.36S 19.09E, 10.vii.1994; 1♂, Molenaars River, Du Toitskloof, 33.44S 19.08E, 24.v.1994, G. Ractliffe (SAMC); 4♂, 5♀, Franschhoek Pass, 33.55S 19.05E, 23.v.1993; 1♂, 3♀, Kristalkloof, Riversdale, 33.58S 21.13E, 1.vii.1995; 2♂, 8♀, Herrie's Drift, Meiringspoort, 33.26S 22.34E, 2.vii.1995; 1♂, Uitspandrift, Meiringspoort, 33.25S 22.34E, 2.vii.1995; all M.D. Picker & D.M. Stevens (SAMC) unless otherwise stated.

Aphanicercopsis outeniquae Barnard

Aphanicercopsis outeniquae Barnard, 1934: 535.

Remarks. In contrast to the other species of *Aphanicercopsis*, *A. outeniquae* has its distribution centred in the southern Western Cape Province, in the Outeniqua, Tsitsikamma, Langeberg, and Langkloof Mountains. It most closely resembles *A. tabularis*, but is clearly separable on features of the epiproct.

Material examined. SOUTH AFRICA: *Western Cape Province*, 5♂, 4♀, Garcia Pass, Riversdale district, 33.58S 21.13E, 1.vii.1995, D. M. Stevens & M. D. Picker (SAMC); 21♂, 19♀, Ysternek Nature Reserve, Prince Alfred's Pass, 33.57S 23.06E, 3.xii.1994, D. M. Stevens & M. D. Picker (SAMC); 3♂, 3♀, Grootvadersbosch River, Grootvadersbosch Nature Reserve, Boosmansbos Wilderness Area, 33.52S 20.23E, 10.ii.1996, D. M. Stevens (SAMC); 1♂, Gouna Forest, Knysna district, 33.58S 23.03E, 2.iii.1996, D. M. Stevens (SAMC); 6♂, Kom Se Pad, 9 km E Brak River, Knysna district, 33.58S 23.03E, 6.iii.1996, D. M. Stevens (SAMC); 1♂, 3♀, Tsitsikamma Coastal Park, Storms River, 33.58S 23.48E, 7.xii.1979, J. G. H. Londt (MPIL).

Aphanicercopsis tabularis Barnard

Aphanicercopsis tabularis Barnard, 1934: 535.

Description of larva. Appearing smooth, with small stout bristles on thorax and abdomen (Figs 2.20, 2.2C-E).

Size (mm). Larvae small to medium-sized; body length male 5.6; female 6.7 (6.7 - 6.8).

Head. Reddish-brown, with irregular dark patches; three small faint black ocelli; compound eyes black, very small; antennal segments with whorl of fine short hairs on distal margins.

Thorax. *Prothorax* pale-brown, with irregular dark patterning; pronotum width (male 0.8 mm and female 0.9 mm) similar to that of head (male 0.8 mm and female 0.9 mm) and mesonotum widths; pronotum not extending laterally beyond margins of prothorax; short stout bristles on margin. *Mesothorax and metathorax* pale-brown with irregular dark markings; row of very short, stout bristles just posterior to anterior margin of mesothorax; conspicuous medial patch of bristles on anterior part of metathorax.

Wingpads. Smooth, lacking enlarged setae.

Legs. Yellow-brown, with stout bristles dorsally and ventrally; glabrous stripe laterally; femur with long, stout bristles dorsally, but with fine setae laterally.

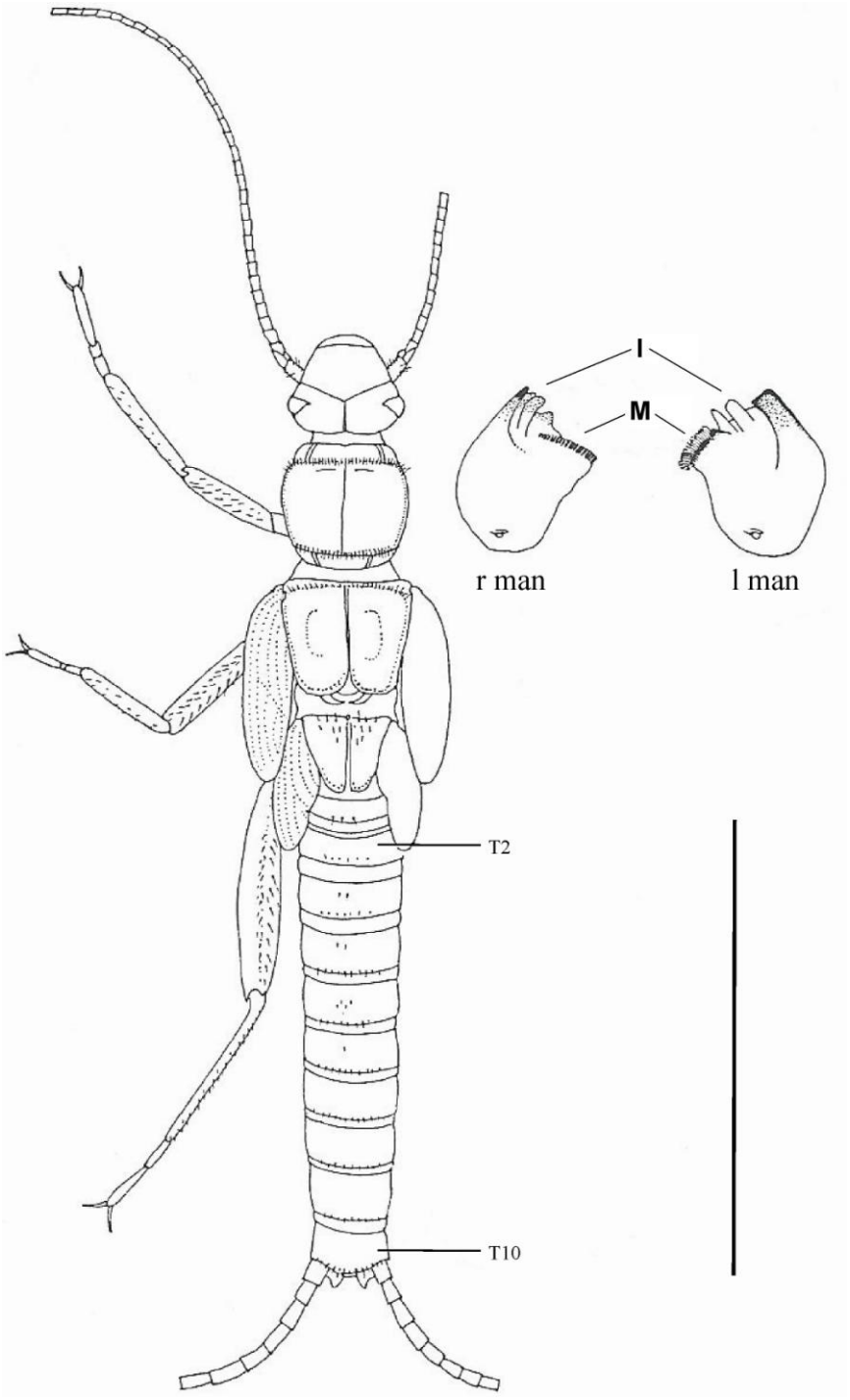


Fig. 2.20. Dorsal view of final instar (black-wingpad) larva of *Aphanicercopsis tabularis* (Pipe track, Twelve Apostles, Peninsula). Abbreviations: I = incisor; l man = left mandible; M = molar; r man = right mandible; T2 = tergite 2; T10 = tergite 10. Scale bar = 2 mm.

Abdomen. Brown to orange-brown; medial third of tergite margins with a row of short, stout bristles; small group of bristles medially on most segments; in most species sternites bearing well developed setae (especially on segments 8-10), often with conspicuous hair sockets on sternite 10 (Fig. 2.2C-E); pleurites evident on first 3-4 segments (Table 2.2).

Cerci. Whorl of short bristles on posterior margin of segments.

Remarks. This species is a Cape Peninsula endemic, where it is widespread. The Peninsula also supports the endemic *Aphanicercella clavata* sp. n. A specimen of *A. denticulata* from Nonna Kloof, Worcester was inadvertently included by Barnard (1934) in a listing of *A. tabularis* localities, although the specimen label clearly identifies it as *A. denticulata*. Larvae of three of the four known species can be separated using medium-sized to large larvae. Species differ both in the number and position of abdominal setae. *Aphanicercopsis tabularis* is the most hirsute, having higher setal counts both dorsally and ventrally on nearly all segments. *Aphanicercopsis outeniquae* Barnard, the smallest species (female body length 5.2 mm) has the lowest setal counts on all segments, and ventral setation only begins on segments 5/6/7 (Fig. 2.2B). *Aphanicercopsis denticulata* (Tillyard), the largest of these three species (female body length 7.5 mm) has setal counts intermediate to those of the above two species, with ventral setation starting on segment 6 (Fig. 2.2A). The pattern of setae is also useful in distinguishing the species, with *A. tabularis* having additional patches of stout spines dorsally and ventrally, which are rare in *A. denticulata* and absent in *A. outeniquae*. The genus is unusual in having somewhat variable pleurite counts for the species, a feature also useful in separating the species (Table 2.2).

Material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, 1♀, Tafelberg Road, Table Mountain, Cape Town, 33.57S 18.25E, 15.v.1993; 1♀, Cecilia State Forest, Cape Peninsula, 34.01S 18.25E, 25.vi.1993; 4♂, 3♀, Silvermine Nature Reserve, Cape Peninsula, 34.05S 18.23E, 6.vi.1993; 1♂, 5♀, Boyes Drive, Cape Peninsula, 34.07S 18.27E, 25.iv.1993; 27♂, 19♀, Slangolie Ravine, Twelve Apostles, Cape Peninsula, 33.58S 18.24E, 21.vi.1993; 26♂, 12♀, Pipe Track, Twelve Apostles, Cape Peninsula, 33.58S 18.24E, 21.vi.1993; 1♂ larva, 2 ♀ larvae, Pipe track, Twelve Apostles, Peninsula, 33.58S 18.22E, 21.vi.1993, D.M. Stevens Private Collection; all D. M. Stevens (SAMC) unless otherwise stated.

Genus ***Desmonemoura*** Tillyard

Desmonemoura Tillyard, 1931: 126.

Type species: *Desmonemoura pulchellum* Tillyard, 1931: 126, by original designation.

***Desmonemoura brevis* sp. n.**, Figs 2.21-2.24

Diagnosis

In *D. brevis* males, the cerci and the processes of pleurites 10 are about half the length of those of *D. pulchellum*. In *D. pulchellum* females, the lobe on sternite 7 is much larger and is more swollen laterally than that of *D. brevis.*

Description

Male. Size. Body length 4.5 ± 1.1 mm, n = 21 (holotype = 4.3 mm). Males are significantly smaller than females (t-test, $P < 0.005$).

Male genitalia. (Figs 2.21, 2.22, 2.24). Tergite 9 bears two posteriorly directed elbow-shaped processes terminating in small spine at posteromedial angle; heavily sclerotized except for median membranous patch. Lateral dorsal plates of tergite 10 sclerotized, bearing a heavily sclerotized small spine proximally; fused medially. Epiproct slender with convex, denticulate lateral margins; tapers distally to rounded apex; attached basally to triangular, apically notched, sclerotized median dorsal plate of tergite 10. Process of pleurite 10 glabrous, reaching almost to the epiproct and terminating in a very short pale spine. Cerci setose and equal in length to the process of pleurite 10. Sternite 9 triangular and elongate with subterminal lateral bulges. Paraprocts with primary supporting struts broad, continuous with the transverse rods, parallel to each other for about two-thirds of their length before forming a medial convexity and diverging distally to a spatulate apex. Medial secondary supporting struts of the paraprocts originate at the junction of the transverse rods with the primary supporting struts, are more slender, shorter and more heavily sclerotized than the primary supporting struts, form the medial margin of a very thin transparent membrane of which the primary supporting struts form the lateral margin, and are fused to each other and to the lateral rods basally and terminate in a spatulate apex. Transverse rods of the paraprocts falciform, terminating acutely.

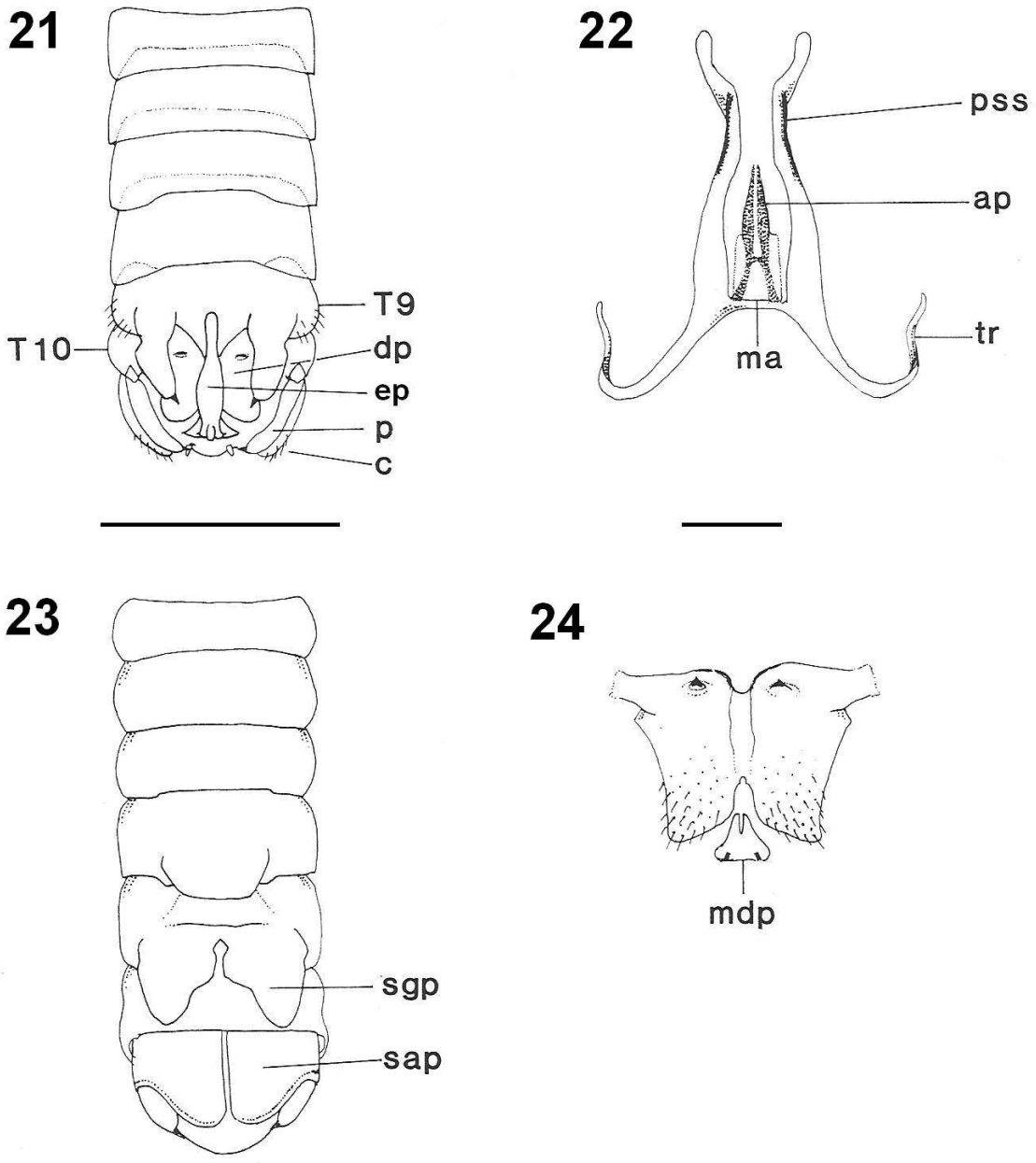
Female. Size. Body length 5.2 ± 1.0 mm, n = 21.

Female genitalia. (Fig. 2.23). Sternites 1-6 unsclerotized. Sternite 7 unsclerotized in proximal half, with distal half lightly sclerotized (yellow-brown) and swollen to form a broad, and usually slightly gibbous posterior margin, median lobe two-thirds the width of the sternite. Sternite 8 lightly sclerotized, the concave proximal half forming a broad transverse groove, and the distal half produced into a deep, triangularly-excised bilobate subgenital plate, with medial margins of excision bearing a mediad convexity. Sternites 9 and 10 unsclerotized.

Larva. Larval taxonomic characters such as abdominal setation patterns concur with those given for the genus.

Etymology. Named for the shortness (Latin: *brevis*) of the processes of pleurite 10 and cerci relative to those of *D. pulchellum*.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, ’10 km east off R29 along Spekboomdraai road, N of Oudtshoorn, 33.32S 22.23E, 4.xii.1994, M.D. Picker & D.M. Stevens' (SAMC). Paratypes, 14♂, 14♀, same data as holotype.



Figs 2.21-2.24. *Desmonemoura brevis*. **21**, male genitalia, dorsal view; **22**, paraprocts; **23**, female genitalia, ventral view; **24**, dorsal plates of tergite 10. Abbreviations: ap – arch process; c – cercus; dp – dorsal plates of tergite 9; ep – epiproct; ma – median arch; mdp – median dorsal plate of tergite 10; p – process of pleurite 10; pss – primary supporting strut; sap – subanal plate; sgp – subgenital plate; T9 & T10 – tergites 9 & 10; tr – transverse rod. Scale bars: **21** & **23** = 1 mm, **22** & **24** = 0.1 mm.

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 3♂, 6♀, Tweede River, Swartberg Pass, nr Prince Albert, 33.18S 22.03E, 4.xii.1994, M.D. Picker & D.M. Stevens (SAMC); 1♂, Swartberg Pass, 12 km west on road to Die Hel, 33.22S 21.51E, 4.xii.1994, M.D. Picker & D.M. Stevens (SAMC); 3♂, between Bergplaas and Kleinplaat, 33.53S 22.40E, 4.12.1979, J. Illies (MPIL).

Desmonemoura pulchellum Tillyard

Desmonemoura pulchellum Tillyard, 1931: 126.

Description of larva. Appearing smooth, but covered evenly in fine hairs with some short setae on abdomen (Figs 2.25, 2.1D).

Size (mm). Medium-sized to large larvae; body length male 6.3, female 5.5.

Head. Pale-brown; three distinct ocelli; compound eyes large, prominent, black; antennal segments each with a whorl of short hair.

Thorax. *Prothorax* pale-brown; pronotum width (male 0.9 mm, female 0.8 mm) equal to head (male 0.9 mm, female 0.8 mm) and mesonotum widths; marginal bristles short; pronotum extended laterally beyond margins of prothorax. *Mesothorax and metathorax* pale-brown, with short setae on anterior margins.

Wingpads. Conspicuously banded in cream and dark-brown in older larvae, and although appearing smooth, are covered in fine setae.

Legs. Pale-yellow; covered in short setae laterally, with a thin glabrous stripe on the lateral surface of the tibiae and femora; numerous enlarged setae on dorsolateral aspect of femora.

Abdomen. Yellow-brown; appearing smooth, but bearing a few stout setae on the posterior margins of segments, with a few scattered spines medially on some segments (Fig. 2.1D,G); pleurites evident on first five abdominal segments.

Cerci. Whorl of short bristles on posterior margin of segments; lateral bristles slightly longer than medial ones.

Material examined. SOUTH AFRICA: *Western Cape Province*, 1♂, 1♀, Molenaars River, Du Toitskloof, 33.44S 19.07E, 03.xi.1992, G. Ractliffe (PSPC).

Remarks. *D. pulchellum* is most common in the south-western Western Cape Province, but is recorded from the Tsitsikamma Mountains at the border of the Western and Eastern Cape Provinces, while *D. brevis* has only been recorded from the Groot Swartberg and Outeniqua Mountains. The former is the more common species. Both species have summer emergence patterns. The holotype slide (wings only) is erroneously labelled '*Desmonemoura pulchella*'.

Type material examined. Holotype ♂, SOUTH AFRICA: *Western Cape Province*, '*Desmonemoura pulchella*. Banhoek. 7.x.29. K.H. B.' Slide, wings only (SAMC).

Additional material examined. SOUTH AFRICA: *Western Cape Province*, 3♂, Groot Drakenstein, 33.56S 18.58E, 25.x.1933, K.H. Barnard & H.G. Wood (SAMC); 1♂, 1♀, Algeria, Cederberg, 32.22S 19.04E, 2.xi.1982, M.D. Picker (SAMC); 4♂, 6♀, same data but 13.xi.1994, D.M. Stevens (SAMC); 6♂, Du Toitskloof, 33.44S 19.08E, 23.xi.1993, D.M. Stevens (SAMC); 1♂, 1 ♀, Swartboskloof, 33.57S 18.56E, M.D. Picker (SAMC); 5♂, Upper Berg River, 33.52S 18.59E, 5.xi.1994, K. Snaddon (SAMC); 1♀, Molenaars River, Du Toitskloof, 33.44S 19.08E, 6.x.1994, G. Ractliffe (SAMC). *Eastern Cape Province*, 2♂, 1♀, Vark River, Tsitsikamma Mountains, 33.57S 23.40E, 3.xii.1979, J. Illies (MPIL); 5♂, 4♀, Bloukranz River, Tsitsikamma Mountains, 33.57S 23.38E, 3.xii.1979, J. Illies (MPIL).

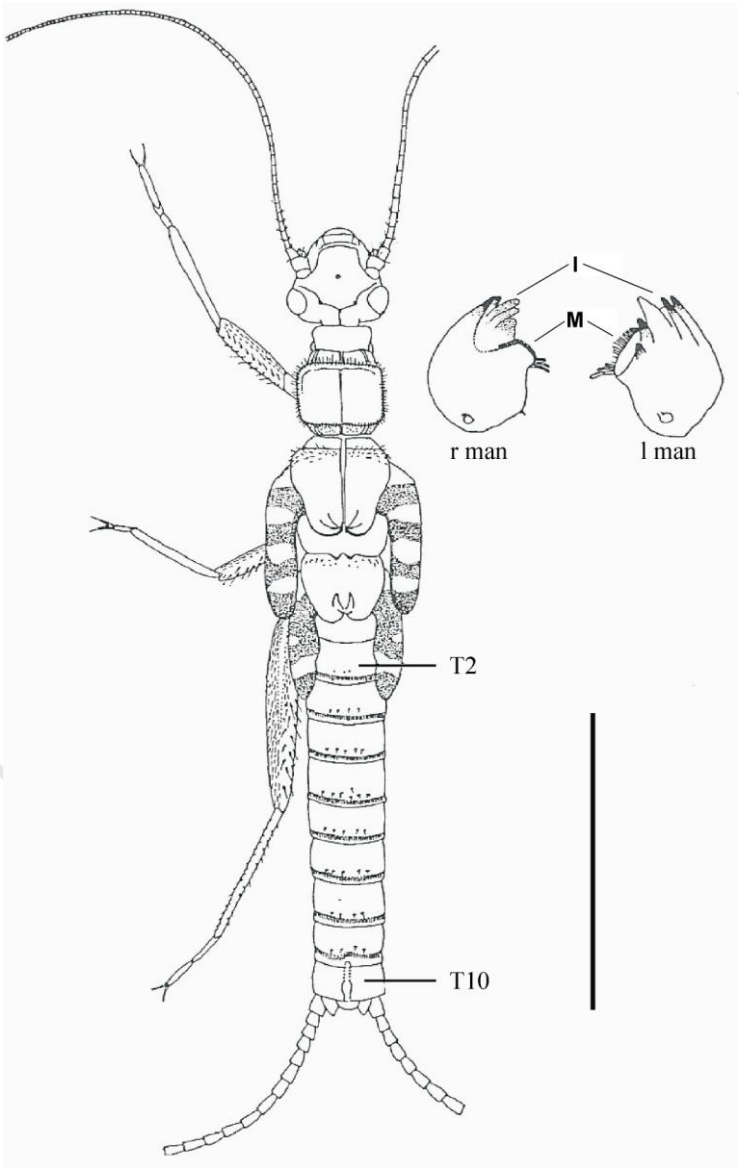


Fig. 2.25. Dorsal view of final instar (black-wingpad) larva of *Desmonemoura pulchellum* (Molenaars River, Du Toitskloof). Abbreviations: I = incisor; l man = left mandible; M = molar; r man = right mandible; T2 = tergite 2; T10 = tergite 10. Scale bar = 2 mm.

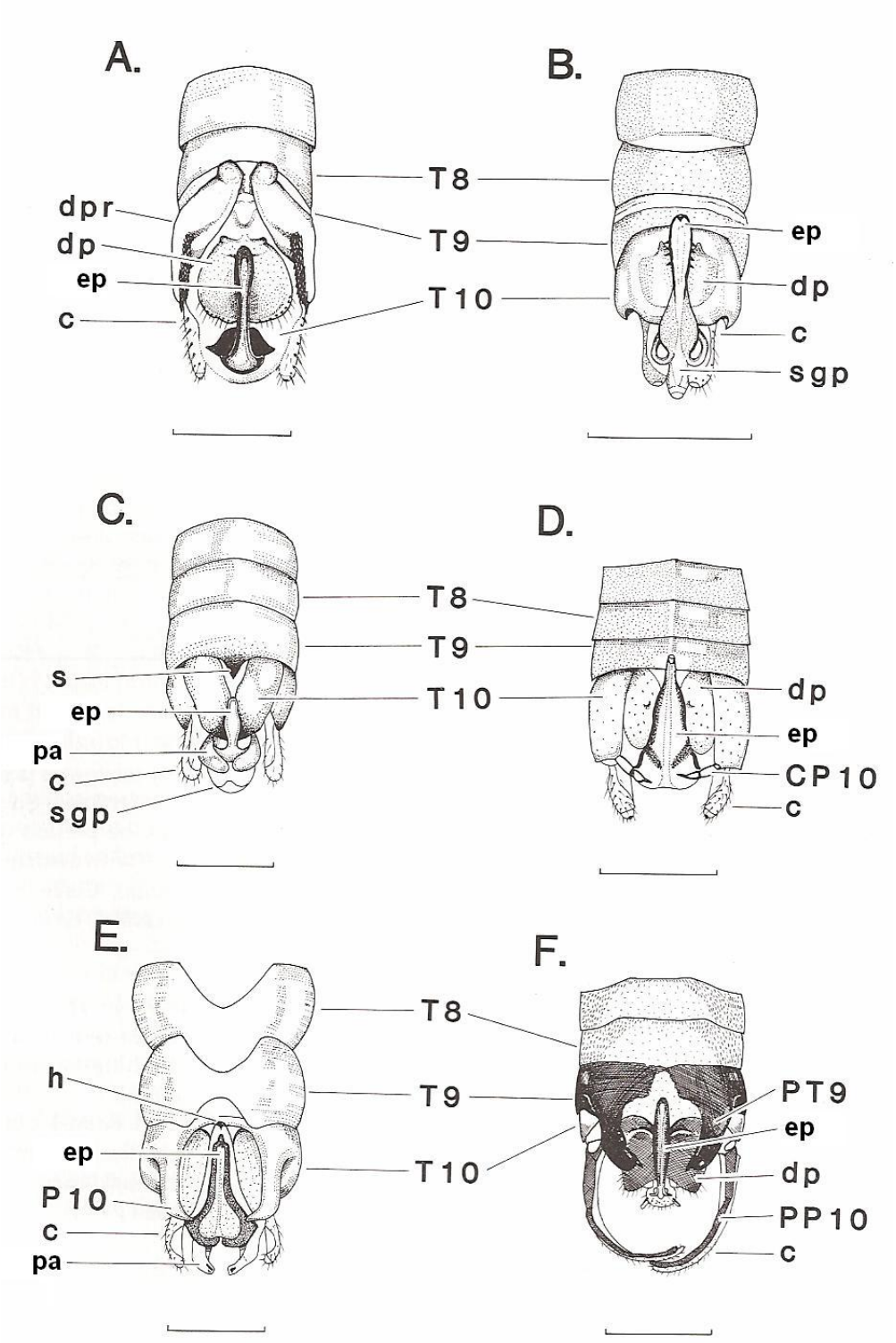


Fig. 2.26. External genitalia of male Notonemouridae genera (dorsal view). **A**, *Aphanicerca capensis* Tillyard (Platteklip Stream, Table Mountain); **B**, *Aphanicercopsis tabularis* Barnard (Silvermine Stream, Cape Peninsula); **C**, *Afronemoura spinulata* (Balinsky) (Hogsback, Eastern Cape); **D**, *Aphanicercella barnardi* complex Tillyard (Citrusdal, Western Cape); **E**, *Balinskycercella gudu* (Balinsky) (Mont-aux-Sources, KwaZulu-Natal Drakensberg); **F**, *Desmonemoura pulchellum* Tillyard (Du Toit's Kloof, Western Cape). Abbreviations: c = circus; CP10 – clasper of pleurite 10; dp = dorsal plate; dpr = dorsal process; ep = epiproct; h = hook; P10 = process of pleurite 10; pa = paraproct; PT9 = process of tergite 9; s = spine; sgp = subgenital plate; T8-T10 = tergites 8-10. Scale bars = 0.5 mm.

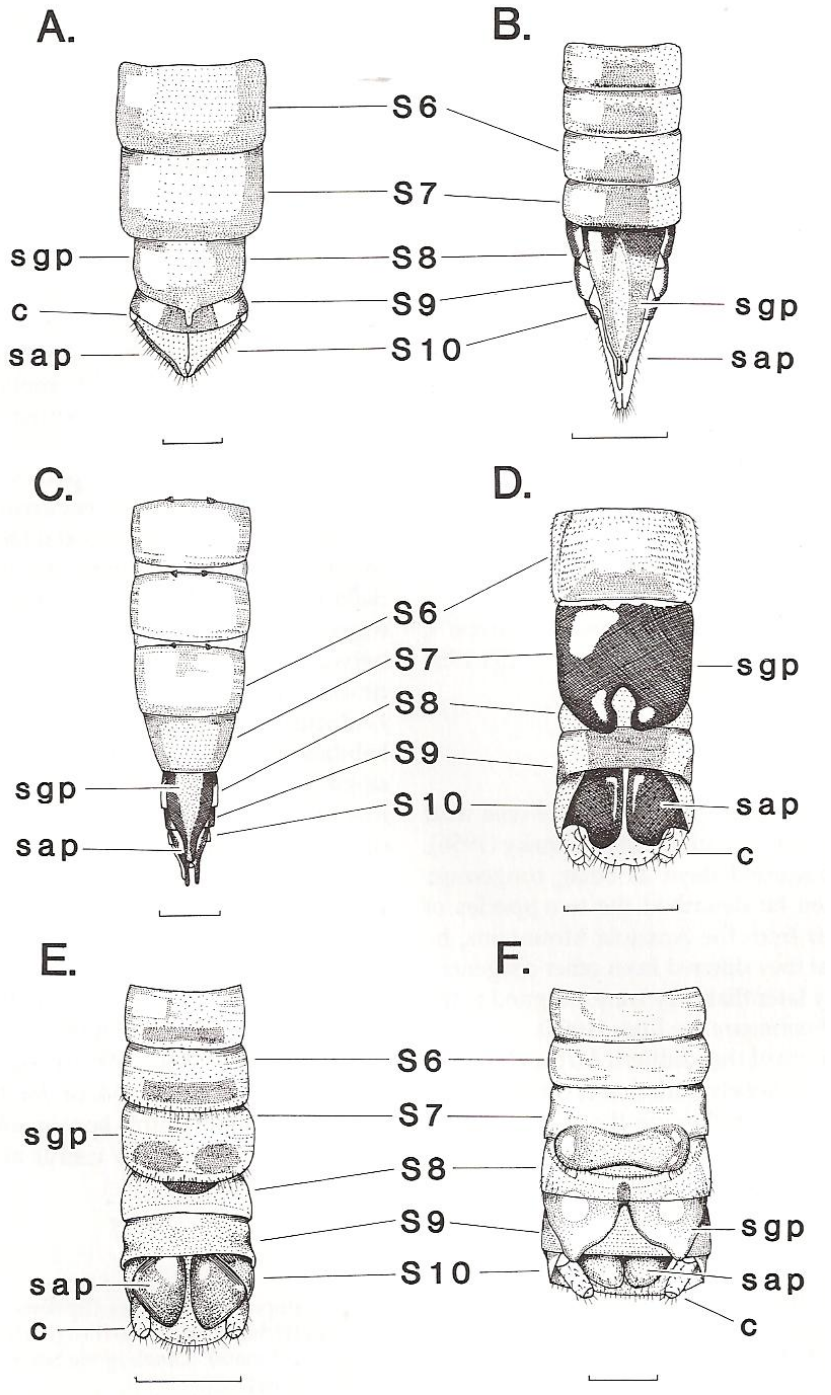


Fig. 2.27. External genitalia of female Notonemouridae genera (ventral view). **A**, *Aphanicerca capensis* Tillyard (Platteklip Stream, Table Mountain); **B**, *Aphanicercopsis tabularis* Barnard (Slangolie Ravine, Table Mountain); **C**, *Afronemoura amatolae* (Balinsky) (Hogsback, Eastern Cape); **D**, *Aphanicercella barnardi* complex Tillyard (Platteklip Stream, Table Mountain); **E**, *Balinskycercella gudu* (Balinsky) (Mont-aux-Sources, KwaZulu-Natal Drakensberg); **F**, *Desmonemoura pulchellum* Tillyard (Swartboskloof, Stellenbosch). Abbreviations: c = circus; sap = subanal plate; sgp = subgenital plate; S6-S10 = sternites 6-10. Scale bars = 0.5 mm.

IDENTIFICATION KEYS

Separate keys to males, females and larvae of the six genera of the Notonemouridae of southern Africa are provided, followed by keys to males of all described species.

Key to genera

Males

1. Wings strongly banded transversely, cerci and processes of pleurite 10 much longer than the epiproct (Fig. 2.26F) ……...…….…. *Desmonemoura* Tillyard
- Wings not banded transversely, cerci and processes of pleurite 10 (if present) short ……..… 2
2. Bifid dorsal process on tergite 9 and distinct clear patch in middle of forewing (at origin of MA_1, MA_2 and Cu_1 with RS) (Fig. 2.26A) …..…….……..……. *Aphanicerca* Tillyard
- No bifid dorsal process on tergite 9; clear patch on forewing present or absent 3
3. One or two small median spines on posterior edge of tergite 9 (Fig. 2.26C) …….. *Afronemoura* Illies
- No spines on posterior edge of tergite 9 ……...……... 4
4. Anterior apex of median plate of tergite 10 bent upward and produced into a recurved hook which may be bifid, clear patch in middle of forewing (at junction of MA_1, MA_2 and Cu_1 with RS) ……..……...…….…. *Balinskycercella* gen. n.
- Anterior apex of median plate of tergite 10 not bent upward into a hook, no clear patch ..… 5
5. Distal margins of pleurite 10 elongated to form arm-like claspers, subgenital plate short and rounded (Fig. 2.26D) ……..……..…….…… *Aphanicercella* Tillyard
- Distal margins of pleurite 10 not modified to form claspers, subgenital plate elongate (Fig. 2.26B) ……..…….. *Aphanicercopsis* Barnard

Females

1. Sternite 8 forms subgenital plate ……..…..…….. 2
- Sternite 7 forms subgenital plate ……...……..…… 5
2. Wings strongly banded transversely, subgenital plate bilobate with a deep triangular incision posteriorly, sternite 7 bears a transverse hollow cylindrical structure (Fig. 2.27F) …….. ……..…… *Desmonemoura* Tillyard
- Wings not banded, no deep incision in subgenital plate, no structure on sternite 7 …… 3
3. Clear patch in middle of forewings (at junction of MA_1, MA_2 and Cu_1 with RS), cerci short and cylindrical, subgenital plate short or only moderately elongate (Fig. 2.27A) …….. ……..… *Aphanicerca* Tillyard
- No clear patch in middle of forewings, cerci very short and conical, subgenital plate elongated to form an ovipositor-like structure ……..…….……..… 4

4. Distal tip of subgenital plate slender and obscured by hairs, subgenital plate brown (Fig. 2.27C) …….…… *Afronemoura* Illies
- Distal tip of subgenital plate robust and glabrous, subgenital plate cream (Fig. 2.27B) ……..…….. *Aphanicercopsis* Barnard
5. Clear patch in middle of forewing (at junction of MA_1, MA_2 and Cu_1 with RS) (Fig. 2.27E) …….. *Balinskycercella* gen. n.
- No clear patch in middle of forewing (Fig. 2.27D) …..…….. *Aphanicercella* Tillyard

Larvae

1. Conspicuous tuft of coarse setae situated one third of the way up the antennae, on closer examination comprising two groups of setae located approximately on segments 26 and 27; pleurites on abdominal segments 1-5, body appearing smooth, but bearing fine short translucent setae; mandibular formula 4:6 (Fig. 2.5) ……..... *Afronemoura* Illies
- No tufts of setae on antennae 2
2. Enlarged medial hairs extending halfway up the antennae; compound eyes very small; body virtually free of setae; pleurites on abdominal segments 1-5/5.5; mandibular formula 5:6 (Fig. 2.10) ..... *Aphanicerca* Tillyard
- No medial enlarged antennal hairs 3
3. Body covered in numerous long setae 4
- Body appears smooth; setae short and inconspicuous where present 5
4. Complete whorls of long setae on all abdominal segments; broken midventrally; pleurites on abdominal segments 1-7/7.5; wingpads evenly covered in long bristles; mandibular formula 6:6 (Fig. 2.4) ..... *Balinskycercella* gen. n.
- Incomplete whorls of setae on abdominal segments; dorsal setae situated medially and not reaching lateral margins (exceptions have complete whorls restricted to segments 8-10); pleurites on abdominal segments 1-6/6.5; mandibular formula 4:5 (Fig. 2.17) ……. ..... *Aphanicercella* Tillyard
5. Abdominal segments 1-3/4 having pleurites; prothorax appearing circular; of equal length and width; curved lateral margins; eyes very small; mandibular formula 4:4 (Fig. 2.20) …… ..... *Aphanicercopsis* Barnard
- Abdominal segments 1-5/6 having pleurites; prothorax rectangular; wider than long; lateral margins straight; eyes round and very large; wingpads strikingly barred in more mature larvae; occasionally with slightly enlarged medial hairs on first few antennal segments; mandibular formula 5:6 (Fig. 2.25) ..... *Desmonemoura* Tillyard

Key to males of *Afronemoura*

1. One medial spine on posterior margin of tergite 9 ……..…..…. *A. spinulata* (Balinsky)
- Two medial spines on posterior margin of tergite 9 ……..……..……. 2
2. Posterior margin of tergite 9 convex; two spines widely separated .... *A. amatolae* (Balinsky)
- Posterior margin of tergite 9 bilaterally concave; two spines close together and connected by a sclerotized band (Fig. 2.6) ……..…….. *A. stuckenbergi* sp. n.

Key to males of *Aphanicerca*

1. Epiproct bears a denticulate convexity on anterior face in lateral view ……..…..…..…… 2
- No convexity on anterior face of epiproct ……..……..……..……. 6
2. Lobes of dorsal process of tergite 9 long, directed posteroventrally proximally and then gradually curving strongly dorsad (Fig. 2.11) ....……..……..…… *A. gnua* sp. n.
- Lobes of dorsal process of tergite 9 not curving strongly dorsad, but may be curved or bent mediad or be subparallel …....……... 3
3. Distal third of lobes of dorsal process of tergite 9 curved strongly mediad, and no ridge of spinules present ……..……..……..…….. *A. lyrata* Barnard
- Distal third of lobes of dorsal process of tergite 9 not curved strongly mediad, although may be elbow shaped, subparallel, or have a slight mediad curve over entire length ……..… 4
4. Lobes of dorsal process of tergite 9 bear a very conspicuous ridge of dorsal or medial spinules distally (Fig. 8) ……. 5
- Lobes of dorsal process of tergite 9 slender and widely separated distal to the base, becoming subparallel or with a slight mediad curve, with an inconspicuous row of minute dorsal spinules over almost the entire length ……..…….. *A. bicornis* Barnard
5. Lobes of dorsal process of tergite 9 bear a very dense and intensely sclerotized dorsal ridge of spinules in the distal half, and may be twisted at the origin of that ridge ……...…….. ….……..……..……..……. *A. capensis* Tillyard
- Lobes of dorsal process of tergite 9 gently sigmoid with a medial ridge of spinules originating slightly distal to the second curvature of the lobe (Fig. 2.14) ….. *A. chanae* sp. n.
6. Lobes of dorsal process of tergite 9 apically truncate and recurved mediad and dorsad .. ……..……..……..……. *A. uncinata* Barnard
- Lobes of dorsal process of tergite 9 apically acute and not recurved ……..…… 7
7. Three to four sharp denticles on medial distal margin of lobes of dorsal process of tergite 9 ….……..……..……..… *A. tereta* Barnard

- No denticles on medial distal margin of lobes of dorsal process of tergite 9 ……..…….. ……. *A. bovina* Barnard

Key to male *Aphanicercella* species

1. Epiproct apically incised ……...…….…… 2
- Epiproct not apically incised ……..……...…… 4
2. Medial margins of epiproct incision convex; width of incision equal to length of lateral margin of epiproct; clasper longer than, or equal in length to dorsomedial margin of pleurite ……..……...…… *A. nigra* Barnard
- Medial margins of epiproct incision concave; width of incision about half or less than half the length of lateral margin of the epiproct; clasper shorter than dorsomedial margin of pleurite …….. 3
3. Width of epiproct incision about half the length of lateral margin of epiproct; anterior margin of epiproct forms a heavily sclerotized broad band; lateral dorsal plates not fused anteriorly ……...……..…… *A. quadrata* Barnard
- Width of epiproct incision about one-quarter the length of lateral margin of epiproct; anterior margin of epiproct not heavily sclerotized; lateral dorsal plates fused anteriorly ……..…… *A. bifurcata* Barnard
4. Epiproct apically truncate with a subapical V-shaped sclerotized transverse strip; epiproct bears no minute apical ventral projection …..……....…… *A. scutata* Barnard
- Epiproct apically acute, rounded, or sub-truncate without a subapical v-shaped sclerotized transverse strip; epiproct bears minute apical ventral projection ……..…. 5
5. Median arch comprises only one process ……...…….... 6
- Median arch comprises more than one process …….…….… 8
6. Basal supporting process of paraproct free standing distal to its base, is elongate, and its apex bulges medially; clasper is equal in length to the dorsal margin of pleurite 10 ……...… ……...……..…… *A. clavata* sp. n.
- Basal supporting process of paraproct firmly adherent to base of primary supporting strut of paraproct, short, and apically rounded without bulging; clasper is half to three-quarters the length of the dorsal margin of pleurite ……..…….…… 7
7. Epiproct triangular with rounded apex; clasper's apical point originates eccentrically; median extension of median dorsal plate of tergite 10 elongate; arch process narrow but stout basally and abruptly changes angle and narrows into an acuminate extension distally; transverse rod bi-apically rounded, but sclerotized strut within terminates acutely ……..... ……..…… *A. bullata* sp. n.

- Epiproct with lateral margin becoming convex and then concave distally, terminating acutely; clasper apical point originates centrally; median extension of median dorsal plate of tergite 10 short; arch process short, thin, and spinous; transverse rod bi-apically very broadly rounded ……...…… *A. cassida* Barnard

8. Basal supporting process of paraproct present ……..…….…… 9

- Basal supporting process of paraproct absent or vestigial …….. *A. flabellata* sp. n.

9. Basal supporting process of paraproct very large, fused to base of arch process, and free standing distally; clasper apical point originates anteriorly ……... *A. barnardi* Tillyard

- Basal supporting process of paraproct small, not fused to arch process, and not free standing distally; clasper apical point originates centrally …….…… 10

10. Spinous part of arch process caudally directed; transverse rod bi-apically acute; primary and medial secondary supporting struts of paraproct joined by a transverse sclerotized band ….. ……..……..... *A. securata* sp. n.

- Spinous part of arch process dorsally directed; transverse rod bi-apically rounded, but sclerotized strut within terminates acutely; primary and medial secondary supporting struts of paraproct joined by an oblique sclerotized band ……..…….. *A. spatulata* sp. n.

Key to males of *Aphanicercopsis*

1. Epiproct with lateral basal expansions …..……...……. 2

- Epiproct without lateral basal expansions ……...……... 3

2. Lateral sclerotized struts of epiproct separated except apically; posterior angle of median dorsal plate of tergite 10 wide and rounded ……...…. *A. tabularis* Barnard

- Lateral sclerotized struts of epiproct meet at an acute angle just proximal to the lateral basal expansion; posterior angle of median dorsal plate of tergite 10 acute and narrow …….…..…… *A. outeniquae* Barnard

3. Epiproct broad basally and narrowing abruptly distally, narrow subapical distal third denticulate ……..…….……..……. *A. hawaquae* Barnard

- Epiproct linguiform, lateral margins denticulate over middle third …… ……...… *A. denticulata* Barnard

Key to males of *Balinskycercella*

1. Apical hook of median plate of tergite 10 bifid …….……...…. *B. fontium* (Balinsky)

- Apical hook of median plate of tergite 10 single ……..…….. 2

2. Transverse rods of paraproct spatulate apically; epiproct broadens towards apex; clear patch on forewings ……..……....…….…..... *B. tugelae* (Balinsky)
- Transverse rods of paraproct apically narrow and rounded; epiproct almost parallel-sided over apical half; no clear patch on forewings ……..…….. *B. gudu* (Balinsky)

Key to males of *Desmonemoura*

1. Cerci and processes of pleurite 10 very long, extending about twice the distance from their bases to the epiproct ……...…….. *D. pulchellum* Tillyard
- Cerci and processes of pleurite 10 extend almost equal to the distance from their bases to the epiproct (Fig. 2.21) …....…… *D. brevis* sp. n.

Chapter 3
Cryptic speciation in a South African stonefly (Plecoptera: Notonemouridae: *Aphanicerca capensis* Tillyard): evidence from morphology, distribution, mating behaviour and mtDNA

One of the great challenges in studying biodiversity is that of distinguishing geographically distributed morphological variation from true differentiated lineages that may comprise a new, and often overlooked, species complex. In this study numerous lines of evidence are examined within a practical application of the unified (general lineage) species concept in a notonemourid stonefly, *Aphanicerca capensis* Tillyard from the Cape Folded Mountains of South Africa. These lines of evidence included assessments of: allopatric fragmentation, genetic structuring, intrinsic reproductive isolation (four types – syntopic, sympatric, and complete and incomplete premating isolation during mate choice trials), morphological phenetic distinguishability, morphological diagnosability, monophyly and reciprocal monophyly. Two out of the ten lines of evidence provided parallel lines of support for all 12 morphogroups as independently evolving metapopulation lineages (i.e. species), namely morphological phenetic distinguishability and male morphological diagnosability. Sole reliance on any one of the other criteria failed to delimit all 12 morphogroups as species. Morphology alone was sufficient to differentiate between these new species, but the additional lines of evidence afforded added support for species delimitation. Eight of the 12 species were successfully delineated using the criterion of monophyly. Analysis of morphometric characters provided further support for the evolutionary relationships among these new species and draws attention to the characters that delimit members of this species complex. Of interest is the finding that the *Aphanicerca* COI gene tree and species tree (as hypothesized from morphological relationships) were incongruent; moreover, the COI gene did not appear to be an efficient 'barcode' molecular marker for this group. Because syntopic distinct morphogroups shared haplotypes, it is clear that the sole use of genetic distance alone would be inappropriate in species delimitation in this species complex. Reproductive cohesion appeared to be incomplete in the recently separated allopatric species of the *A. capensis* complex, but species unity was maintained in sympatric situations. Rates of change in mate recognition systems in the *A. capensis* complex appeared to lag behind those of morphological and genetic divergence in vicariant speciation. In addition, there was also evidence for the distribution of spatially structured morphological lineages across the Cape Folded Mountains, evidence of mitochondrial introgression (possibly historical) or incomplete lineage sorting (or both) within the species complex, and evidence of a centre of origin of the species complex in the central region of the Cape Folded Mountains.

Key words: Notonemouridae, *Aphanicerca capensis*, species complex, morphometrics, cytochrome oxidase I, mitochondrial DNA, species delimitation

INTRODUCTION

Of the 16 extant families of the small insect order Plecoptera, only two occur in southern Africa, namely the Perlidae and the relictual family Notonemouridae (Balinsky 1962; Zwick 1973). The Notonemouridae have a Gondwanan distribution with 31 described species in six

genera (Picker & Stevens 1999) occurring in southern Africa, and the remaining 90 species in Australia, New Zealand, Madagascar and South America (Fochetti & Tierno de Figueroa 2008). Notonemouridae are restricted to cold, low order, fast-flowing streams with stony substrates.

The South African genus *Aphanicerca* Tillyard was erected by Tillyard (1931) (from specimens collected mostly by K.H. Barnard), and is particularly interesting as it initially comprised representatives of what later were divided into three distinct genera. Also interesting is the unique bifid dorsal process of the male tergite 9 which shows taxonomically useful variation in shape and size. When describing the type species *A. capensis*, Tillyard erected two subgenera within *Aphanicerca*, with the subgenus *Aphanicerca* containing *A. capensis* (type locality Table Mountain), and the second subgenus, *Aphanicercella* Tillyard containing *Aphanicerca denticulata* Tillyard (later *Aphanicercopsis denticulata* (Tillyard)), and *Aphanicerca barnardi* Tillyard (later *Aphanicercella barnardi* Tillyard). Barnard (1934) removed the subgenus category and re-described *Aphanicerca capensis*, and additionally described *A. uncinata*, *A. lyrata*, *A. bicornis*, *A. bovina* and *A. tereta*. Of importance was the recognition by Barnard of allopatric "varieties" of *A. capensis* from Wellington, Montagu Pass and Tulbagh (Fig. 7 in Barnard 1934), based on the shape of the dorsal process of tergite 9 of males, and subgenital plates of females. He stated however, that the slight variation observed in male and female genitalia did not justify assigning varietal names to them. Barnard recorded his variety α from the northern Hottentots Holland Mountains (Wellington, Klein Drakenstein, Worcester, Tulbagh, Franschhoek Pass) and the Cederberg Mountains; his variety β was recorded from Tulbagh, and also from the Gydo Pass north of Ceres (Skurweberge) (Barnard 1936), and variety γ (females only) from the southern (Kleinmond) and northern (Landdrost) Hottentots Holland Mountains, and the Riviersonderend Mountains. In addition, he recorded only males from the Outeniqua Mountains (Barnard 1934, 1936). Through extensive collection and examination of specimens, it became evident that these different varieties warranted closer examination (Picker & Stevens 1999). In this contribution, this complex of morphologically similar stoneflies is examined in the context of possible cryptic speciation.

The Cape Floristic Region (CFR) of South Africa, which encompasses the distribution of most of the notonemourid stoneflies, is characterized by a high degree of plant species endemism and diversity (Goldblatt & Manning 2002), especially the south-west region (Simmons & Cowling 1996). Furthermore, diversity of the freshwater fauna (invertebrates, fish and amphibia) of the CFR is high, with about 64% endemism (Wishart & Day 2002); it is interesting to note however that the terrestrial plant-associated insect diversity in the same region is relatively low, with reasons discussed by Giliomee (2003). Diverse groups of faunal endemics and cryptic species include cicadas (Price *et al*. 2007), isopods (Gouws *et al*. 2004),

velvet worms (Daniels *et al*., in press), chameleons (Tolley *et al*. 2006, 2008), fish (Skelton *et al*. 1995; Swartz *et al*. 2007), amphibia (Drinkrow & Cherry 1995) and many others (Picker 1999). Numerous factors, including climatic and geophysical, have been posited as playing a causative role in the high floral and faunal endemism and biodiversity, including rainfall patterns and the topographically complex intersection of the Western and Southern Folded Mountains (*sensu* Kleynhans *et al*. 2005) (Stuckenberg 1962). The highest terrain diversity within the Cape Folded Mountains (CFM) (*sensu* Kleynhans *et al*. 2005) occurs in the Hottentots Holland area (Deacon *et al*. 1992). Whilst species richness of Afrotemperate plant clades does not appear to be associated with habitat heterogeneity, but rather with clade age and distribution range (Gehrke & Linder 2008), a strong relationship does exist between palaeorelictual invertebrate distributions and topographical features in this region; the largest number of records (55%) and the highest species density (0.024 per km^{-2}) are found within the CFM compared to other areas (Day 2005).

The centre of diversity for the African Notonemouridae is the mountainous region of the CFR, and in particular the south-western area of this region. Of the six notonemourid stonefly genera, *Aphanicerca*, *Aphanicercopsis* Barnard and *Desmonemoura* Tillyard are endemic to the CFM in the south-western and southern region of the Western Cape and a short distance into the Eastern Cape Province (Stevens & Picker 1995). Stoneflies are weak fliers and dependant on cool microhabitats for survival, resulting in poor vagility, and are thus ideal candidates for vicariant allopatric speciation. In a study of leuctrid stonefly dispersal (Macneale *et al*. 2005), most adults were caught within 50 metres of the natal stream, while a small percentage had dispersed between 500 and an estimated 640 metres. Although dispersal across nearby watersheds is therefore possible, Macneale *et al*. (2005) also pointed out that other studies on a stonefly (Hughes *et al*. 1999) and a net-winged midge (Wishart & Hughes 2001) have suggested that steep terrain separating streams may act as a physical barrier to dispersal. It can therefore be hypothesized that the combination of 1) poor vagility, 2) cycles of climatic change during the Plio-Pleistocene (Deacon 1983) (other authors though, claim climatic stability during this time (Price *et al*. 2007)), and 3) the potential for multiple refugia in the complex topography of the CFM, are together conducive to isolation of populations. Together with the antiquity of the group, these features are likely to result in divergent lineages across this region (Stuckenberg 1962; Hendey 1983a; Barraclough 2006).

Cryptic species have been reported in a diverse array of taxa that display conservative morphological evolution or have recently diverged (Bickford *et al*. 2006). Morphological stasis though, does not always signify recent speciation (Bickford *et al*. 2006). Criteria for the delimitation of biological species can come from numerous sources, typically morphology and

genetic markers, but in general, multiple and independent data sources are recommended for species delimitation (Ross 1974; Crowe 1999; Sites & Marshall 2004; Coyne & Orr 2004; Knowles & Carstens 2007; Petersen *et al.* 2007). In Plecoptera, male genitalic morphology is more useful than that of the female in separating species, and the Notonemouridae are no exception. Picker (1980) used allozymes, male and female internal and external genitalic characters and egg morphology as evidence for a species complex in southern African populations of *Neoperla spio* (Perlidae). Intersexual communication employed in courtship is another data source used in differentiating species within the suborder Arctoperlaria; for example, the structure of the species specific (Stewart 1997) drumming (mate recognition) calls of *Isoperla* is important in distinguishing species (Tierno de Figueroa & Luzón-Ortega 2002; Tierno de Figueroa & Sánchez-Ortega 1999). Baumann & Kondratieff (2008) resolved the *Alloperla severa* Hagen (Chloroperlidae) species complex based on male epiproct morphology.

Because of the pronounced endemism in the CFM, it is likely that further notonemourid species remain to be discovered in remote mountain streams. The application of molecular and behavioural techniques among morphologically conservative taxa is likely to reveal additional (cryptic) species. For example, a recent phylogeographic study of the notonemourid stonefly *Aphanicercella cassida* Barnard, based on mitochondrial DNA, has shown this species to comprise two geographically and genetically distinct populations, a northern Mpumalanga Province population, and a southern Western Cape Province population, which may prove to be cryptic species pending further research (van Alphen-Stahl, unpubl.). In another solely molecular study on defining functional units for lotic ecosystem biodiversity conservation, Wishart (2002) compared three of the *A. capensis* populations, concluding that three varieties (those of Table Mountain; Jonkershoek + Bain's Kloof; and Garcia's Pass + Swellendam) should not be considered as separate species, but rather as evolutionary significant units. He did not, however, compare the adults morphologically, only using molecular data from larval collections. The varieties sampled by Wishart (2002) were the morphogroups ***C*** (Table Mountain), ***Z*** (Hottentots Holland northern) and ***L*** (Langeberg) (Fig. 3.1) respectively in the present study.

This study of speciation within the *Aphanicerca capensis* species complex is conceived within an *a priori* morphological framework hypothesis. At this point the term "species complex" is used for convenience, prior to species delimitation decisions resulting from the analyses employed here. From Barnard (1934) and personal observation, a morphological definition that set the species complex apart from the other species within the genus was established. Within this definition, morphological differences between populations were used to hypothesize the existence of 12 species. Some of these populations are allopatric, some

parapatric, others sympatric and some even syntopic. In this study morphologically united populations are called "morphogroups" to highlight the fact that they are established on morphological or morphometric characters until the conclusion of the study when the term "species" can be conferred if additional supportive lines of evidence can be found. Within this framework, the unified species concept (also called the general lineage species concept; de Queiroz 1998, 1999, 2007) which recognizes the common element in all previous species concepts that species are segments of separately evolving metapopulation lineages (de Queiroz 2007), is then applied. While the primary criterion of a species is that it is a segment of a separately evolving metapopulation lineage, this concept regards the different lines of evidence, of separately evolving lineages, as secondary species criteria. The application of this concept in delimiting species is to demonstrate lines of evidence as secondary criteria to infer that a metapopulation is a separately evolving lineage. The aim here is to present a morphological hypothesis to define the morphogroups or species and then to garner additional support through biological, morphometric and preliminary molecular data to reach a decision on species delimitation. To this end, species boundaries between populations of *A. capensis* were explored using: 1) gross external morphology of males and females, 2) multivariate analyses of linear measurements of male external genitalia, 3) distributional patterns, 4) mate choice trials, and 5) mitochondrial DNA sequence data analysis. The use of reproductive isolation, as evidence of individuated evolutionary histories, as used in this study does not imply adherence to the theoretical definition of the Biological Species Concept (*sensu* Mayr 1942, 1970) over any other concept, because any species concept would agree that there can be no doubt that observed reproductive isolation (pre-zygotic or post-zygotic) of sympatric populations provides an operational criterion for species delimitation. Reproductive isolation can be inferred by the presence of multiple sympatric morphogroups, by the lack of observed field intermediates or by controlled laboratory mating trials which are rarely carried out (e.g. Picker 1980; Dagley *et al*. 1994; Stevens & Picker 1999; Chapter 2 of this thesis; Prendini *et al*. 2005).

Morphometric analyses are commonly used to investigate species complexes or the existence of separate lineages in diverse taxa of animals and plants, often as part of a total evidence approach, for example, braconid parasitic wasps (Baylac *et al*. 2003), beetles (Chown & Stamhuis 1992; Gómez-Zurita *et al*. 2007), moths (Althoff *et al*. 2001), caddisflies (Bálint *et al*. 2008), cladocera (Kappes & Sinsch 2002), spiders (Bond & Stockman 2008), ticks (Hutcheson *et al*. 1995), river crabs (Stewart 1997), fish (Zaki *et al*. 2008), snakes (Malhotra & Thorpe 2004), Ranunculaceae (bugbane plants) (Compton & Hedderson 1997), and seagrass (Campey *et al*. 2000). The occurrence of similar yet morphologically distinct groups of organisms implies within-group morphological stability in time and space. This finding would be consistent with a scenario of grouped populations as segments of separately evolving metapopulation lineages,

and would therefore be informative as secondary evidence of speciation. Morphological characters represent a broad sampling of the nuclear genome and are complimented by mitochondrial DNA data in a combined analysis, especially useful in closely related species when morphological differences may be relatively minor as in cryptic species.

Phylogeographic techniques can also be highly informative in tests of species delimitation. Phylogeography examines the principles and processes shaping the distribution in time and space of closely related species (Avise 2000). This is achieved through analysis of the geographical distribution of genealogical lineages (Emerson & Hewitt 2005). The mitochondrial genome has generally been favoured over the nuclear genome in phylogeographic studies (Beheregaray 2008). Avise *et al*. (1987) list features of mitochondrial DNA (mtDNA) that make it ideal for studies of phylogeography; these include lack of recombination through maternal inheritance (some paternal is possible but insignificant (Avise 2000)), and more rapid evolution than the nuclear genome, which is due to both reduced effective population size (N_e) and higher mutation rates (Ballard & Whitlock 2004). Despite these and other advantages, there are also drawbacks to the sole use of mitochondrial DNA in phylogeographic studies (Ballard & Whitlock 2004). These include physical linkage of markers resulting in a lack of independent replicated data, mutation rate heterogeneity among lineages, paralogy, incomplete lineage sorting and introgression. Nonetheless, mtDNA has proved very popular and useful, largely due to the small N_e resulting in a four fold more rapid time to coalescence than nuclear genes (Wiens & Penkrot 2002). Because it is widely used in speciation and phylogeographic studies (Beheregaray 2008), the mtDNA cytochrome oxidase subunit I (COI) gene was used in this study. It is important for comparative purposes on a global scale and across the entire spectrum of insect taxa, that one or a few markers are routinely used in insect molecular systematics to maximize compatibility with previous research (Caterino *et al*. 2000), and to enable better understanding of ordinal and lower relationships. COI is one of the three most commonly sequenced genes, the other two being COII and 16S rDNA (Caterino *et al*. 2000). The COI marker was selected also because this is the gene chosen as the DNA barcode marker (Hebert *et al*. 2003), and as such its utility in the southern African Notonemouridae needs to be evaluated in this light.

The controversial concept of DNA barcoding was introduced as the taxonomy of the future, but it has been shown to fail at delimiting species at the level where it would be most useful: where divergence is recent among species and may not be readily distinguished by morphological characters (Sperling 2003). Molecular data can however support species delimitation hypotheses through inferring separately evolving lineages by, for example, genetic distance (Petersen *et al*. 2007; but see Ferguson 2002), monophyly (Donoghue 1985; Mishler

1985; Mishler & Theriot 2000), reciprocal monophyly (Avise 2000), and allopatric fragmentation (Templeton 1989, 2001). In the present study standard phylogenetic techniques (maximum parsimony, maximum likelihood and Bayesian Inference) are used to discover secondary support for monophyly and reciprocal monophyly among the identified morphogroups. These techniques are more appropriate for among-species rather than within-species analyses because of the reticulate nature of relationships within the latter. They also assume a dichotomous branching pattern. In the case of a single species with gene flow between populations, phylogenetic techniques may result in widespread nonmonophyly. In this way, the standard phylogenetic trees (cladogram) may be useful in assessing species status by the degree of monophyly and reciprocal monophyly. In contrast, network phylogeographic methods allow visualization of reticulate relationships between populations within a species. Then, a network with similar topology to the cladogram is useful corroboration of limited or no gene flow between populations with respect to the genetic marker used. The second phylogeographic approach used in this study, nested clade analysis as elaborated on later, builds on the network approach by incorporating geographical distribution data, and may lead to inferences consistent with speciation, thereby providing further secondary support to the primary morphogroup species hypothesis. Considering that there is no sharp demarcation between population biology and phylogenetic biology in terms of genetic history (Maddison 1995), both phylogenetic and phylogeographic techniques are used concurrently in this study, an approach that is useful because recently speciated or incipient species are evolutionarily positioned where both methodologies may help to resolve relationships.

The primary aims of this chapter are: 1) to examine *Aphanicerca capensis* (*sensu lato*) morphometrically, morphologically, reproductively (using mate choice trials), and genetically using mitochondrial DNA (cytochrome oxidase subunit I) to determine whether or not a species complex exists using the unified (general lineage) species concept, and in so doing, to determine species boundaries within this species complex; 2) if shown that a species complex exists, to provide a means to correctly assign individuals to one of the newly discovered species; and 3) to evaluate the utility of the COI gene as a DNA barcode for the genus *Aphanicerca*, and by extrapolation, more generally for the southern African Notonemouridae. Additional aims are to examine the relationship between mate recognition systems and morphological and genetic divergence in the *A. capensis* species complex, and to determine possible processes that shaped regional speciation within the *A. capensis* species complex using the constructed phylogenies.

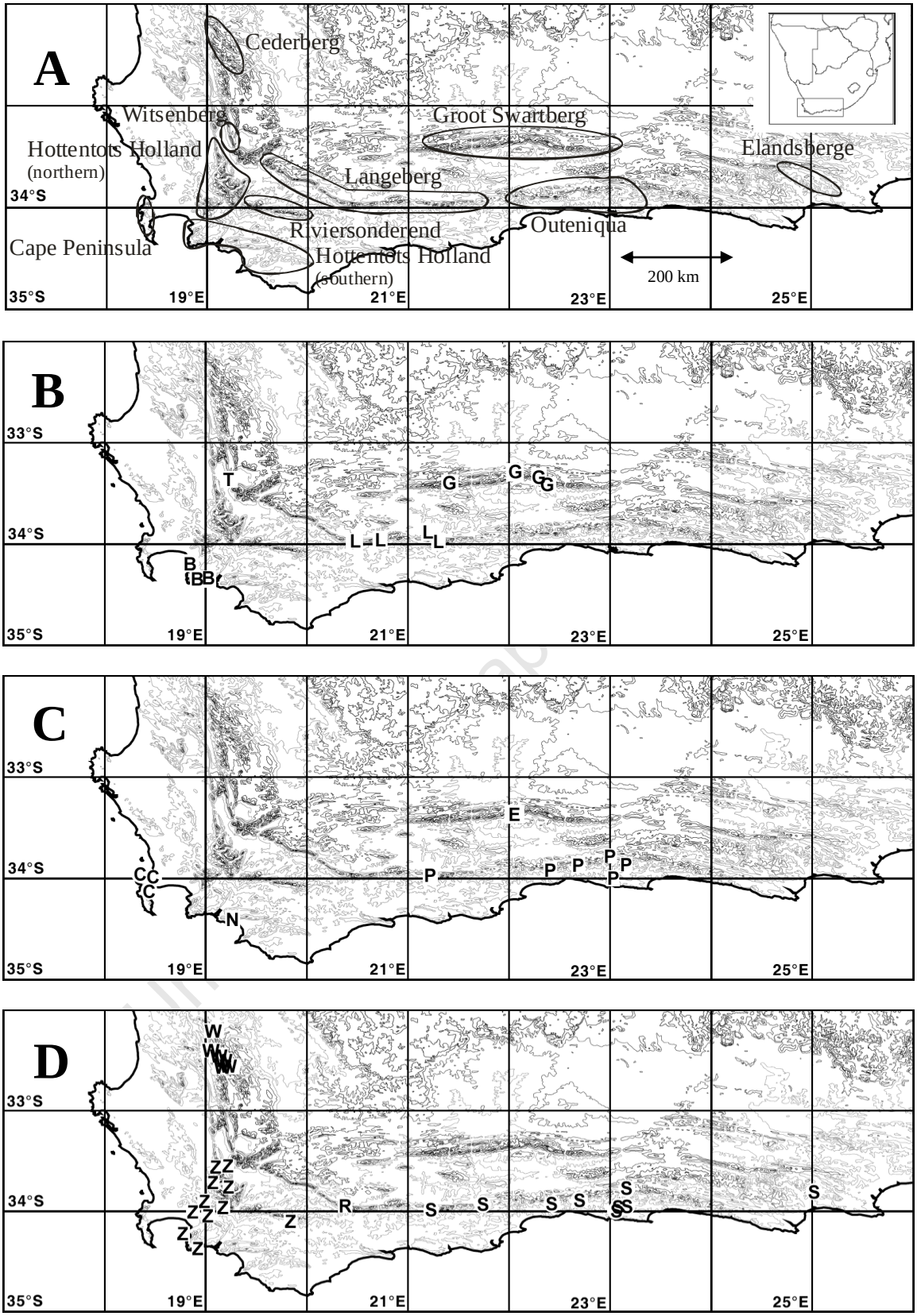


Fig. 3.1. Localities of the 12 *Aphanicerca capensis* species complex morphogroups in the Cape Folded Mountains of South Africa. Contours of higher lying ground are shown. **A**, Schematic outlines of all sampled mountain ranges, with inset showing area in context; **B**, localities of morphogroups ***B***, ***G***, ***L*** and ***T***; **C**, localities of morphogroups ***C***, ***E***, ***N*** and ***P***; **D**, localities of morphogroups ***R***, ***S***, ***W*** and ***Z***.

Table 3.1. Localities of morphogroups (MG) of the *Aphanicerca capensis* species complex. M = localities from which individuals were used for morphometric studies. H-H = Hottentots Holland Mountains. Collector D.M. Stevens in all cases.

MG	Latitude	Longitude	Locality	Mountain range	
B	-34.207200	18.833100	Clarence Drive, monument site 10 km N of Rooiels	H-H (southern)	M
B	-34.330262	18.991217	Faerie Glen Picnic Site, Kleinmond	H-H (southern)	
B	-34.352300	18.927000	Harold Porter Botanic Reserve, Betty's Bay	H-H (southern)	M
C	-33.987460	18.437190	Boschenheuwel Arboretum, Kirstenbosch	Cape Peninsula	
C	-34.123700	18.449500	Boyes Drive, Kalk Bay	Cape Peninsula	
C	-33.997800	18.425700	Cecilia State Forest, Cape Peninsula	Cape Peninsula	
C	-33.943300	18.419400	Gardens, Table Mountain	Cape Peninsula	M
C	-33.987600	18.434900	Liesbeeck River, Kirstenbosch	Cape Peninsula	M
C	-33.970400	18.386000	Pipe Track, Cape Peninsula	Cape Peninsula	
C	-33.955700	18.415900	Platteklip Gorge, Tafelberg Rd, Table Mountain	Cape Peninsula	
C	-34.100100	18.429300	Silvermine Nature Reserve, Steenberg	Cape Peninsula	
C	-33.981900	18.424400	Skeleton Gorge, Kirstenbosch, Table Mountain	Cape Peninsula	
C	-33.977700	18.385100	Slangolie Ravine, Twelve Apostles	Cape Peninsula	
C	-33.967920	18.382010	Theresa Avenue, Camps Bay	Cape Peninsula	
E	-33.357400	22.058500	Boegoekloof, Swartberg pass	Groot Swartberg	M
G	-33.299600	22.050100	Malvadraai, Swartberg Pass	Groot Swartberg	M
G	-33.391800	22.355900	Oudemuragie road, near Meiringspoort	Groot Swartberg	M
G	-33.411200	22.354100	Oudemuragie road, near Meiringspoort	Groot Swartberg	M
G	-33.413400	22.383000	Oudemuragie road, near Meiringspoort	Groot Swartberg	
G	-33.394300	21.399200	Seweweekspoort	Groot Swartberg	M
G	-33.405500	21.400500	Seweweekspoort	Groot Swartberg	
G	-33.412100	21.408700	Seweweekspoort	Groot Swartberg	
L	-33.985800	21.227300	Garcia's Pass, 13.5 km N of Riversdale on R323	Langeberg	M
L	-33.968000	21.219700	Garcia's Pass, 16.2 km N of Riversdale on R323	Langeberg	M
L	-33.958600	21.230400	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	
L	-33.996900	20.445300	Marloth Nature Reserve, Swellendam	Langeberg	M
L	-33.999000	20.456500	Marloth Nature Reserve, Swellendam	Langeberg	
L	-33.956900	21.216100	Sleeping Beauty Trail, Garcia's Pass, Riversdale	Langeberg	
L	-33.982738	20.708599	Tradouw Pass	Langeberg	
N	-34.390000	19.269100	Fernkloof Nature Reserve, Hermanus	H-H (southern)	
N	-34.393900	19.276100	Fernkloof Nature Reserve, Hermanus	H-H (southern)	M
N	-34.398479	19.273004	Fernkloof Nature Reserve, Hermanus	H-H (southern)	M
P	-33.872275	22.687287	Bergplaas-Kleinplaat road, NE of George	Outeniqua Mts	M
P	-33.884300	22.689300	Bergplaas Forest, road to Klipplaat, N of Knysna	Outeniqua Mts	
P	-33.990700	23.040700	Gouna pump station, Knysna	Outeniqua Mts	M
P	-33.907175	22.418134	Keur River Bridge, Montagu Pass, George	Outeniqua Mts	M
P	-33.958600	21.230400	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	M
P	-33.860994	23.171860	Prince Alfred's Pass, N of Knysna	Outeniqua Mts	M
P	-33.766000	23.005100	Road to George from Prince Alfred's Pass	Outeniqua Mts	
R	-33.932800	20.380900	Bergheim, between Montagu and Barrydale	Langeberg	M
R	-33.918500	20.378800	Ravenna, between Montagu and Barrydale	Langeberg	M
S	-33.872275	22.687287	Bergplaas-Kleinplaat road, NE of George	Outeniqua Mts	
S	-33.906700	22.419100	Keur River Bridge, Montagu Pass, George	Outeniqua Mts	
S	-33.947500	23.141100	Kom se Pad, Gouna Forest, Knysna	Outeniqua Mts	
S	-33.958600	21.230400	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	M
S	-33.783867	25.019421	Otterford Forestry Station, Hankey, Elandsberge	Elandsberge	
S	-33.756219	23.159235	Prince Alfred's Pass, a few km's S of Avontuur	Outeniqua Mts	M
S	-33.919800	21.742100	Cloete's Pass, NW of Mossel Bay	Langeberg	

Table 3.1. Continued.

MG	Latitude	Longitude	Locality	Mountain range	
S	-33.950977	23.064429	Terblans Walk, Gouna Forest, N of Knysna	Outeniqua Mts	
S	-33.933325	23.163417	Ysternek N Reserve, Prince Alfred's Pass, Knysna	Outeniqua Mts	M
T	-33.382737	19.213298	Witsenberg Game Park, near Wolseley	Witsenberg	M
W	-32.374100	19.062000	Algeria, Cederberg	Cederberg	M
W	-32.425600	19.131800	11.2 km S of Algeria forest station	Cederberg	M
W	-32.454900	19.169600	Eikeboom, 16.4 km S of Algeria, Cederberg	Cederberg	
W	-32.175700	19.063800	Fortyn se Kloof, Jeep track south of Pakhuis Pass	Cederberg	M
W	-32.501528	19.155643	Sneeuberg, Cederberg	Cederberg	
W	-32.522500	19.164900	Trib of Driehoekrivier, Eikeboom, Koerasieberg	Cederberg	M
Z	-33.592800	19.123600	Bain's Kloof Pass, cement wall bridge	H-H (northern)	
Z	-33.645158	19.070927	Bain's Kloof Pass, 1st stream N of Wellington	H-H (northern)	
Z	-33.592100	19.125000	Bain's Kloof Pass, concrete channel	H-H (northern)	
Z	-33.547060	19.163000	Bain's Kloof, Bastiaanskloof River	H-H (northern)	
Z	-33.594720	19.121140	Bain's Kloof, sharp bend	H-H (northern)	
Z	-33.555860	19.149920	Bain's Kloof, Steenbok Park	H-H (northern)	
Z	-33.601820	19.110870	Bain's Kloof, stream under road near concrete bin	H-H (northern)	
Z	-34.200000	18.766700	Clarence Drive, N of Rooiels	H-H (southern)	
Z	-33.948057	19.168624	Franschhoek Pass, Du Toit's River Bridge	H-H (northern)	
Z	-33.641300	19.104100	Gawie se Water, Bain's Kloof	H-H (northern)	
Z	-34.352300	18.927000	Harold Porter Botanic Reserve, Betty's Bay	H-H (southern)	M
Z	-33.993700	18.974900	Jonkershoek Nature Reserve, Stellenbosch	H-H (northern)	
Z	-33.989800	18.956900	Jonkershoek Nature Reserve, Stellenbosch	H-H (northern)	
Z	-33.989100	18.968400	Jonkershoek Nature Reserve, Stellenbosch	H-H (northern)	
Z	-33.966464	18.926315	Assegaaibos Nature Reserve, Stellenbosch	H-H (northern)	M
Z	-33.722100	19.182100	Klip River, trib of Molenaars, Du Toit's Kloof Pass	H-H (northern)	
Z	-34.346690	18.930410	Leopard's Kloof, Harold Porter, Betty's Bay	H-H (southern)	
Z	-34.082000	19.829100	Oubos farm, Riviersonderend	Riviersonderend	M
Z	-33.900000	18.950000	Pniel, near Boschendal	H-H (northern)	M
Z	-33.991700	18.954200	Swartboskloof, Stellenbosch	H-H (northern)	

MATERIALS AND METHODS

Sample collection and identification

Lower order streams were sampled from 78 localities across the Western and Eastern Cape Provinces in all major mountain ranges (Fig. 3.1; Table 3.1) where the genus *Aphanicerca* is known to occur (Stevens & Picker 1995; Picker & Stevens 1999; Chapter 2 of this thesis) Collecting effort was less intense in the Eastern Cape Province due to practical difficulties and the rarity of the genus in that area. Adults were collected live by hand from stream boulders and leaf packs or occasionally by sweeping the riparian vegetation, and then killed and stored in 70% or absolute ethanol. *A. capensis*-like individuals of both sexes were separated from other species of *Aphanicerca* using the keys and descriptions in Stevens & Picker (1995) and Picker & Stevens (1999). These were then sorted into morphologically distinguishable groups using both male and female characters. The male characters used were the length and shape of the dorsal process of tergite 9, and the length of the spinous part of the same process. Females were

characterized based on the shape of the subgenital plate, where possible. Notonemourid female morphology is generally more conservative than that of males, and females sometimes had to be assigned to a morphogroup based on syntopic association with the male. Individuals were subjectively assigned to groups after an initial examination under the stereo microscope. Where sampled populations were morphologically quite similar (although nevertheless distinguishable), but geographically distant and separated by unsuitable habitat (low lying ground), they were assigned to separate groups. Twelve such groups were distinguished, and were assigned the alphabetical identifiers ***B***, ***C***, ***E***, ***G***, ***L***, ***N***, ***P***, ***R***, ***S***, ***T***, ***W*** and ***Z*** (see Table 3.1 for sample localities). Three localities, namely Bain's Kloof Pass (northern Hottentots Holland Mountains), Boegoekloof (Swartberg Pass, Groot Swartberg) and Fernkloof Nature Reserve (southern Hottentots Holland Mountains) each had one additional morphogroup present, but these were collected in such small numbers that no decision could be made as to their affinities, so they were excluded from further study. Nevertheless, it is important to recognize the potential presence of multiple morphogroups at these and other sites.

The morphological features defining the *A. capensis* species complex were ascertained from the re-description of the type species *A. capensis* by Barnard (1934) and from examination of sampled specimens. The males of this species complex could be distinguished from males of other *Aphanicerca* species by the possession of both of the following two features: the dorsal lobe of tergite 9 is divided into two stout posteriorly directed processes, each of which bears a distal mediodorsal band of posteriorly directed heavily sclerotized spinules, and the scimitar-like epiproct bears a heavily sclerotized convexity with denticles in the middle of each margin of the concave anterior face (Fig. 3.2). This convexity also occurs prominently in *A. lyrata* Barnard, and weakly in *A. bicornis* Barnard). The females were defined as having a short subgenital plate relative to other congeners, with or without a median modification such as a notch or a process. Eggs and larvae which might yield additional diagnostic characters were not used in the analysis.

Morphometric analysis

The characters used for morphometrics were those showing sufficient variability between morphogroups and relative constancy within morphogroups based on subjective *a priori* assessments. Within morphogroup variability was observed (Figs 3.3, 3.4), but was less than between morphogroup variation. Because body length can vary substantially with abdominal contraction in preserved specimens, body size parameters used were those of the more sclerotized portions of the body, namely pronotum width (pnw) and head capsule width (hcw). Morphometric characters for male genitalia were: distance between the apices of the dorsal processes of tergite nine (T9) (adp), T9 dorsal process length (dp), length of the spinous part of

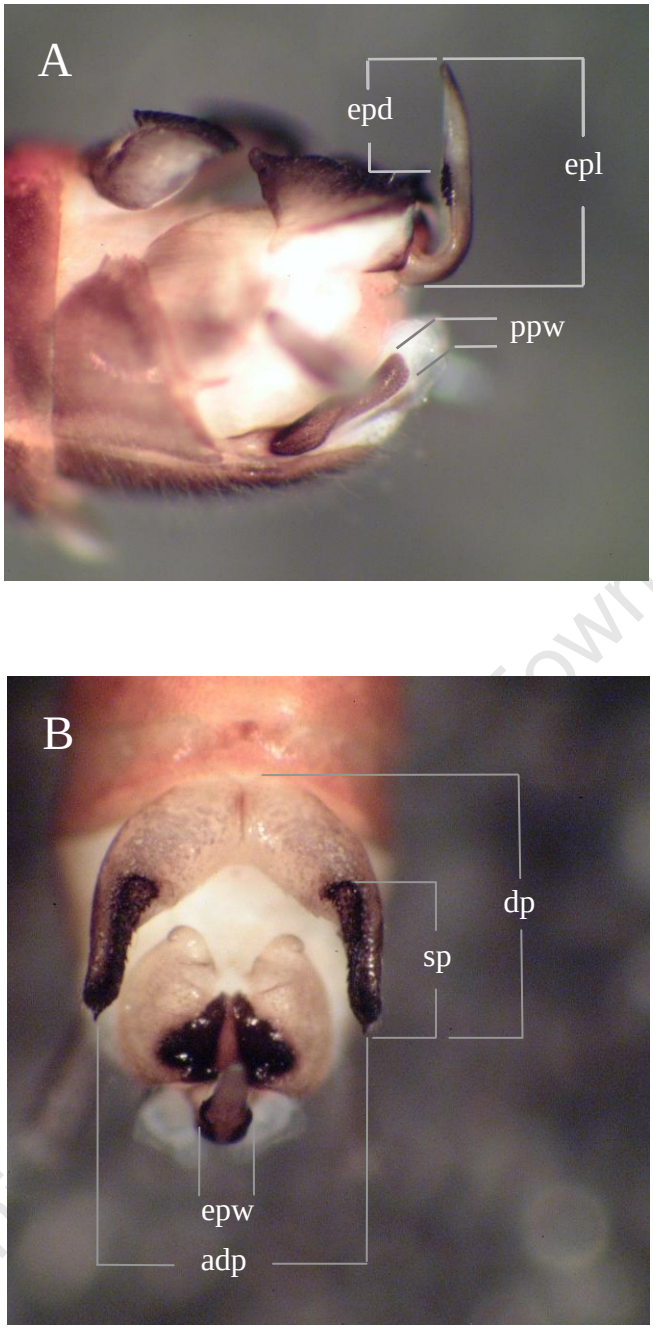


Fig. 3.2. Morphometric variables; **A**, lateral view of male postabdomen; **B**, dorsal view of same. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes. Additional variables not figured are the head capsule width and the pronotum width.

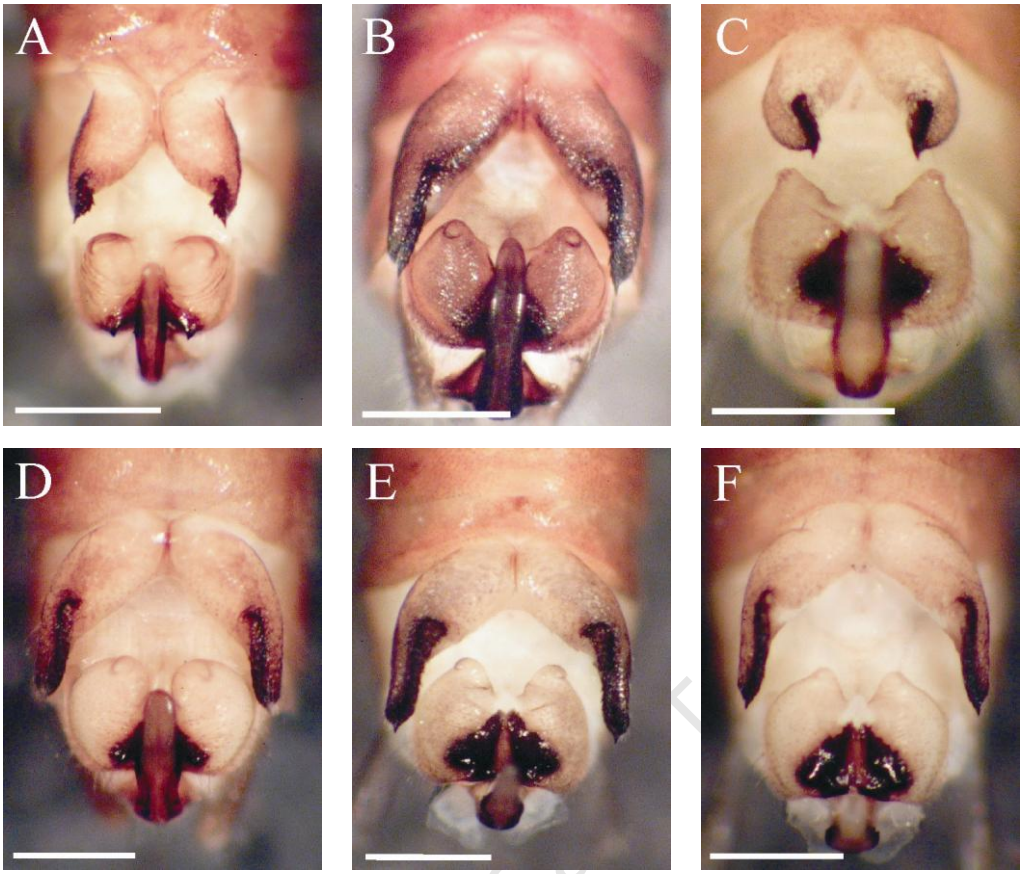


Fig. 3.3. Dorsal view of male postabdomen showing the morphology of the tergite 9 dorsal process lobes of 12 morphogroups of the *Aphanicerca capensis* species complex. More than one representative for some morphogroups have been included to illustrate intra-morphogroup variation. Data given for each figure: Morphogroup (bold italics), locality, mountain range. **A**, ***B***, Betty's Bay, Hottentots Holland (southern); **B**, ***C***, Table Mountain, Cape Peninsula Mountain Chain; **C**, ***E***, Boegoekloof Swartberg Pass, Groot Swartberg; **D**, ***G***, Malvadraai Swartberg Pass, Groot Swartberg; **E**, ***G***, Oudemuragie road near Meiringspoort, Groot Swartberg; **F**, ***G***, Seweweekspoort, Groot Swartberg; **G**, ***L***, Kristalkloof Garcia's Pass, Langeberg; **H**, ***L***, Tradouw Pass, Langeberg; **I**, ***N***, Fernkloof Nature Reserve Hermanus, Hottentots Holland (southern); **J**, ***P***, Bergplaas-Kleinplaat road NE George, Outeniqua; **K**, ***P***, Bergplaas-Kleinplaat road NE George, Outeniqua; **L**, ***P***, Montagu Pass George, Outeniqua; **M**, ***P***, Montagu Pass George, Outeniqua; **N**, ***P***, Kristalkloof Garcia's Pass, Langeberg; **O**, ***R***, Ravenna between Montagu and Barrydale, Langeberg; **P**, ***R***, Ravenna between Montagu and Barrydale, Langeberg; **Q**, ***S***, Bergplaas-Kleinplaat road NE George, Outeniqua; **R**, ***S***, Prince Alfred's Pass N Knysna, Outeniqua; **S**, ***T***, Witsenberg Game Farm near Wolseley, Witsenberg; **T**, ***T***, Witsenberg Game Farm near Wolseley, Witsenberg; **U**, ***W***, 11.2km S Algeria forest station, Cederberg; **V**, ***W***, 11.2km S Algeria forest station, Cederberg; **W**, ***Z***, Jonkershoek Stellenbosch, Hottentots Holland (northern); **X**, ***Z***, Bain's Kloof Pass, Hottentots Holland (northern); **Y**, ***Z***, Betty's Bay, Hottentots Holland (southern); **Z**, ***Z***, Oubos farm, Riviersonderend; **AA**, ***Z***, Du Toit's Kloof Pass, Hottentots Holland (northern). Scale bar = 0.3 mm.

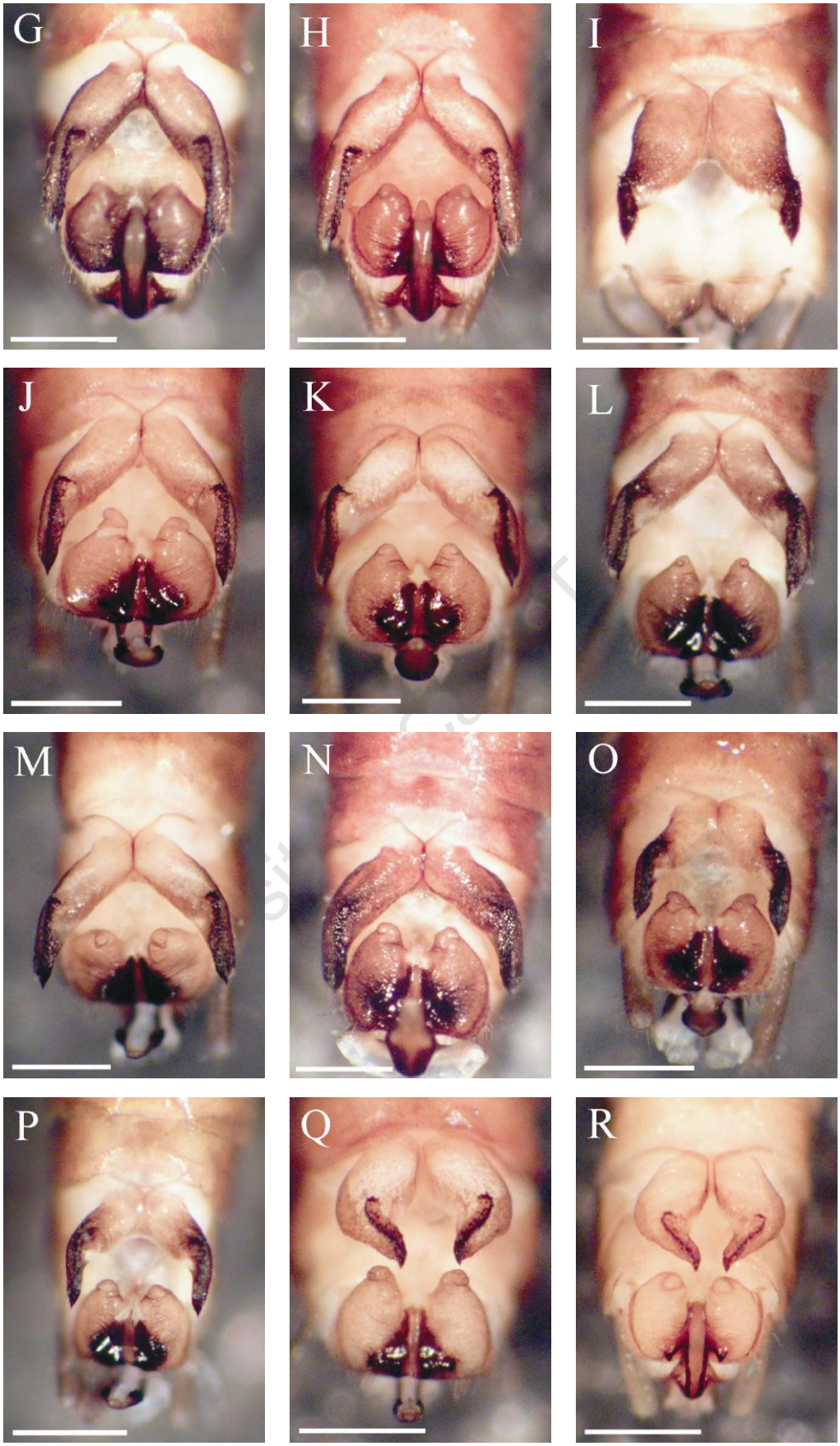


Fig. 3.3. Continued.

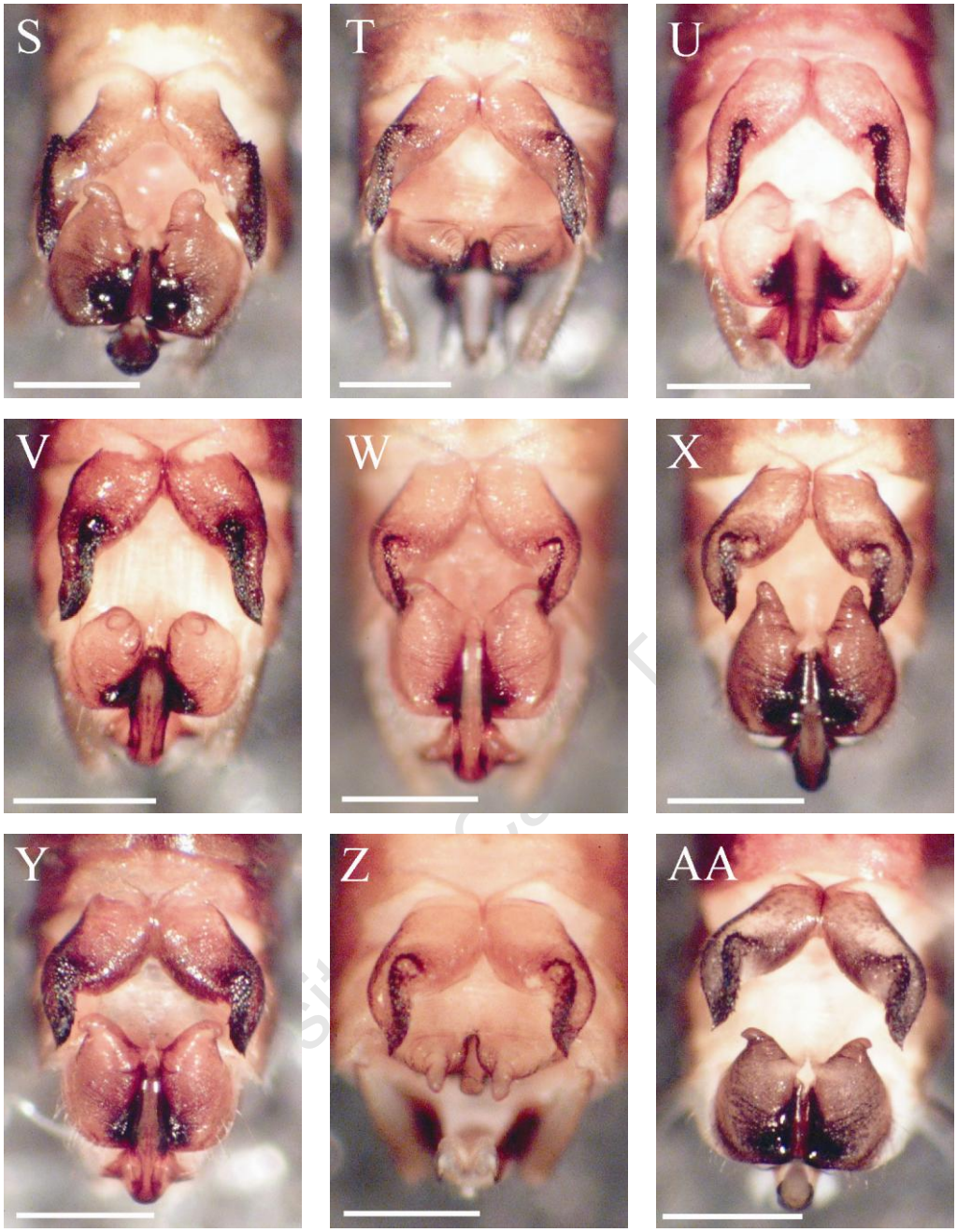


Fig. 3.3. Continued.

T9 dorsal processes (sp), width of the distal extremity of the lateral sclerotized plate of the paraprocts (ppw), epiproct length (epl), distance from apex of epiproct to the first (i.e. most distal or apical) denticle (epd), and epiproct width at the widest point of the denticulate region (epw) (Fig. 3.2). Measurements were made using a Wild stereo microscope with an ocular micrometer. Nine variables were measured on 217 individuals. Only males were used in this analysis. All morphometric analyses were performed using STATISTICA® (StatSoft, Inc. 2004). Dorsal views of male genitalia of 12 morphogroups (and variations thereof) are depicted in Fig. 3.3. Female genitalia were not used in the morphometric analysis because they often do not show diagnostic features, and were characterized on morphological appearance (Fig. 3.4).

Fig. 3.4. *Aphanicerca capensis* species complex female ventral abdomen showing pattern of sclerotization. Data given for each figure: Morphogroup (bold italics), locality, mountain range. **A**, ***B***, Betty's Bay, Hottentots Holland (southern); **B**, ***C***, Table Mountain, Cape Peninsula Mountain Chain; **C**, ***E***, Boegoekloof Swartberg Pass, Groot Swartberg; **D**, ***G***, Malvadraai Swartberg Pass, Groot Swartberg; **E**, ***L***, Tradouw Pass, Langeberg; **F**, ***N***, Fernkloof Nature Reserve Hermanus, Hottentots Holland (southern); **G**, ***P***, Montagu Pass George, Outeniqua; **H**, ***R***, Ravenna between Montagu and Barrydale, Langeberg; **I**, ***S***, Bergplaas-Kleinplaat road NE George, Outeniqua; **J**, ***T***, Witsenberg Game Farm near Wolseley, Witsenberg; **K**, ***W***, 11.2km S Algeria forest station, Cederberg; **L**, ***Z***, Bain's Kloof Pass, Hottentots Holland (northern). Scale bar = 1 mm.

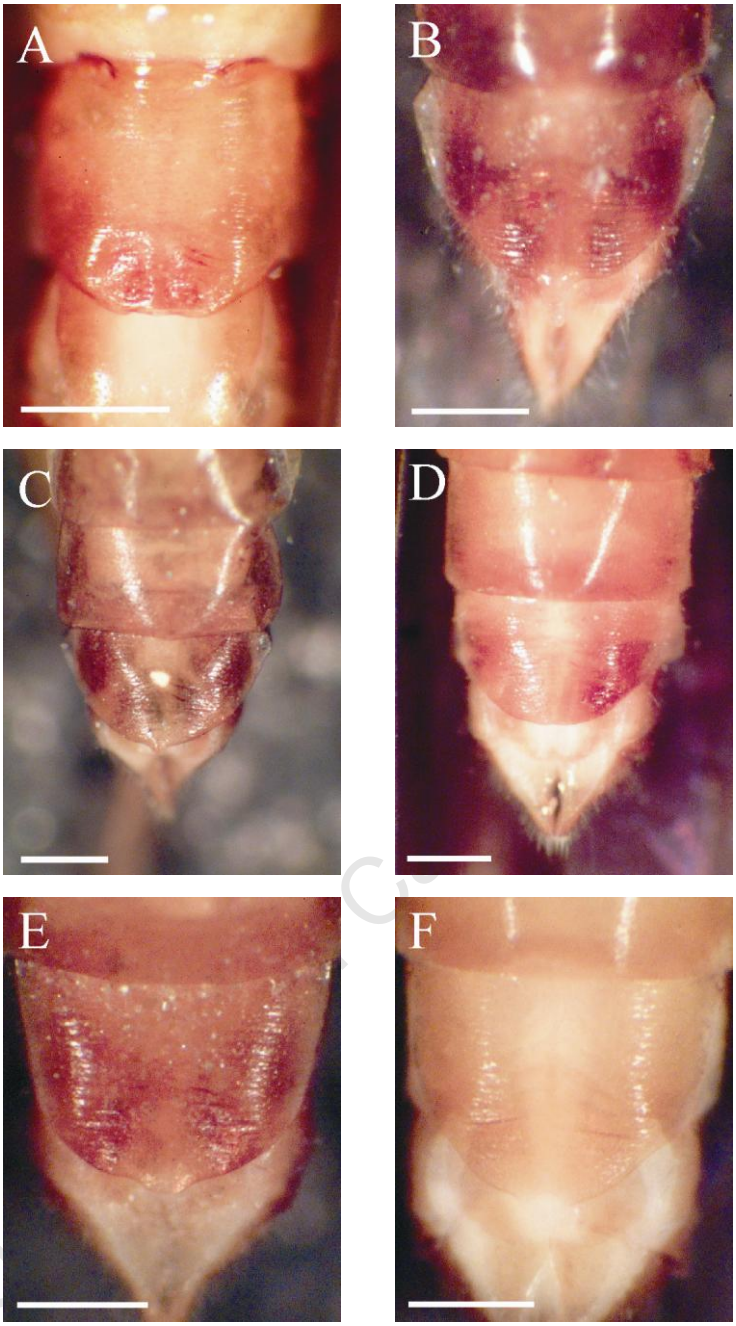


Fig. 3.5. *Aphanicerca capensis* species complex female ventral postabdomen showing subgenital plate (sternite 8). Data given for each figure: Morphogroup (bold italics), locality, mountain range. **A**, ***B***, Betty's Bay, Hottentots Holland (southern); **B**, ***C***, Table Mountain, Cape Peninsula Mountain Chain; **C**, ***E***, Boegoekloof Swartberg Pass, Groot Swartberg; **D**, ***G***, Malvadraai Swartberg Pass, Groot Swartberg; **E**, ***G***, Seweweekspoort, Groot Swartberg; **F**, ***G***, Oudemuragie road, Groot Swartberg; **G**, ***L***, Tradouw Pass, Langeberg; **H**, ***L***, Garcia's Pass, Langeberg; **I**, ***L***, Swellendam, Langeberg; **J**, ***N***, Fernkloof Nature Reserve Hermanus, Hottentots Holland (southern); **K**, ***P***, Montagu Pass, Outeniqua; **L**, ***P***, Bergplaas-Kleinplaat road NE George, Outeniqua; **M**, ***R***, Ravenna between Montagu and Barrydale, Langeberg; **N**, ***S***, Bergplaas-Kleinplaat road NE George, Outeniqua; **O**, ***S***, Kristalkloof Garcia's Pass, Langeberg; **P**, ***T***, Witsenberg Game Farm near Wolseley, Witsenberg; **Q, R, S**, all ***W***, 11.2km S Algeria forest station, Cederberg; **T**, ***Z***, Bain's Kloof Pass, Hottentots Holland (northern); **U, V**, both ***Z***, Jonkershoek Stellenbosch, Hottentots Holland (northern). Scale bar = 0.3 mm.

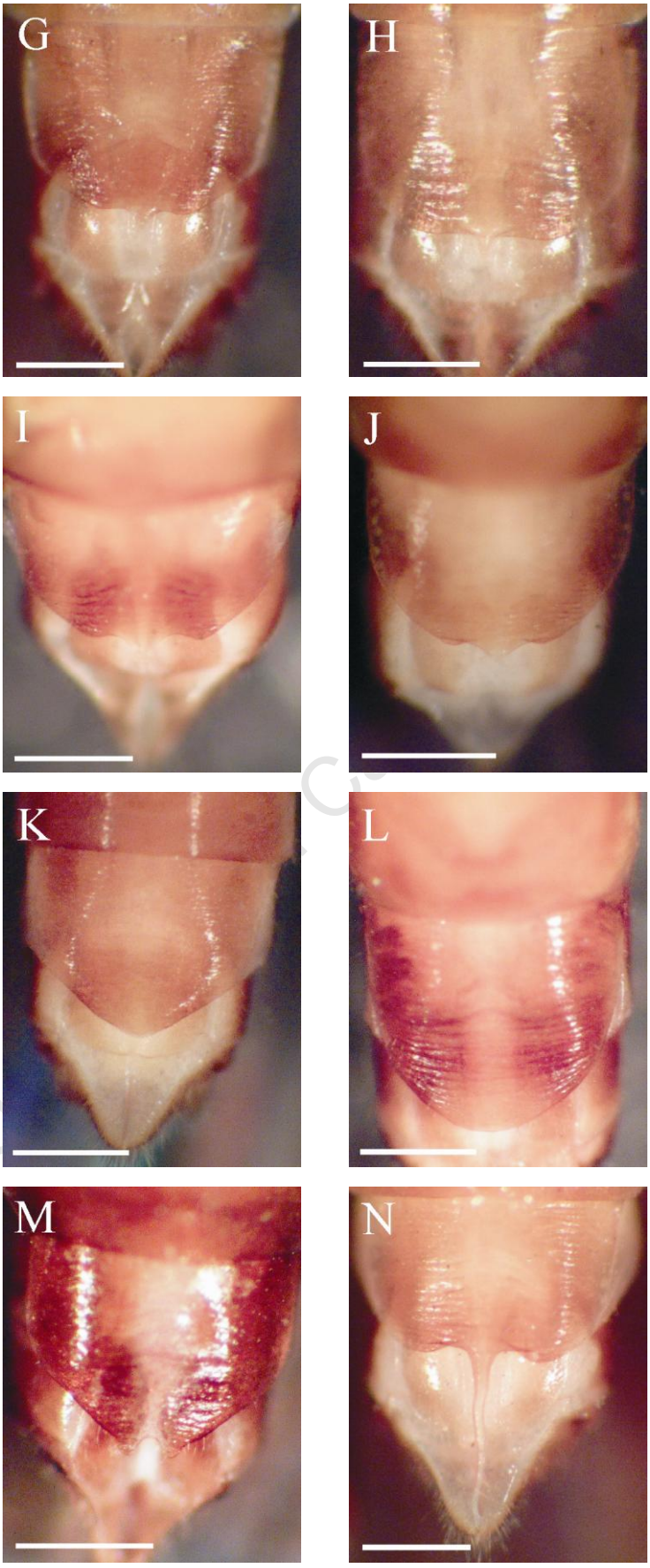


Fig. 3.5. Continued.

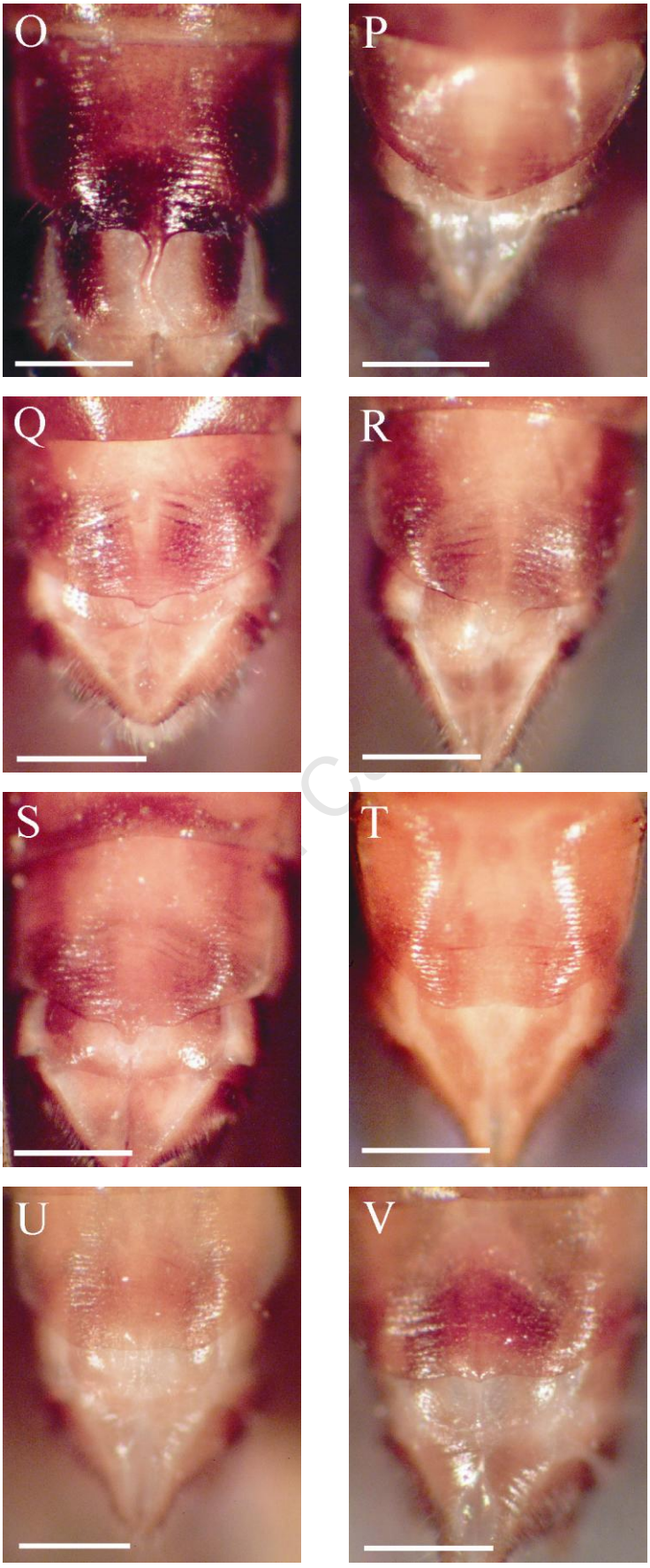


Fig. 3.5. Continued.

Variables were not standardized for body size because a) this is a useful character in itself and untransformed values provided good discrimination, and b) the goal was to find useful morphological data for discrimination that would also benefit field workers. Ratios were used to eliminate body size when discriminating between morphogroups ***P*** and ***T*** and have been used successfully in providing discriminating features in other notonemourids (McLellan 2005).

All nine variables showed departure from normality (Shapiro-Wilks' W test, $P < 0.05$), although histograms showed that this was not a major departure. Plots of the means for selected variables across morphogroups against the respective standard deviations showed no obvious correlations, indicating that homogeneity of variances was adequate for analysis of variance and other multivariate analyses. A multivariate analysis of variance (MANOVA) was conducted on the nine variables between the 12 morphogroups, with three aims: to ascertain whether the morphogroups could be separated on these variables, to determine which variables contributed to the separation of groups, and to determine which variables differed significantly between morphogroups using the *post hoc* multiple comparison Tukey Unequal N Honest Significant Difference (HSD) test. In order to assess whether the different populations could be grouped meaningfully by fewer variables, a principal components analysis (PCA) was performed. A variance maximizing (varimax normalised) rotation did not improve interpretability and therefore unrotated component loadings were used. PCA was used as it can be performed on non-normally distributed data (Quinn & Keough 2002). A PCA serves to reduce the number of correlated variables (in this case, nine) to a lesser, more easily visualized and explained number of uncorrelated variables (factors or components). It also is useful in detecting structure in the data (StatSoft, Inc. 2004). Essentially, components extracted from the raw data account for less and less variance with each successive component (StatSoft, Inc. 2004). Ideally, the first two or three components should be sufficient to explain most of the variability in the data (Manly 1986). There is no rule as to how many components to retain, although the most commonly used method is the Kaiser criterion. Because the variance of each original variable in a correlation matrix was standardized to equal one in this study (measurement minus sample mean divided by the sample standard deviation), only principal components (PC's) with eigenvalues (the variance extracted by each new factor) greater than one were retained to explain the data. This Kaiser criterion ensures that a component extracts at least as much as the equivalent of the variance of one of the original variables (StatSoft, Inc. 2004). In this study, the Kaiser criterion suggested that two principal components should be retained, as the first three eigenvalues were 4.574, 2.363 and 0.705 (Table 3.4).

In order to identify which variables are most useful in discriminating between the morphogroups of the *A. capensis* species complex, a discriminant function analysis (DFA) was

performed on the raw data. Although the assumptions of homogeneity of variances and normality apply to DFA, minor violations of the former are not important, and violations of normality may still result in trustworthy significance tests (StatSoft, Inc. 2004). Morphogroup ***P*** has one outlier in eight of the nine variables. Outliers in small sample sizes may greatly bias the mean and substantially increase the variance (StatSoft, Inc. 2004). Although DFA significance tests may suffer erroneous results if one group in the study contains a few extreme outliers (StatSoft, Inc. 2004), test runs with this individual excluded (a method recommended by StatSoft, Inc. 2004), showed very similar results to analyses in which it was included. Therefore the analysis was run with this individual included.

Distribution

The Mantel test in Arlequin version 3.11 (Excoffier *et al.* 2005) was used to test for correlation between morphometric and geographic distance matrices (20000 permutations). Morphometric similarity matrix values for morphogroups, as used in the cluster analysis, were used for the Mantel test by assigning a morphogroup value to each of the 40 individuals used in the mtDNA analysis. These 40 specimens also provided the locality data used for the geographic distance matrix. Geographic distance was calculated using Geographic Distance Matrix Generator version 1.2.1 (Ersts 2007). The rejection of the null hypothesis of no correlation would suggest the existence of a single species that showed minor variation along one or many clines. Acceptance of the null hypothesis would suggest the existence of multiple non-interbreeding species in the groups sampled.

To visually examine the relationship between morphology and distribution, the means of the principal components were plotted together with the name of the mountain range/s for each morphogroup (Fig. 3.8). Mountain ranges were also mapped onto a cluster analysis performed on the means of the raw morphometric data of the 12 morphogroups using generalized Euclidian distance (Fowler *et al.* 1998) and a complete linkage algorithm (Fig. 3.9).

Mate choice

The choice of species to use in these trials was governed by availability, and the seasonality of emergence. The type species of the genus and of the species complex, *A. capensis* (morphogroup ***C***, Cape Peninsula) was used because it was readily available, close geographical proximity to the laboratory, and its important status in the taxonomy of the genus. Because individuals were field-collected, it was not known whether males or females were virginal, but all males made attempts to mate. A potential problem may be with females, since if they only mate once might show rejection on physiological rather than genetic grounds. One male of a selected morphogroup was placed in a Petri dish with a female of the same morphogroup and

with a female of a second morphogroup (or previously described species in the case of controls). In a separate Petri dish, the male of the second morphogroup or species used in the trial was placed with a female of his own morphogroup or species and a female of the first morphogroup. In that way, depending on specimen availability, mating preference in both directions could be tested. A small piece of the wing tip of one morphogroup was cut off to enable identification without disturbing the mating. Mating was assumed to occur if the tip of the male abdomen had ceased probing the female abdomen, and appeared locked in position with the posterodorsal male abdomen (epiproct and paraprocts) engaging the posteroventral female abdomen. At this stage the male abdomen could be seen pulsating. In addition to the trials, two control experiments were conducted. In the first control, morphogroup ***C*** males from the type locality Table Mountain (Cape Peninsula) were placed with female morphogroup ***C*** and female *A. bicornis* from Stellenbosch, a species morphologically very distinct from the *A. capensis* species complex. There was insufficient material to do the reverse experiment with *A. bicornis* males. The second control was between Cape Peninsula morphogroup ***C*** and *A. bovina* (Stellenbosch), in both directions. Experimental trials were conducted between the following morphogroups: ***C*** and ***Z*** (Stellenbosch), ***C*** and ***Z*** (Bain's Kloof), ***C*** and ***W*** (Cederberg), and syntopic morphogroups ***B*** and ***Z*** from Betty's Bay (some morphogroups occur at more than one locality). Wing tips were not cut in the Cape Peninsula - Cederberg trial as the females are distinguishable macroscopically on sclerotization patterns of the ventral abdomen, and on distal femoral colouration. Because the two Betty's Bay morphogroups are syntopic (from the same reach of the same stream) and therefore presumably employ positive assortative mating in nature, cross-morphogroup mating in the Petri dish would signify experimental failure due to introduced biases such as stress, unnatural proximity to foreign species, elimination of escape routes, adverse effects of cutting wing tips and unnatural microclimate. Therefore, this experimental trial also served as a control trial. The assumption of positive assortative mating in nature rests on the absence of morphologically intermediate forms between these two morphologically distinct forms. To test the null hypothesis that mating between and within the tested morphogroups is random, the Fisher's Exact Test chi-square statistic, which is used for small sample sizes (Quinn & Keough 2002), was calculated using STATISTICA®.

Mitochondrial DNA (COI)*: *DNA Extraction, PCR amplification and DNA sequencing

The stoneflies used in the molecular analysis are listed in Table 3.12. Only males were used (because they are generally easier to distinguish morphologically to morphogroup), except for females ***S***E1 and ***S***L4a, the latter being collected *in copula* with male ***S***L3. The female of the ***S*** morphogroup is also easily distinguished. Total genomic DNA was extracted from a small piece of thoracic muscle tissue from each stonefly. The stonefly tissue was homogenized using a plastic pestle in a 1.5ml microcentrifuge tube containing 350 µl 2x CTAB buffer (cetyltrimethyl

ammonium bromide) (modified CTAB extraction, Doyle & Doyle 1987). One µl of proteinase K (10 mg/ml) was added. The sample was then incubated at 60°C for 2 hours, 350 µl 24:1 chloroform:isoamylalcohol added, vortexed, then centrifuged for four minutes at 13000 rpm. The supernatant (300 µl) was transferred to a new microcentrifuge tube, precipitated with 300 µl ice-cold isopropanol, and then frozen overnight. The pelleted mix was then centrifuged for 25 minutes at 13000 rpm, supernatant discarded, washed with 100 µl ice-cold 96% ethanol, and centrifuged again for 5 minutes at 13000 rpm. The supernatant was discarded, the pellet dried in a desiccation jar for 2 hours and then dissolved in 50 µl sterile distilled water for 30 minutes.

A 557 base pair (bp) fragment of the mtDNA cytochrome oxidase subunit I (COI) gene was amplified from each individual DNA extract using forward (LCO1490: 5′-GGTCAA CAAATCATAAAGATATTGG-3′) and reverse (HCO2198: 5′-TAAACTTCAGGGTGACC AAAAAATCA-3′) primers designed to amplify a 710-bp fragment (Folmer *et al.* 1994), with poor quality sequence ends resulting in the shorter useful fragments. The 30 µl polymerase chain reaction (PCR) volume contained 3 µl 10 x NH_4 buffer, 3 µl 25 mM $MgCl_2$, 1.2 µl each of dATP, dCTP, dGTP and dTTp (each 25 mM), 17.65 µl sterile distilled water, 1 µl of each 10 µM primer, 0.15 µl of 5 units / ml Taq (*Thermus aquaticus*) DNA polymerase, and 3 µl of stonefly DNA. The amplification parameters used for 35 cycles on a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems) were as follows: an initial denaturing step of 95°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 1.5 min. This was followed by a final extension step of 72°C for 7 min. The resultant amplified DNA concentrations were estimated by running 3 µl of PCR product on a 1% agarose gel (with ethidium bromide) next to a marker and then visualized under ultraviolet light. The PCR double stranded products were purified using the QIAquick PCR purification kit (Qiagen). Direct sequencing of both strands of the purified PCR product was achieved by first cycle-sequencing on a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems) using the following in 10 µl reactions (BigDye® Terminator v3.1, Applied Biosystems): 2 µl Terminator Ready Reaction premix, 1 µl BigDye® 5x sequencing buffer, 0.16 µl primer (10 µM), 2.84 ml double distilled water and 1-4 µl DNA. The cycle-sequencing routine was 30 cycles of 96°C for 15s, 50°C for 15s and 60°C for 4 min. Sequencing reactions were then run on an ABI 3100 genetic analyzer at the Core DNA Sequencing Facility at the University of Stellenbosch, South Africa.

Sequence alignment and data analyses

The analysis comprised 40 individuals of the *A. capensis* species complex, representing 12 morphogroups, as well as six outgroup species. The outgroup comprised one individual of each of six other *Aphanicerca* species, of which *A. bainii* sp. n. is undescribed. Material for the remaining two species of the genus, *A. gnua* Picker & Stevens and *A. tereta* Barnard, was not

available. Trees were rooted on *A. bicornis*, as preliminary analysis of the genus with *Neoperla* sp. (Perlidae) as the outgroup placed the present outgroup as basal to the ingroup in this study. COI sequences were assembled from forward and reverse trace files and aligned using CLC Gene Workbench 2 (CLC bio, Aarhus, Denmark). The alignment was checked by eye and was straight forward as there were no indels or missing data. Nucleotide composition was determined using MEGA version 4.0 (Tamura *et al.* 2007). DnaSP version 4.10.9 (Rozas *et al.* 2003) was used to separate the 40 individual sequences into 27 haplotypes which were used for all subsequent analyses. Maximum parsimony (MP) phylogenetic analysis was performed using the WinClada version 1.00.08 (Nixon 2002) interface of NONA version 2.0 (Goloboff 1999). Heuristic search options used were: 4000 "max trees to keep" (= "hold" in NONA), 3000 "replications" (= "mult*N"), one "starting trees per rep" (= "hold/"), and "multiple TBR + TBR" (= "mult*max*"). Uninformative sites were excluded from consistency index (Ci) and retention index (Ri) calculations. Confidence was assessed using WinClada by 1000 bootstrap "replications", 10 "search reps", one "starting tree per rep", "don't do max*" and 100 "max trees". The most appropriate model of DNA substitution and associated parameters were estimated using the Akaike Information Criterion (AIC) (Akaike 1973) implemented in MODELTEST version 3.06 (Posada & Crandall 1998) in tandem with PAUP* version 4.0b10 for Windows® (Swofford 2002). These parameters were used to produce a maximum likelihood tree using PhyML version 2.4.4 (Guindon & Gascuel 2003). Branch support was estimated by 1000 non-parametric bootstrap pseudoreplicates (Felsenstein 1985). A Bayesian approach to phylogenetic inference (BI) was conducted using MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001). The General Time Reversible (GTR) model with a proportion of invariable sites (+ I) and a gamma distribution (+ gamma) of substitutions was chosen as it is similar to the TVM + I + gamma (transversional model) selected by MODELTEST, the latter having seven, and the former eight, free parameters (Posada 2005). The proportion of invariable sites (0.5801) and the gamma distribution shape parameter (α = 0.9462) priors were fixed, using the values obtained from MODELTEST. The default uninformative (flat Dirichlet) priors were used for estimation of base substitution rates and nucleotide frequencies. Five Metropolis-coupled MCMC chains (one cold and four heated) were employed for each of the two simultaneous runs, with three million generations per run. Trees were sampled every 100 generations. The first 7500 samples (25%) were discarded as burnin resulting in 22501 trees per run. Stationarity was assumed to have been achieved when the average standard deviation of split frequencies between the two runs was less than 0.01 and was not decreasing further (0.00497 at termination), and by examining the generation – log likelihood plot, chain mixing, and the potential scale reduction factor. Trees from MrBayes and PhyML throughout were produced in TreeView version 1.6.6 (Page 1996) and modified in Corel®Draw™ version 11 (Corel, Ottowa, Canada).

DnaSP version 4.10.9 (Rozas *et al.* 2003), was used to perform the neutrality tests of Fu & Li (1993). Fu & Li's D* and F* tests test the hypothesis that mutations in the region of study are selectively neutral, which they do by comparing the numbers of mutations in internal and external branches of the phylogenetic tree with their expectations under selective neutrality (Fu & Li 1993). The D* test statistic is based on the differences between the number of mutations appearing only once among the sequences, and the total number of mutations. The F* test statistic is based on the differences between number of mutations appearing only once among the sequences, and the average number of nucleotide differences between pairs of sequences (DnaSP version 4.10.9). Using a neutral marker in phylogeographic analyses is desirable (Ballard & Whitlock 2004) because of potential confounding effects of different selection pressures among populations.

MODELTEST was run a second time, now excluding the outgroup to obtain the value of the gamma distribution shape parameter appropriate for the 27 haplotypes. Arlequin version 3.11 (Excoffier *et al.* 2005) was used to examine genetic differentiation within and among morphogroup populations of the *A. capensis* species complex. Tamura-Nei distances with a gamma distribution shape parameter $\alpha = 0.145$ (the value obtained from the second MODELTEST run) were used for these analyses, which comprised haplotype and nucleotide diversity indices, analysis of molecular variance (AMOVA) to assess inter-population genetic differentiation, population pairwise F_{ST}'s, and exact tests of population differentiation. Significance was tested using 20022 permutations for the AMOVA and population pairwise F_{ST}'s, and 100000 Markov chain steps and 10000 dememorisation steps for the exact tests of population differentiation. F_{ST} values are actually Φ_{ST} values which are analogous to Wright's F-statistics and used for both diploid and haploid DNA data (Weir & Cockerham 1984; Excoffier *et al.* 1992). The maximum value of Φ is one; when there is polymorphism present, Φ will be less than one (Kalinowski 2002). Arlequin's Mantel test (20000 permutations), was used to test for a correlation between genetic distance (F_{ST} matrix) and morphometric similarity (squared Euclidean distance matrix) between morphogroups. The Mantel test was also used on the 40 individuals sampled for mtDNA to test for correlation between matrices of both uncorrected and corrected (Tamura-Nei, $\alpha = 0.145$) distances and morphometric similarity, and also between uncorrected and corrected distances and geographic distance. Morphometric similarity matrix values for morphogroups, as used in the morphometric analysis, were used for this analysis by assigning the morphogroup values to the individuals belonging to that morphogroup. These Mantel tests were performed in order to test for morphological stasis in the face of genetic divergence, which would provide support for cryptic species hypotheses, and to test whether genetic divergence was associated with geographic distance which would suggest lack of gene flow and isolation by distance or

allopatric fragmentation, which would favour speciation. Geographic distance was calculated using Geographic Distance Matrix Generator version 1.2.1 (Ersts 2007).

Because networks provide better estimates of genealogical relationships among closely related lineages than traditional phylogenetic methods (Clement *et al.* 2000), a haplotype network was constructed using TCS version 1.21 (Clement *et al.* 2000). TCS uses a "statistical parsimony" approach, whereby the probability of parsimony (Templeton *et al.* 1992) is calculated for all pairwise absolute distance comparisons, until the probability exceeds 0.95 (Clement *et al.* 2000). The number of mutational differences at that point defines the maximum number of mutational connections allowed in the resulting network. Although the statistical parsimony approach of TCS may under some circumstances produce an inaccurate network compared to the median-joining approach of NETWORK (Bandelt *et al.* 1999) (Cassens *et al.* 2005), results from both programs were almost identical, so only the former is reproduced here. This network was subjected to the nesting procedure outlined in Templeton *et al.* (1987) and Crandall (1996), followed by cladistic nested analysis using GeoDis version 2.5 (Posada *et al.* 2000). This nested clade analysis tests the null hypothesis of no association between genetic structure and geographical distribution using permutation tests (1 million permutations in this analysis) and, for clades where significant association occurs, Templeton's (1998) inference key (version of 11 November 2005 downloaded at http://darwin.uvigo.es) provides historical processes which may have given rise to those patterns. The analysis generates a clade distance statistic (D_C), which measures the geographical range of the specified clade (the average distance of a haplotype from the centre of its clade), and a nested clade distance statistic (D_N) which measures the average distance of a clade from the average geographic centre of all clades nested within the immediate higher clade (Templeton 2001). In addition, an interior-tip statistic (I-T), which represents the average distance between interior and tip clades, is calculated. This mainly corresponds to an old-young contrast, and to a common-rare contrast (Posada *et al.* 2000). The purpose of this analysis was to discover genetic structure and geographical patterns that were suggestive of isolation by distance or allopatric fragmentation which would favour hypotheses of speciation. NCA has been criticized because of difficulty in maintaining objectivity in working through the inference key resulting in incorrect conclusions, thereby prompting the development of automated methods (Panchal & Beaumont 2007). Further criticisms include the lack of statistical confidence of the phylogeographic conclusions drawn from the key (Knowles & Maddison 2002).

RESULTS

Morphometric analysis

Multivariate analysis of variance

Morphogroups were significantly different from each other with respect to the nine variables (Appendix 3.1) used in this analysis (MANOVA, Wilks' Lambda < 0.001, F = 87.1769, effect (morphogroup) df = 108, error df = 1432.142, $P < 0.001$). The mean, standard error of the mean, and standard deviation of each variable across all groups are presented in Appendix 3.2. The univariate results from the multivariate analysis are presented in Table 3.2. All variables were significantly different between morphogroups ($P < 0.01$) (Table 3.2). Every morphogroup differed from every other morphogroup in at least two variables ($P < 0.05$, Unequal N Tukey HSD test; Table 3.3). The groups differing in only two variables were ***T*** and ***G*** (differ in sp and epw), and ***N*** and ***W*** (differ in sp and ppw). No variable differed across all groups. The main variables that differed between the most groups were, in order of importance: dp, sp, adp, epl, and epw (Table 3.3; Fig. 3.2).

Principal components analysis

The first principal component (PC) explained 50.6% of the variance, and the first two PC's 76.8% (Table 3.4). Interpreting the first two principal components allowed the nine variable data to be summarized by two independent variables (Fig. 3.6). This was done by examining the component loadings which are correlations between the variables and the components (StatSoft, Inc. 2004).

All PC1 variables, except ppw, had fairly high positive component loadings, especially dp, hcw, adp, sp and pnw, which are all over 0.7 (Table 3.5). Only two PC2 variables had component loadings greater (absolute value) than 0.7, ppw which is negative and epw which is positive (Table 3.5). PC1 was therefore a measure of body size, dorsal process size, and to a lesser extent, epiproct length. PC2 was a contrast between epiproct width and paraproct apex width, and to a lesser extent epiproct length. Individuals with a high PC1 value were large, with long and widely divergent dorsal processes and long epiprocts. Individuals with a high PC2 value had broad and often fairly short epiprocts, and narrow paraproct apices. Individuals with high PC1 and PC2 values tended to be large, with long and widely divergent dorsal processes, had narrow paraproct apices, and broad epiprocts of intermediate length.

Table 3.2. Univariate results from the MANOVA of the nine morphometric variables across the 12 *Aphanicerca capensis* (*sensu lato*) morphogroups. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes.

	df	pnw SS	pnw MS	pnw F	pnw *P*
Between groups	12	142.9865	11.9155	5550.0303	0.00
Within groups	203	0.4358	0.0021		
Total	215	143.4223			

	df	dp SS	dp MS	dp F	dp *P*
Between groups	12	35.9914	2.9993	4466.8080	0.00
Within groups	203	0.1363	0.0007		
Total	215	36.1277			

	df	epl SS	epl MS	epl F	epl *P*
Between groups	12	17.8432	1.4869	6515.2742	0.00
Within groups	203	0.0463	0.0002		
Total	215	17.8895			

	df	hcw SS	hcw MS	hcw F	hcw *P*
Between groups	12	195.4939	16.2912	9515.4414	0.00
Within groups	203	0.3476	0.0017		
Total	215	195.8415			

	df	sp SS	sp MS	sp F	sp *P*
Between groups	12	10.1815	0.8485	2173.8984	0.00
Within groups	203	0.0792	0.0004		
Total	215	10.2607			

	df	epd SS	epd MS	epd F	epd *P*
Between groups	12	4.5869	0.3822	2262.8825	0.00
Within groups	203	0.0343	0.0002		
Total	215	4.6212			

	df	adp SS	adp MS	adp F	adp *P*
Between groups	12	31.7852	2.6488	1194.0686	0.00
Within groups	203	0.4503	0.0022		
Total	215	32.2355			

	df	ppw SS	ppw MS	ppw F	ppw *P*
Between groups	12	0.6510	0.0543	1072.7114	0.00
Within groups	203	0.0103	0.0001		
Total	215	0.6613			

	df	epw SS	epw MS	epw F	epw *P*
Between groups	12	1.9060	0.1588	3086.5848	0.00
Within groups	203	0.0104	0.0001		
Total	215	1.9165			

Table 3.3. *Post hoc* Unequal N Tukey HSD test results from the MANOVA, showing which morphometric variables are significantly different (grey blocks) ($P < 0.05$) between morphogroups (MG). Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes. Columns are arranged from left to right with decreasing frequency of significance between MG's, and rows from top to bottom with decreasing frequency of significant variables per MG.

MG pairs	Morphometric variables								
	dp	sp	adp	epl	epw	epd	ppw	hcw	pnw
CR	■	■	■	■	■	■	■	■	■
CS	■	■	■	■	■	■	■	■	■
LR	■	■	■	■	■	■	■	■	■
BC	■	■	■	■	■	■	■	■	
BT	■	■	■	■	■	■	■	■	
CE	■	■	■	■		■	■	■	■
EG	■	■	■	■	■	■		■	■
EL	■	■	■	■		■	■	■	■
ET	■	■	■	■	■	■		■	■
EZ	■	■	■	■		■	■	■	■
NR	■	■		■	■	■	■	■	■
RT	■	■	■	■	■	■		■	■
ST	■	■	■		■	■	■	■	■
BG	■	■	■	■	■	■		■	
BL	■	■	■	■	■	■		■	
BR	■	■	■		■		■	■	■
BZ	■	■	■	■	■	■		■	
CW	■		■	■		■	■	■	■
EN	■		■	■		■	■	■	■
EP	■	■	■	■	■			■	■
EW	■	■	■	■		■		■	■
GR	■	■	■	■		■		■	■
GS	■	■	■		■	■	■	■	
GZ	■	■	■	■	■	■	■		
PR	■	■	■	■	■			■	■
PW	■	■	■	■	■			■	■
PZ	■	■	■	■	■	■	■		
SZ	■	■	■	■	■	■		■	
TW	■	■	■		■	■		■	■
TZ	■	■	■	■	■	■	■		
BN	■	■	■	■		■	■		
BP	■	■	■	■	■	■			
BW	■	■	■	■	■	■			
CN	■	■	■	■		■	■		
CT	■	■		■	■	■	■		
ES	■	■		■			■	■	■
LW	■	■	■				■	■	■
NP	■	■	■	■	■		■		
NT	■	■	■		■		■	■	
RS		■	■	■	■		■	■	
RZ				■	■	■	■	■	■
WZ		■		■		■	■	■	■
BE	■		■				■	■	■
CZ	■	■	■	■			■		
GN	■	■	■		■		■		
GW	■		■		■			■	■
LS	■	■	■		■	■			
LZ	■	■	■	■		■			
PS	■	■	■		■		■		
SW	■	■	■		■		■		
CG				■	■	■	■		
CL		■		■		■	■		
CP				■	■	■	■		
ER	■	■	■		■				
GL	■	■			■		■		
LP	■			■	■		■		
RW				■	■	■		■	
BS		■	■	■					
GP				■	■	■			
LN	■	■	■						
LT	■				■		■		
NS	■		■	■					
NZ	■	■				■			
PT					■	■		■	
GT		■			■				
NW		■					■		

Table 3.4. PCA eigenvalues and percentage explained variance of all nine principal components.

Component	Eigenvalue	% Total variance	Cumulative %
1	4.573681	50.82	50.82
2	2.363102	26.26	77.08
3	0.705158	7.84	84.91
4	0.520273	5.78	90.69
5	0.271510	3.02	93.71
6	0.248650	2.76	96.47
7	0.132272	1.47	97.94
8	0.120326	1.34	99.28
9	0.065027	0.72	100.00

Table 3.5. Unrotated component loadings for the first two principal components (PC) of the *A. capensis* species complex morphometric data. Variables for each PC are arranged from highest to lowest loading. Component loadings with absolute values greater than 0.7 are bold and italicised. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes.

Variable	Component 1	Variable	Component 2
dp	***0.882141***	ppw	***-0.828805***
hcw	***0.862077***	epw	***0.729903***
adp	***0.818060***	epl	-0.651717
sp	***0.807953***	epd	-0.555245
pnw	***0.779456***	sp	0.406449
epl	0.658334	adp	0.315682
epd	0.608327	dp	0.281569
epw	0.532076	hcw	-0.183151
ppw	0.190267	pnw	-0.180875

Table 3.6. Discriminant Function Analysis. Standardized discriminant function (DF) coefficients for the eight significant DF's. CP = cumulative proportion of variance. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes.

	DF 1	DF 2	DF 3	DF 4	DF 5	DF 6	DF 7	DF 8
pnw	0.05196	-0.090963	0.063422	0.203665	0.208314	-0.038008	-0.506453	-0.169322
hcw	-0.32800	-0.005386	-0.211763	0.479217	-0.960866	-0.682554	0.516818	0.108280
adp	0.33556	0.077812	-0.745061	0.110866	0.402871	-0.715425	-0.456798	0.220171
dp	0.23595	0.781636	1.328334	0.191322	0.247888	0.432658	0.459510	-0.215776
sp	0.47731	-0.122875	-0.708426	-0.931946	-0.431419	-0.268004	-0.333505	-0.025086
ppw	-0.29620	0.356400	0.053898	0.067446	-0.355416	0.249207	-0.750268	-0.112652
epl	-0.33069	0.196259	-0.323123	-0.231694	0.254233	0.554259	0.289443	1.036299
epd	0.07745	0.238871	-0.482307	0.271420	0.194436	0.080068	0.184744	-0.970671
epw	0.67584	-0.464513	-0.070992	0.378038	-0.075831	0.524425	-0.086792	0.092564
CP	0.58031	0.794047	0.902100	0.943987	0.966016	0.983265	0.993121	0.999769

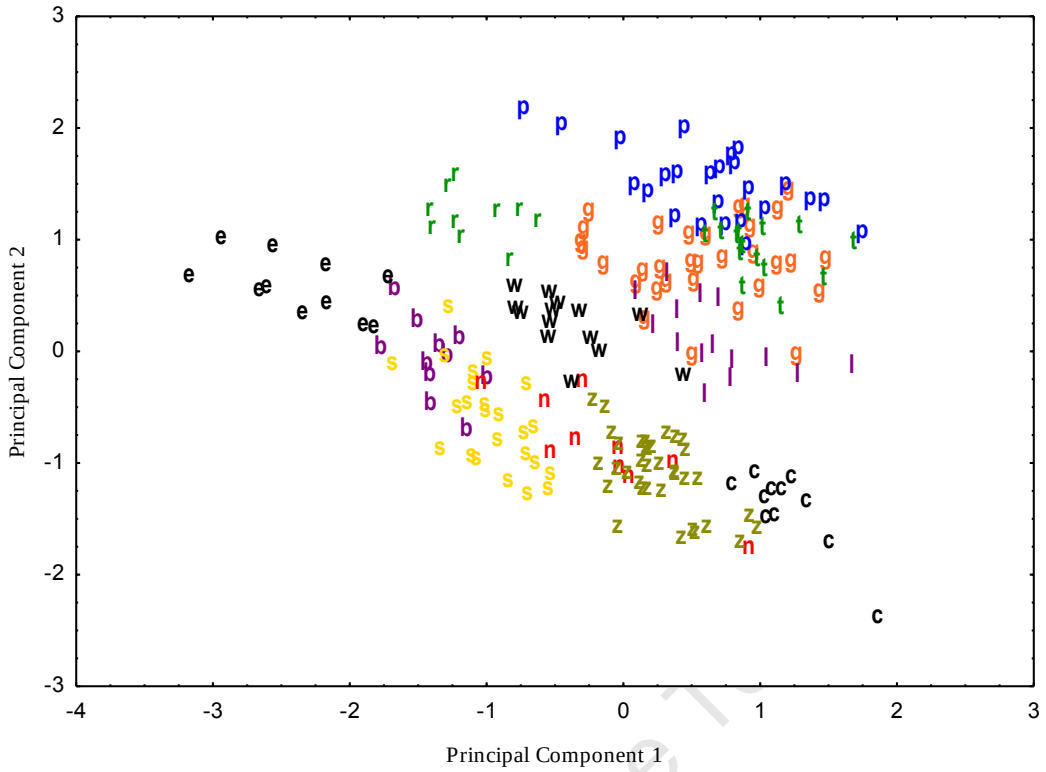


Fig. 3.6. Scatterplot of PC1 and PC2 values in the principal component (PC) analysis of the *Aphanicerca capensis* species complex morphometric data. Letters correspond to the 12 morphogroups.

The scatterplot of PC1 and PC2 (Fig. 3.6) showed clearly that the summarized morphometric variables had conferred a grouping structure on the various populations or morphogroups of the *A. capensis* species complex. Each population was restricted to an easily demarcated small area of the graph, and not widely dispersed, although there was overlap in most of the groupings. A few of the groupings showed no (or slight) overlap, while the remainder showed moderate to extensive overlap.

Discriminant function analysis

The discriminant function analysis resulted in overall highly significant discrimination between morphogroups (Wilks' Lambda = 0.00011, approx $F_{99,1386}$ = 37.108, P = 0.0000). The Wilks' Lambda statistic ranged from 0 (perfect discrimination), to 1 (no discrimination). Significant (all P = 0.000 except for pnw which was not significant) partial Wilks' Lambda values for each variable ordered the contribution to the discrimination within the model as a whole, from most to least, as follows: epw, dp, sp, adp, ppw, epd, epl, hcw and pnw. The first two, epw and dp, had very similar partial Wilks' Lambdas, and contributed substantially more than the rest, with sp and adp also important contributors relative to the remaining five. With

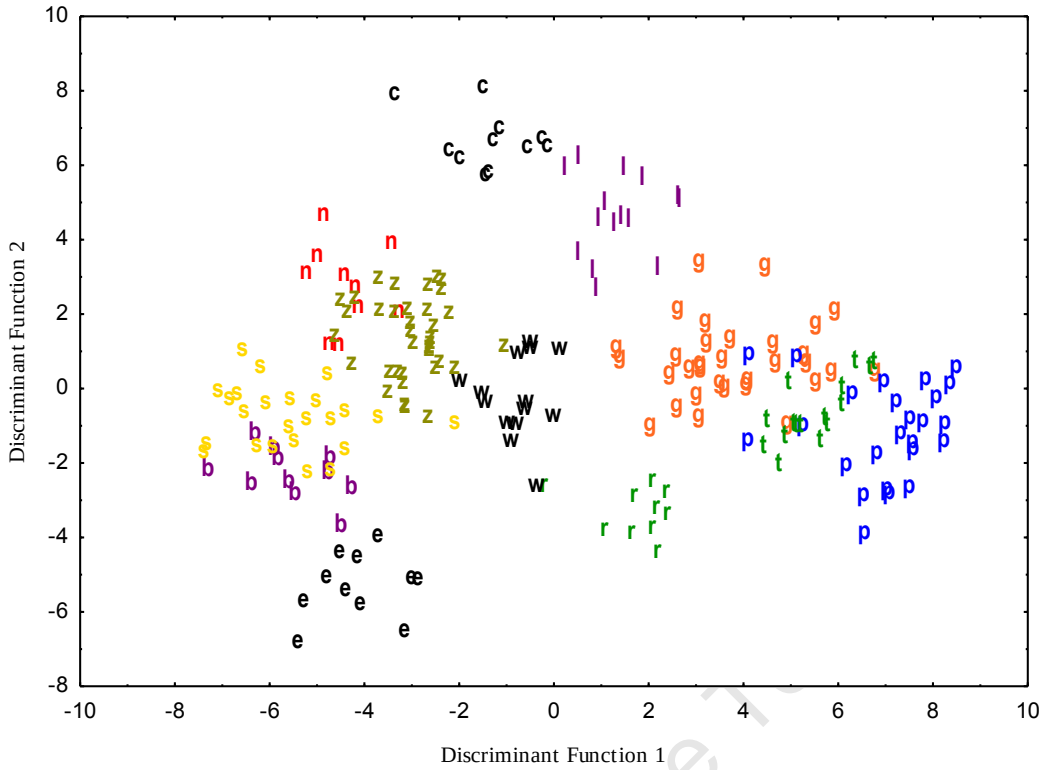
hcw contributing the least of the significant variables, and pnw being non-significant, one can conclude that genitalic variables were more important than body size variables in discriminatory power. However, in the PCA, hcw and pnw were important contributors to PC1 but not PC2, and epw was not important in PC1 but was in PC2.

Nine discriminant functions (DF's) (and canonical roots) were derived, of which the first eight were significant (P = 0.0000). Examining the standardized absolute value of coefficients (Table 3.6), the first DF was weighted most heavily by epw and sp, the second DF mainly by dp and epw; and the third DF mainly by dp, adp and sp. The first DF explained 58% of the variance or discriminatory power, the first two DF's 79.4%, the first three 90.2% and the first four 94.4%.

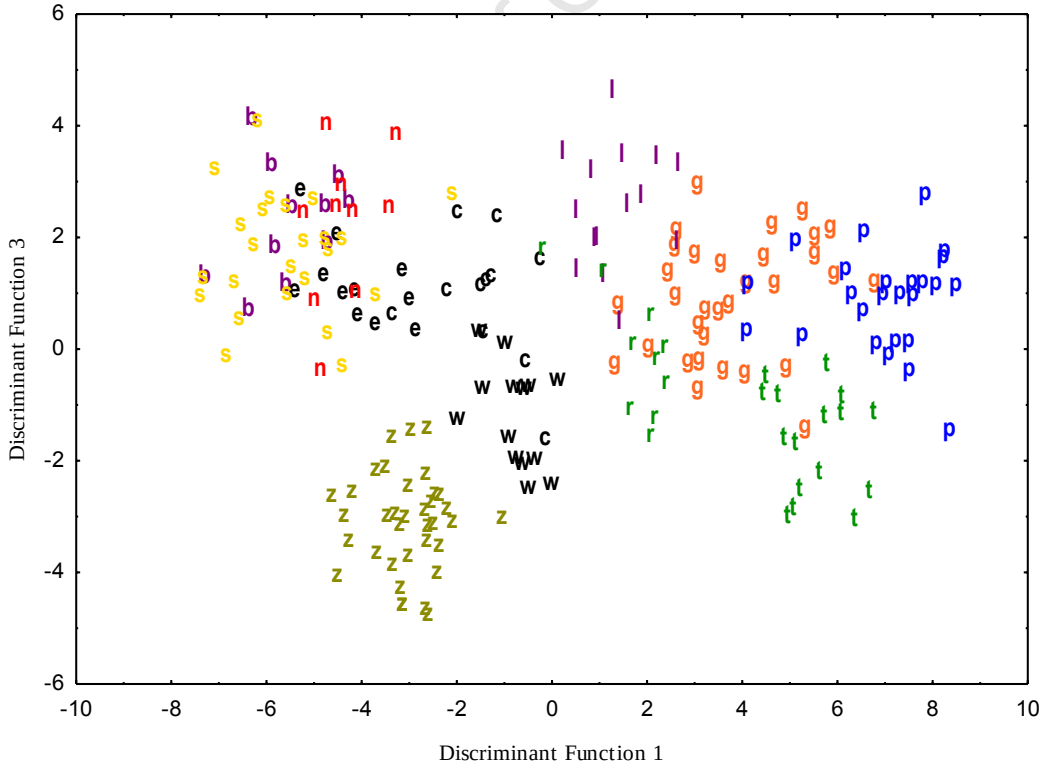
The discriminant function analysis plot of discriminant function 1 and discriminant function 2 (Fig. 3.7a) resulted in a graphical representation similar in grouping structure to that produced from the PCA. Discriminant functions 1 and 2 together provided clear discrimination of most morphogroups, with the remaining morphogroups well grouped although showing some degree of morphometric similarity (Fig. 3.7a). DF1 and DF3 together additionally isolated morphogroup ***Z*** from all the others (Fig. 3.7b). The *post hoc* classifications (Table 3.7) showed that the problem morphogroups were ***G*** which was similar to ***P*** with which one individual was misclassified, ***S*** which was misclassified with ***B*** on one occasion although they were easily distinguishable on the shape of the dorsal process, and ***P*** which had two individuals misclassified as belonging to morphogroups that were similar, one as ***G*** and one as ***T***. *Post hoc* classifications should not be regarded as an indication of how successful the discrimination analysis is, because it remains untested on new individuals (*a priori* classifications) (StatSoft, Inc. 2004).

Categorizing future collections

How are these results useful besides providing evidence for separately evolving lineages? A situation may arise when stoneflies are collected in the field, but the collector is unclear from the species descriptions as to which morphogroup of the *A. capensis* complex the specimens belong. To solve this problem, these results of the DFA are used to apply a standard formula. The results in Table 3.8 from the DFA in this study can help decide to which MG to assign the individuals. The collector will need to measure the specimens for the variables used in this study, read off the respective constant (c_i) and weight (w_i) for each variable for each MG in turn from Table 3.8 in this study, and then enter the measurement, the constant and the weight for each variable into the standard discriminant function formula to obtain a value S_i, the classification score. This



A



B

Fig. 3.7. Scatterplot of discriminant functions (DF's) of the *Aphanicerca capensis* species complex morphometric data. Letters correspond to the 12 morphogroups. **A**, DF's 1 and 2; **B**, DF's 1 and 3.

Table 3.7. *Post hoc* classifications from the discriminant function analysis. Rows show observed classifications. Numbers on the diagonal show the number of individuals that were correctly classified. Misclassifications of the morphogroup in the column on the left are shown in the same row.

Morphogroup	*n*	Percent Correct	***B***	***C***	***E***	***G***	***L***	***N***	***P***	***R***	***S***	***T***	***W***	***Z***
B	11	100.0	11	0	0	0	0	0	0	0	0	0	0	0
C	11	100.0	0	11	0	0	0	0	0	0	0	0	0	0
E	11	100.0	0	0	11	0	0	0	0	0	0	0	0	0
G	32	96.9	0	0	0	31	0	0	1	0	0	0	0	0
L	15	95.8	0	0	0	0	15	0	0	0	0	0	0	0
N	10	100.0	0	0	0	0	0	10	0	0	0	0	0	0
P	25	88.0	0	0	0	2	0	0	22	0	0	1	0	0
R	10	100.0	0	0	0	0	0	0	0	10	0	0	0	0
S	24	100.0	1	0	0	0	0	0	0	0	23	0	0	0
T	16	100.0	0	0	0	0	0	0	0	0	0	16	0	0
W	14	100.0	0	0	0	0	0	0	0	0	0	0	14	0
Z	36	100.0	0	0	0	0	0	0	0	0	0	0	0	36

Table 3.8. Discriminant function analysis of raw morphometric data. Classification functions used to classify new cases into morphogroup membership. First column = variables; first row = morphogroup. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes.

	B	***C***	***E***	***G***	***L***	***N***
pnw	32.280	8.093	16.332	8.558	1.023	11.961
hcw	468.144	351.058	371.719	367.625	369.113	415.662
adp	41.117	50.959	8.262	93.193	69.789	36.832
dp	-45.065	242.464	-108.174	110.945	234.286	167.240
sp	-271.926	-132.378	-138.041	5.134	13.535	-332.882
ppw	655.753	989.157	325.038	280.863	683.944	754.089
epl	400.795	617.112	472.004	376.601	360.737	575.632
epd	79.045	308.957	149.313	251.875	226.745	184.162
epw	329.593	277.652	684.519	1028.399	350.755	207.654
Constant	-298.287	-422.441	-231.919	-367.761	-365.175	-354.651

	P	***R***	***S***	***T***	***W***	***Z***
pnw	24.910	33.702	-5.881	14.489	-7.400	2.118
hcw	334.702	316.508	455.911	397.279	380.832	439.576
adp	113.298	108.519	-10.341	138.159	104.315	114.598
dp	61.527	-129.691	-13.603	-79.264	-116.420	-158.571
sp	107.717	139.363	-143.712	205.992	122.488	-0.781
ppw	259.832	332.974	785.238	242.169	377.486	690.600
epl	287.581	387.953	549.883	309.542	611.173	584.827
epd	196.301	146.157	66.652	317.171	130.888	370.710
epw	1544.620	1022.743	270.339	1295.472	517.652	409.495
Constant	-389.915	-271.760	-312.145	-407.223	-311.665	-374.677

process is repeated 12 times, once for each MG, using the values in Table 3.8 according to the formula

$$S_i = c_i + w_{i1}(x_1) + w_{i2}(x_2) + \ldots + w_{i9}(x_9)$$

where, S_i is the classification score for morphogroup i; numerals 1 to 9 are the morphometric variables; c_i is a constant for the i'th morphogroup (Table 3.8); w_{ij} is the weight for the j'th variable in the computation of the classification score for the i'th morphogroup (Table 3.8); and x_j is the observed value for the respective individual for the j'th variable (StatSoft, Inc. 2004).

The classification score, S_i, is computed for each morphogroup in turn. The individual stonefly measured by the collector is then assigned to the morphogroup with the highest S_i score. An example will help to clarify this process:

The collector starts by calculating the S_i score for MG ***B***. The morphometric variable measurements for one newly measured individual would be substituted, between the parentheses, into the equation (constant and weight values were taken from column ***B*** in Table 3.8):

$$S_B = -298.287 + 32.280(pnw) + 468.144(hcw) + 41.117(adp) - 45.065(dp) - 271.926(sp) + 655.753(ppw) + 400.795(epl) + 79.045(epd) + 329.593(epw).$$

This process is now repeated for the remaining 11 morphogroups, and the individual is assigned to the MG with the highest S_i score. The geographic distribution would also add weight to the decision. The best way to assess the success of the formula is to test it on new individuals where the MG to which they belong is not in doubt. Note that a stonefly of a hitherto undiscovered MG would be erroneously assigned to an existing MG. For this reason, if a new MG is suspected, a large sample should be included in a new combined analysis with all existing morphogroups.

Female morphology

Unfortunately, the familial characteristic of morphological conservatism amongst female Notonemouridae applies well to the genus *Aphanicerca*. The most common generalised shape of the female subgenital plate was a convex posterior margin with an additional small median convexity. This pattern, with varying degrees of convexity and size and shape of median convexity within and between morphogroups, was found in ***C***, ***E***, ***L***, ***N***, ***W*** and ***Z***, with

occasional occurrences in ***G***. An elongated convex posterior margin with no additional median convexity was characteristic of ***G*** (but see before), ***P***, or ***T***.

The *A. capensis* species complex does include two morphogroups (***R*** – Langeberg, and ***S*** - Langeberg and Outeniqua), with a highly distinctive eighth sternite (S8) (or subgenital plate) (Fig. 3.5M and N,O respectively). The posterior margin of S8 in ***R*** was clearly quite deeply notched, with a terminal bilateral convexity leading into the notch. The only other indentation observed was a shallow depression seen in some individuals of ***G*** (illustrated in Fig. 3.5D-F). Other morphogroups can be separated in conjunction with locality data. ***B*** and ***Z*** shared a distinctive S8, in that the posterior margin was flattened, sometimes with a small median bulge (Fig. 3.5A and T-V respectively). Where they are syntopic (southern Hottentots Holland), they were easily distinguished by the complete sclerotization of sternites in ***B***, and incomplete anterior sclerotization of S3 to S5 in ***Z*** (Fig. 3.4A and L respectively). In addition, the cerci of ***B*** were much smaller than of ***Z***. Sternite sclerotization patterns were also useful in other cases. ***C*** (Cape Peninsula) and ***Z*** (Hottentots Holland and Riviersonderend) shared the incomplete anterior sclerotization of S3 to S5 described above (Fig. 3.4B and L respectively), a pattern not found in the other morphogroups (Fig. 3.4).

Distribution

Morphometrics and geographic distance

There was no correlation between morphological dissimilarity and geographic distance (Mantel test, $P = 0.757$, $r = -0.043$). This implies that it would not be possible to predict morphometric characters given a geographic locality or distance from any other morphogroup. Acceptance of this null hypothesis of no association suggests the existence of multiple non-interbreeding species in the groups sampled. In this scenario, the geographic distribution of morphometric variable size classes was random with respect to other size classes, and therefore maintenance of morphological unity within a morphogroup was not dependant on isolation by geographic distance (i.e. no cline was evident) but rather on reproductive isolation. This is clearly seen in Fig. 3.8 which shows a scatterplot of component value means, with each population labelled with its mountain range group (see Fig. 3.1 for the spatial distribution of mountain ranges).

Distributions of morphogroups across mountain ranges

Of the ten mountain range groups (the nine that were included in the morphometric and genetic analyses, plus the Elandsberge range which was not included), six were represented by a single morphogroup each, while the southern Hottentots Holland was represented by three, Groot Swartberg by two, Outeniqua by two, and the Langeberg by four (Figs 3.1, 3.8, 3.9; Table

3.1). Each mountain group that is home to more than one morphogroup, namely the Langeberg, Outeniqua, Groot Swartberg and Hottentots Holland (southern), was spread over a large area of the PC1 PC2 plot, showing that morphometrics and mountain group were unrelated. However, in the case of a particular morphogroup occurring in more than one mountain group, those montane regions were in near proximity (considering also that the MG may occur in the intervening mountainous territory). Three of the morphogroups, ***P***, ***S*** and ***Z***, are each found in more than one mountain range. Morphogroup ***S*** is found in the Langeberg, Outeniqua and Elandsberge ranges, with different populations of this morphogroup showing slight variation in both male and female morphology (Figs 3.3Q,R, 3.5N,O) which is a reflection of random genetic drift in non-interbreeding populations of the MG. These three mountain ranges run in a west to east continuum. One can then conclude that this morphogroup occurs in one geographical montane feature. Similarly, ***P*** occurs in the almost contiguous Outeniqua and Langeberg Mountains, also with inter-populational slight variation. Morphogroup ***Z*** occurs in the greater Hottentots Holland region (northern and southern) and in the Riviersonderend Mountains, also displaying morphological variability. Again, these mountains are contiguous, with this morphogroup being found in the triangle from Bain's Kloof in the north, to Betty's Bay in the south, and to the Riviersonderend Mountains in the east. Six mountain ranges, namely the Cederberg, Cape Peninsula Mountain Chain, Riviersonderend Mountains, Witsenberg, northern Hottentots Holland and Elandsberge have, as far as current collecting effort has uncovered, one morphogroup each (Fig. 3.8). Most of these are characterised by being isolated from other ranges by unsuitable flat habitat.

Mountain range morphogroup endemics were found to be the rule. Current distribution records categorized all 12 morphogroups as endemic to their respective montane regions. Nine of the 12 morphogroups are endemic to their immediate mountain range (Figs 3.1, 3.8). Morphogroups ***B*** and ***N*** are endemic to the southern Hottentots Holland, ***C*** to the Cape Peninsula, ***E*** and ***G*** to the Groot Swartberg, ***L*** and ***R*** to the Langeberg, ***T*** to the Witsenberg, and ***W*** to the Cederberg. The exceptions, as described above, are ***P***, ***S***, and ***Z*** which each occur in multiple mountain ranges which are geographically proximate.

The Langeberg range is home to four morphogroups of the *A. capensis* species complex which were separated by PC1 and PC2 (Fig. 3.6) and by DF1 and DF2 (Fig. 3.7a) into four distinct non-overlapping morphogroups. Morphogroup ***S*** was syntopic with ***P*** and ***L*** at Riversdale (Kristalkloof). Two sympatric (defined in this context as occurring in the same mountain range) morphogroups have thus far been found in the Groot Swartberg range, namely ***G*** from the Prince Albert side (northern slopes) of the Swartberg Pass, and ***E*** at Boegoekloof on the southern slopes of the same pass (Table 3.9). The southern Hottentots Holland range has

three sympatric morphogroups, namely ***B*** and ***Z*** which are syntopic at Betty's Bay, and ***N*** from Hermanus (Table 3.9). Syntopic morphogroups ***B*** and ***Z*** did not overlap on the PC plot (Fig. 3.6) but ***N*** overlapped with both. There was also clear separation of ***B***, ***Z*** and ***N*** on the DFA plots (Fig. 3.7a,b). The Outeniqua Mountains are home to two morphogroups, ***S*** and ***P*** which are syntopic at Prince Alfred's Pass, Gouna Forest and Bergplaas, all near Knysna, with ***P*** also occurring at Montagu Pass near George. They separated out from each other in both PC1 and PC2. The positive PC1 and positive PC2 quadrant (Fig. 3.6) contained three morphogroups, ***T*** (Witsenberg), ***P*** (Outeniqua Mountains) and ***G*** (Groot Swartberg) that overlapped with each other in both PC1 and PC2.

Morphometric cluster analysis – relationships between morphogroups and mountains

The cluster diagram (Fig. 3.9) shows the relationships between morphogroups (and mountain ranges) according to morphometric similarity (using the means of the raw morphometric data). The groupings correlated well with the groupings in the PCA (Fig. 3.6) and DFA (Fig. 3.7). As in Fig. 3.8, there was no correlation between morphometrics and geographic location. However, three trends are apparent from this analysis. Firstly, syntopic morphogroups (***L***, ***P***, and ***S*** – Langeberg; ***B*** and ***Z*** – southern Hottentots Holland) (Table 3.9) were morphometrically more similar to other morphogroups than to each other, except that ***L*** was equally similar to ***P*** and ***G***. ***L*** was easily identified on the shape of the T9 dorsal process lobes. Secondly, sympatric morphogroups (***E*** and ***G*** – Groot Swartberg; ***R***, ***L***, ***P***, and ***S*** – Langeberg; ***B***, ***N***, and ***Z*** – Hottentots Holland) (Table 3.9) followed that same pattern as syntopic morphogroups, except for ***N*** and ***Z*** which were most similar to each other than to others. However, ***N*** and ***Z*** were also easily distinguishable on the shape of the dorsal process lobes. Thirdly, pairs of mountain ranges that were grouped together by morphologically similar stoneflies were geographically disjunct (except for the ***N*** / ***Z*** pair).

Table. 3.9. Sympatric (found in the same mountain range) and syntopic *A. capensis* species complex morphogroups. Syntopic morphogroups (occurring together in the same stream) within each mountain range are shaded.

Morphogroups	Mountain range / group
P / ***S***	Outeniqua
P / ***S*** / ***L*** / ***R***	Langeberg
B / ***Z*** / ***N***	Hottentots Holland (southern)
E / ***G***	Groot Swartberg

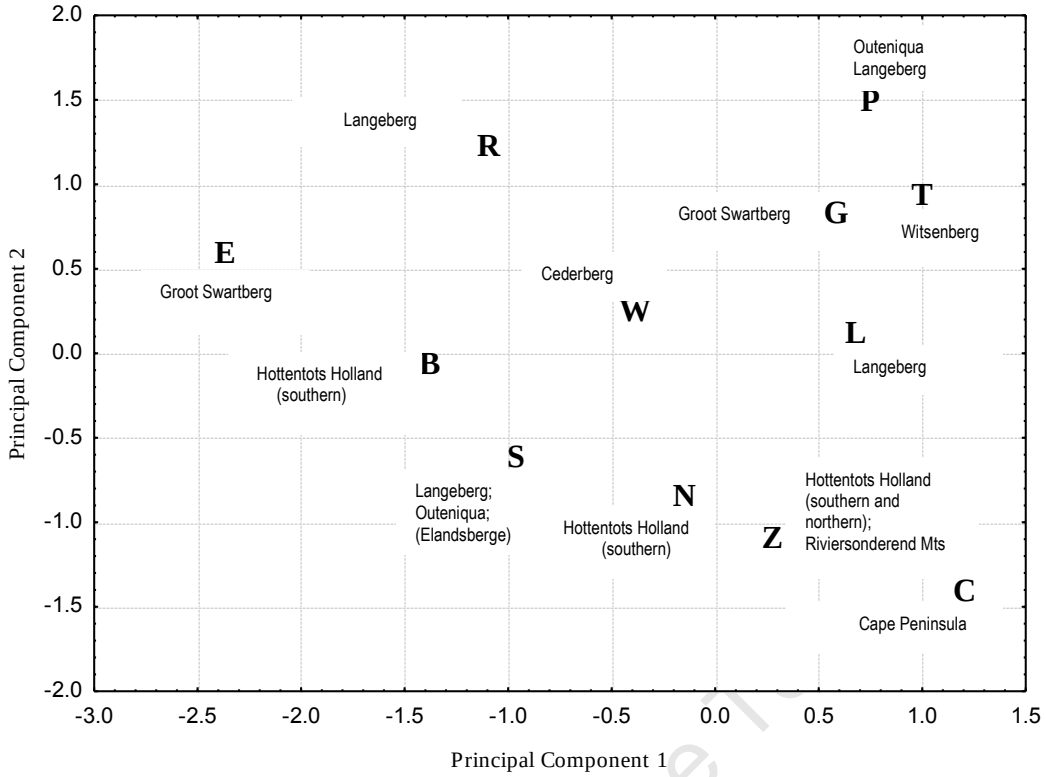


Fig. 3.8. Scatterplot of PC1 and PC2 value means, with each morphogroup and its associated mountain range group from where specimens were obtained for morphometric analysis. The Elandsberge is in parentheses as individuals from there were not used in the morphometric analysis.

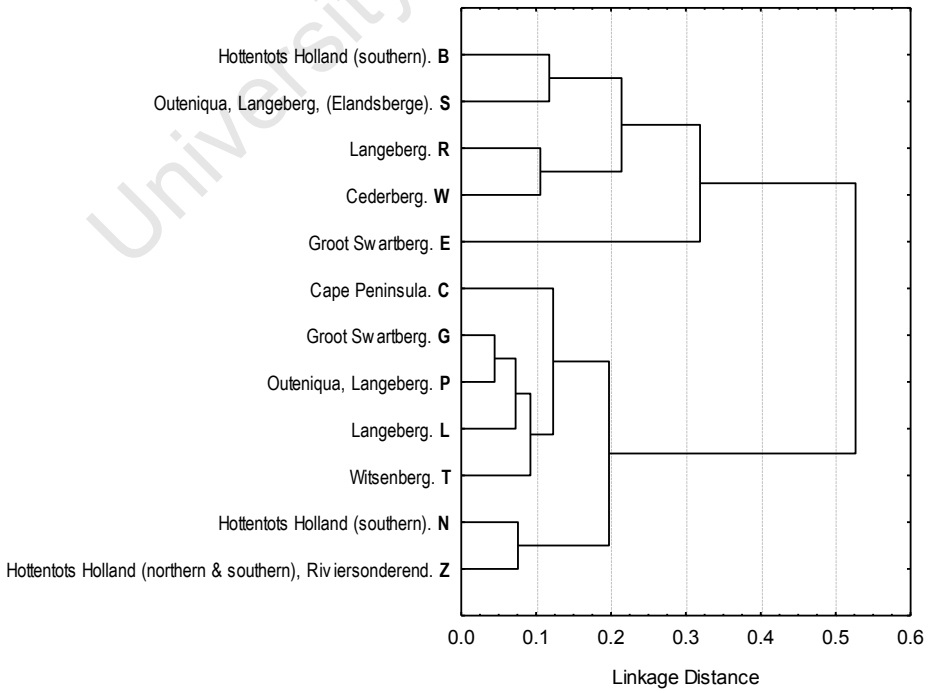


Fig. 3.9. Cluster analysis of means of raw morphometric data using a complete linkage algorithm and Euclidean distance. Mountain ranges are associated with populations. The Elandsberge is in parentheses as individuals from there were not used in the morphometric analysis.

Mate choice

Occurrences of positive assortative mating

Details of the reproductive behaviour observed during mate choice trials were described for *Aphanicercella* in Stevens & Picker (1999), and were similar to those of *Aphanicerca*, so are not repeated here. Drumming, a species specific reproductive communication modality between male and female stoneflies (Stewart 1997), was not observed during the *Aphanicercella* experiments, but isolated episodes were observed in *Aphanicerca* during this study, too few for analysis. The control experiments between the Cape Peninsula morphogroup ***C*** of *A. capensis* and *A. bicornis* and *A. bovina* respectively, showed that positive assortative mating occurred under the experimental conditions ($P < 0.05$, Fisher's Exact Test) (Table 3.10). However, one mating between an *A. capensis* male and an *A. bicornis* female occurred. In experimental trial 1, Cape Peninsula ***C*** males preferentially paired with Cape Peninsula females over Stellenbosch ***Z*** females ($P < 0.01$). Nevertheless, there were four inappropriate pairings, i.e. pairings between, rather than within, morphogroups. The Stellenbosch ***Z*** males however, paired randomly with Cape Peninsula and Stellenbosch females ($P > 0.05$). In experimental trial 2, the Cape Peninsula ***C*** males preferentially paired with Cape Peninsula females over Bain's Kloof ***Z*** females ($P < 0.01$). There was no negative assortative (i.e. inappropriate between group) mating. The Bain's Kloof ***Z*** males paired randomly with Cape Peninsula and Bain's Kloof females ($P > 0.05$). In experimental trial 3, the Cape Peninsula ***C*** males preferentially paired with Cape Peninsula females over Cederberg ***W*** females ($P < 0.01$). There was one inappropriate pairing. The Cederberg ***W*** males paired randomly with Cape Peninsula and Cederberg females ($P > 0.05$). In experimental trial 4, the Betty's Bay ***B*** males preferentially paired with Betty's Bay ***B*** females over Betty's Bay ***Z*** females ($P < 0.05$). There was one inappropriate pairing. The Betty's Bay ***Z*** males preferentially paired with Betty's Bay ***Z*** females over Betty's Bay ***B*** females ($P < 0.01$). There were no inappropriate pairings which indicates that the experimental protocol does not stress the stoneflies into abnormal random mating. This can be deduced because these two forms are syntopic and will therefore not be interbreeding in nature.

To summarise these results: Cape Peninsula ***C*** males preferentially paired with Cape Peninsula females over Stellenbosch ***Z*** females, Bain's Kloof ***Z*** females, and Cederberg ***W*** females; and the Stellenbosch ***Z*** males, Bain's Kloof ***Z*** males, and Cederberg ***W*** males however, showed no discrimination between morphogroups.

Mating trials are designed to assess pre-zygotic isolation only and have no meaning for actual situations where different morphogroups will never meet due to geographic separation. Their value lies in the implication of positive assortative mating. Failure of two morphogroups

Table 3.10. Results of mate choice experiments between 12 morphogroups of the *Aphanicerca capensis* species complex, using the Fisher's Exact Test chi-square statistic in two controls and four experimental trials. Figures in the results blocks are the number of successful matings, with the expected frequencies in parentheses, given the null hypothesis of random mating. A successful mating ended the experiment, and the pair was removed. NS = not significant at the 5% level; d.f. = 1 in all cases; the *n* (the number of experiments using different individuals) is the sum of matings.

Control 1	***C*** ♀ (Cape Peninsula)	*A. bicornis* ♀	Fisher's Exact χ^2	*P*
C ♂ (Cape Peninsula)	9 (5)	1 (5)	6.40	<0.05

Control 2	***C*** ♀ (Cape Peninsula)	*A. bovina* ♀	Fisher's Exact χ^2	*P*
C ♂ (Cape Peninsula)	16 (8)	0 (8)	16.00	<0.01
A. bovina ♂	0 (3)	6 (3)	6.00	<0.05

Trial 1	***C*** ♀ (Cape Peninsula)	***Z*** ♀ (Stellenbosch)	Fisher's Exact χ^2	*P*
C ♂ (Cape Peninsula)	16 (10)	4 (10)	7.20	<0.01
Z ♂ (Stellenbosch)	20 (17)	14 (17)	1.06	NS

Trial 2	***C*** ♀ (Cape Peninsula)	***Z*** ♀ (Bain's Kloof)	Fisher's Exact χ^2	*P*
C ♂ (Cape Peninsula)	12 (6)	0 (6)	12.00	<0.01
Z ♂ (Bain's Kloof)	9 (7.5)	6 (7.5)	0.60	NS

Trial 3	***C*** ♀ (Cape Peninsula)	***W*** ♀ (Cederberg)	Fisher's Exact χ^2	*P*
C ♂ (Cape Peninsula)	15 (8)	1 (8)	12.25	<0.01
W ♂ (Cederberg)	9 (6)	3 (6)	3.00	NS

Trial 4	***B*** ♀ (Betty's Bay)	***Z*** ♀ (Betty's Bay)	Fisher's Exact χ^2	*P*
B ♂ (Betty's Bay)	7 (4)	1 (4)	4.50	<0.05
Z ♂ (Betty's Bay)	0 (7)	14 (7)	14.00	<0.01

to interbreed at the pre-zygotic level, albeit in the laboratory, and subject to control results, can only mean the existence of two separately evolving metapopulation lineages, i.e. species.

Mitochondrial DNA

Species level analysis

Nucleotides, codons and amino acids

The following nucleotide and phylogram results apply to the combined ingroup and outgroup. The first codon position had the most uniform nucleotide composition. The second position had a thymine bias (43.5%), and the third position was A-T rich, as found in many insect orders (Williams *et al*. 2006), at 78.1%, versus 51.8% and 56.9% for the first and second positions respectively. The third codon position showed the highest rate of substitutions, followed by the first position, and with no substitutions at position two. Amino acid sequences were identical throughout all morphogroups, except for the ***P*** morphogroup individual from Kristalkloof (Garcia's Pass, Riversdale, Langeberg) which differed at amino acid 140 out of 185 because of a non-synonymous first codon position adenine to guanine transition at nucleotide position 418 of the sequence, which resulted in the change from methionine to valine. All other substitutions were synonymous.

Cladograms and phylograms

The data set contained 82 parsimony informative sites. Two most parsimonious trees of length 185, with Ci = 71 and Ri = 83, were recovered in the heuristic MP analysis (Figs 3.10, 3.11). The strict consensus tree was one step longer (Fig. 3.12). The best-fit model selected using MODELTEST was TVM+I+G (-lnL = 1790.5494; AIC = 3599.0989), with base frequencies of A: 0.2824, C: 0.2010, G: 0.1703, T: 0.3462. The substitution rate matrix was A-C: 4.6420, A-G: 19.2600, A-T: 1.4347, C-G: 0.0000, C-T: 19.2600, and G-T: 1.0000 (fixed). The proportion of invariable sites was 0.5801 and the gamma distribution shape parameter was 0.9462. The PhyML ML tree had a –lnL of 1786.63623. The ML tree (Fig. 3.10) was identical to one of the two MP trees. The BI tree (Fig. 3.13) was identical to the other MP tree, except that haplotypes 4 and 20 are more closely related to each other than to the rest of the branch in the MP phylogram. The ML tree differed from the MP strict consensus tree in separating haplotypes 21 and 22 from the most terminal polytomy. The BI tree differed from the MP strict consensus in not separating haplotypes 4 and 20 from the most terminal polytomy, and in separating haplotypes 25, 26 and 27 from their most closely related clade. The BI tree differed from the ML tree in uniting haplotypes 4 and 20, in including the clade of haplotypes 21 and 22 in the terminal polytomy, and in separating the clade of haplotypes 25, 26 and 27 from a tritomy.

Table 3.11. The 74 variable sites for 27 haplotypes representing 40 individuals of the *Aphanicerca capensis* species complex COI 557 base pair segment sampled. A dot indicates an identical nucleotide to haplotype 1.

	Position																																							
	1	2	3	7	7	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	1	2	3	7	7	8	1	1	2	2	3	3	4	5	5	5	5	5	6	6	7	7	8	8	0	0	2	2	3	4	5	6	6	7	7	8	8	8	8	8
Haplotype	8	7	6	2	5	7	1	7	0	9	5	6	1	0	1	3	6	9	5	8	4	7	0	3	1	5	2	5	4	1	8	4	7	0	3	2	6	6	6	6
1	C	A	T	T	C	G	T	T	T	A	G	C	T	G	C	A	A	C	T	G	C	C	A	G	A	C	T	A	C	T	G	A	G	A	T	A	C	C	C	C
2	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	T	G	.	.	T	A	.	.	.	G	A	T	C	A	T	G	.	.	C	A	.	.	C	.	G	.	.	.	.	C	A	T	A	G	A	.	.	.	.	.
4	T	.	.	.	T	.	C	C	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	.	.	.	C	.	C	A	.	.	G	.	.	.	.
5	T	.	C	.	T	.	C	.	.	.	A	.	C	.	T	.	.	T	.	A	.	A	.	.	.	.	C	.	.	C	.	.	A	.	.	.	.	.	.	.
6	T	.	C	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	C	.	.	C	.	.	A	.	.	.	.	.	.	.
7	T	.	C	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	G	.	C	.	.	C	.	.	A	.	.	G	.	.	.	.
8	T	G	C	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	C	.	.	C	.	.	A	.	.	G	.	.	.	.
9	T	.	C	.	T	.	C	.	.	.	A	.	C	.	.	.	.	.	.	A	.	A	.	.	.	.	C	.	.	C	.	.	A	.	.	G	.	.	.	.
10	T	.	.	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
11	T	.	.	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	.	.	.	C	.	.	A	.	.	C	.	.	.	.
12	T	.	.	.	T	.	C	.	.	.	A	.	C	.	T	.	G	.	.	A	.	A	.	.	.	T	.	.	.	C	.	.	A	.	.	.	.	.	.	.
13	.	.	.	.	T	.	.	C	.	.	A	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	T	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	.	.	.	C	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	A	.	.	.	.	.	.	.	C	T	C	.	.	A	.	.	.	.	.	.	.
16	.	.	.	C	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	A	.	.	.	.	.	.	.	C	T	C	.	.	A	.	.	.	.	.	.	.
17	.	.	.	C	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	.
18	T	.	.	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	A	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
19	T	.	.	.	T	.	C	.	.	.	A	.	C	.	T	.	.	.	.	A	.	G	.	A	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
20	T	G	.	.	T	.	C	C	.	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
21	T	.	.	.	T	.	C	.	A	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
22	T	.	.	.	T	.	C	.	A	.	A	.	C	.	T	.	.	.	.	A	.	A	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
23	T	.	.	.	T	.	.	.	.	.	A	.	C	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
24	T	.	.	.	T	.	.	.	.	.	A	.	C	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.
25	T	.	.	.	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	A	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	T	.	.	.	.
26	T	.	.	.	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	A	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	.	.
27	T	.	.	C	T	.	.	.	.	.	A	.	C	.	T	.	.	.	.	A	.	.	.	.	.	.	.	T	C	.	.	A	.	.	.	.	.	.	.	.

Table 3.11. Continued

	Position																																							
	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5
	9	9	0	1	2	3	3	4	4	4	4	4	4	7	7	9	0	0	1	1	2	2	2	2	3	5	7	7	9	9	0	0	1	1	1	2	3	5	5	5
Haplotype	1	4	3	2	4	0	9	0	2	5	6	8	2	5	0	2	8	7	8	0	3	6	9	9	9	4	7	5	8	4	7	0	3	9	0	1	5	1	2	3
1	A	A	C	T	T	A	G	T	G	T	C	A	G	A	C	C	T	T	A	A	C	G	T	T	G	G	C	A	A	A	C	T	T	C	T	G	T	T	T	T
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
3	.	G	.	C	.	.	.	.	.	T	.	A	C	A	T	.	.	.	G	.	A	C	.	A	A	A	G	.	G	A	C	.	.	C	A	C	C	C	C	C
4	C	.	.	.	C	.	A	C	.	.	.	.	A	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
5	C	.	.	C	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
6	C	.	.	C	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
7	C	.	.	C	C	.	A	C	.	C	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
8	C	.	.	C	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
9	C	.	.	C	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
10	C	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
11	C	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
12	C	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
13	.	.	.	.	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
15	.	.	T	.	.	.	.	.	A	.	.	.	.	.	A	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
16	.	.	T	.	.	.	.	.	A	.	.	.	.	.	A	.	.	G	.	.	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
17	.	.	T	.	.	.	.	.	A	.	.	.	.	.	A	.	.	G	G	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
18	C	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
19	C	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
20	C	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	G	.	.	.	.	T	.	A	.	.	.	.	.
21	C	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
22	C	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
23	.	.	.	.	C	.	A	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.
24	.	.	.	.	C	.	A	C	.	.	.	.	A	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.
25	.	.	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
26	.	.	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
27	.	.	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.

Table 3.12. Distribution data for the 40 individuals of the *Aphanicerca capensis* species complex sampled for the COI mtDNA analysis. Code = specimen field code; MG = morphogroup; H = haplotype; H-H = Hottentots Holland Mountains.

Code	MG	H	Locality	Mountain range	Latitude	Longitude
D4	***B***	1	Harold Porter Botanic Reserve, Betty's Bay	H-H (southern)	-34.352300	18.927000
D3	***B***	2	Harold Porter Botanic Reserve, Betty's Bay	H-H (southern)	-34.352300	18.927000
A2	***C***	3	Boschenheuwel Arboretum, Kirstenbosch	Cape Peninsula	-33.987460	18.437190
A1	***C***	3	Slangolie Ravine, Twelve Apostles	Cape Peninsula	-33.977700	18.385100
C1	***E***	4	Boegoekloof, Swartberg Pass	Groot Swartberg	-33.357400	22.058500
C2	***E***	4	Boegoekloof, Swartberg Pass	Groot Swartberg	-33.357400	22.058500
J2	***G***	5	Seweweekspoort	Groot Swartberg	-33.412100	21.408700
J1	***G***	6	Seweweekspoort	Groot Swartberg	-33.394300	21.399200
O2	***G***	7	Malvadraai, Swartberg Pass	Groot Swartberg	-33.299600	22.050100
O1	***G***	8	Malvadraai, Swartberg Pass	Groot Swartberg	-33.299600	22.050100
DDD2	***G***	9	Oudemuragie road, near Meiringspoort	Groot Swartberg	-33.391800	22.355900
M2	***P***	17	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	-33.958600	21.230400
I3	***L***	10	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	-33.958600	21.230400
I2	***L***	11	Tradouw Pass	Langeberg	-33.982738	20.708599
I1	***L***	12	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	-33.958600	21.230400
F2	***N***	13	Fernkloof Nature Reserve, Hermanus	H-H (southern)	-34.390000	19.269100
F4	***N***	14	Fernkloof Nature Reserve, Hermanus	H-H (southern)	-34.393900	19.276100
N2	***P***	15	Bergplaas-Kleinplaat road, NE of George	Outeniqua	-33.872275	22.687287
CCC3	***P***	15	Keur River bridge, Montagu Pass, George	Outeniqua	-33.907175	22.418134
N3	***P***	15	Prince Alfred's Pass, N of Knysna	Outeniqua	-33.860994	23.171860
CCC1	***P***	16	Keur River bridge, Montagu Pass, George	Outeniqua	-33.907175	22.418134
P5	***R***	18	Bergheim, between Montagu and Barrydale	Langeberg	-33.932800	20.380900
P1	***R***	19	Ravenna, between Montagu and Barrydale	Langeberg	-33.918500	20.378800
P3	***R***	20	Bergheim, between Montagu and Barrydale	Langeberg	-33.932800	20.380900
P4	***R***	19	Ravenna, between Montagu and Barrydale	Langeberg	-33.918500	20.378800
L4a	***S***	10	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	-33.958600	21.230400
L3	***S***	10	Kristalkloof, Garcia's Pass, near Riversdale	Langeberg	-33.958600	21.230400
L5	***S***	21	Cloete's Pass, NW of Mossel Bay	Langeberg	-33.919800	21.742100
L2	***S***	22	Cloete's Pass, NW of Mossel Bay	Langeberg	-33.919800	21.742100
E6	***S***	21	Kom se Pad, Gouna Forest, Knysna	Outeniqua	-33.947500	23.141100
E1	***S***	21	Keur River bridge, Montagu Pass, George	Outeniqua	-33.907175	22.418134
N4	***P***	15	Prince Alfred's Pass, N of Knysna	Outeniqua	-33.860994	23.171860
G2	***T***	10	Witsenberg Game Park, near Wolseley	Witsenberg	-33.382737	19.213298
G1	***T***	10	Witsenberg Game Park, near Wolseley	Witsenberg	-33.382737	19.213298
H2	***W***	23	11.2 km S of Algeria forest station	Cederberg	-32.425600	19.131800
H3	***W***	24	Eikeboom, 16.4 km S of Algeria, Cederberg	Cederberg	-32.454900	19.169600
B5	***Z***	25	Oubos farm, Riviersonderend	Riviersonderend	-34.082000	19.829100
B1	***Z***	26	Jonkershoek Nature Reserve, Stellenbosch	H-H (northern)	-33.989100	18.968400
B2	***Z***	26	Bain's Kloof Pass, 1st stream N Wellington	H-H (northern)	-33.645158	19.070927
B4	***Z***	27	Oubos farm, Riviersonderend	Riviersonderend	-34.082000	19.829100

Table 3.13. Frequency distribution of the 27 COI haplotypes by locality. The upper-case letter in each cell refers to the morphogroup to which the haplotype at the particular locality belongs.

Locality	Haplotypes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Mountain Range
Betty's Bay	1***B***	1***B***																										
Fernkloof (Hermanus) 1														1***N***														Hottentots Holland (southern)
Fernkloof (Hermanus) 2													1***N***															
Slangolie Ravine			1***C***																									Cape Peninsula Mountain Chain
Kirstenbosch			1***C***																									
Boegoekloof (Swartberg Pass)				2***E***																								
Malvadraai (Swartberg Pass)							1***G***	1***G***																				
Oudemuragie									1***G***																			Groot Swartberg
Seweweekspoort 1						1***G***																						
Seweweekspoort 2					1***G***																							
Kristalkloof, Garcia's Pass (Riversdale)										1***L*** 2***S***		1***L***					1***P***											
Tradouw Pass											1***L***																	
Bergheim (Montague-Barrydale)																		1***R***		1***R***								Langeberg
Ravenna (Montague-Barrydale)																			2***R***									
Cloete's Pass																					1***S***	1***S***						
Bergplaas (N of George)															1***P***													
Montagu Pass (George)															1***P***	1***P***					1***S***							Outeniqua Mts
Prince Alfred's Pass (N of Knysna)															2***P***													
Gouna (Knysna)																					1***S***							
Witsenberg Game Farm										2***T***																		Witsenberg
Eikeboom (S of Algeria)																								1***W***				Cederberg
Algeria south road																							1***W***					
Jonkershoek (Stellenbosch)																										1***Z***		Hottentots Holland (northern)
Bain's Kloof Pass																										1***Z***		
Oubos (Riviersonderend)																									1***Z***		1***Z***	Riviersonderend

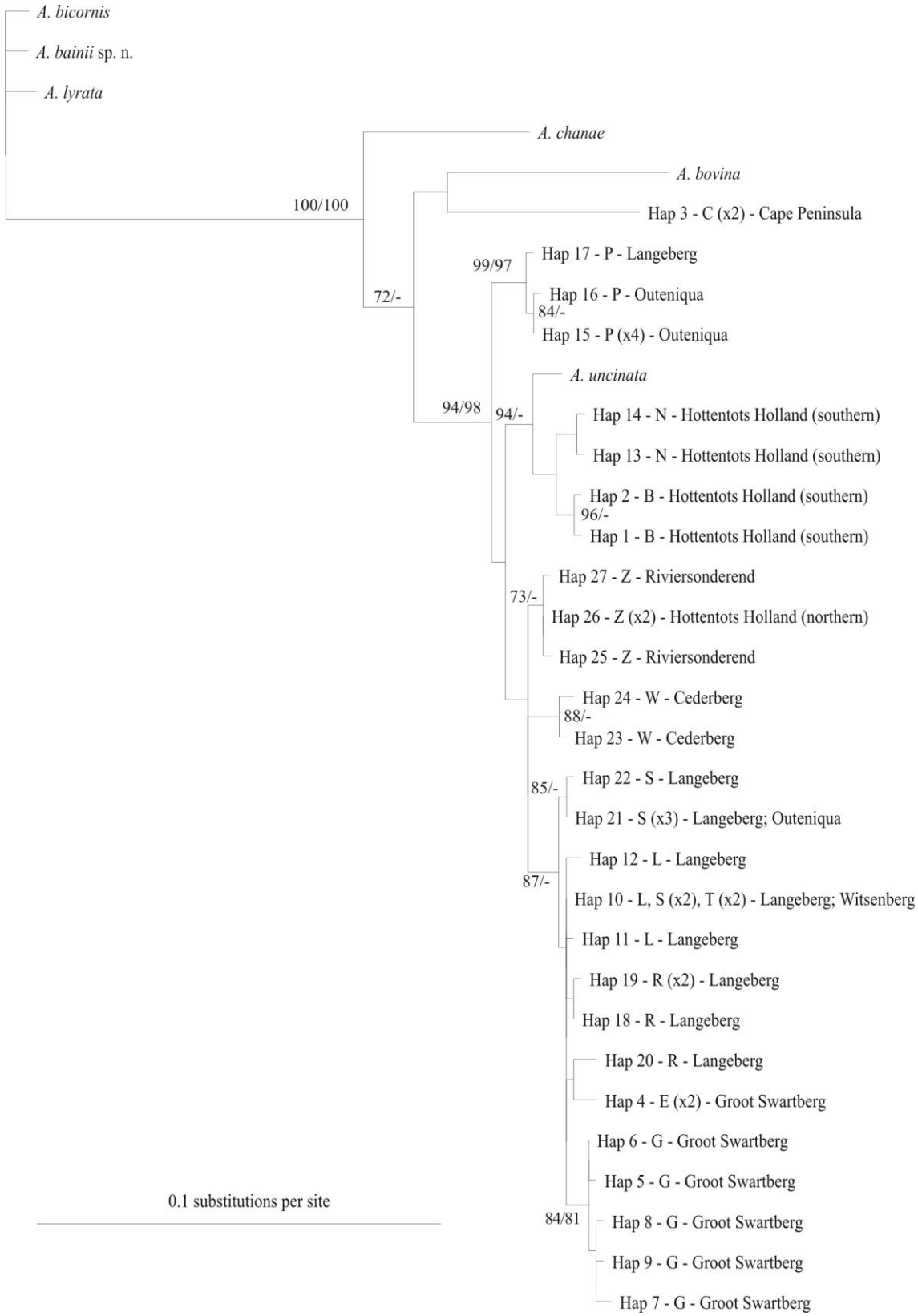


Fig. 3.10. Maximum likelihood phylogram of 27 mtDNA COI haplotypes of the *Aphanicerca capensis* species complex, with six congeneric outgroup species of which one was used as the root. The parameters used corresponded to the TVM + I + gamma model of nucleotide substitution. This topology was identical to one of the two maximum parsimony trees. Bootstrap values of 70% and above are given as ML/MP. Where more than one individual share a haplotype, this in indicated in parentheses. The morphogroup and mountain range locality are given to the right of the haplotype number.

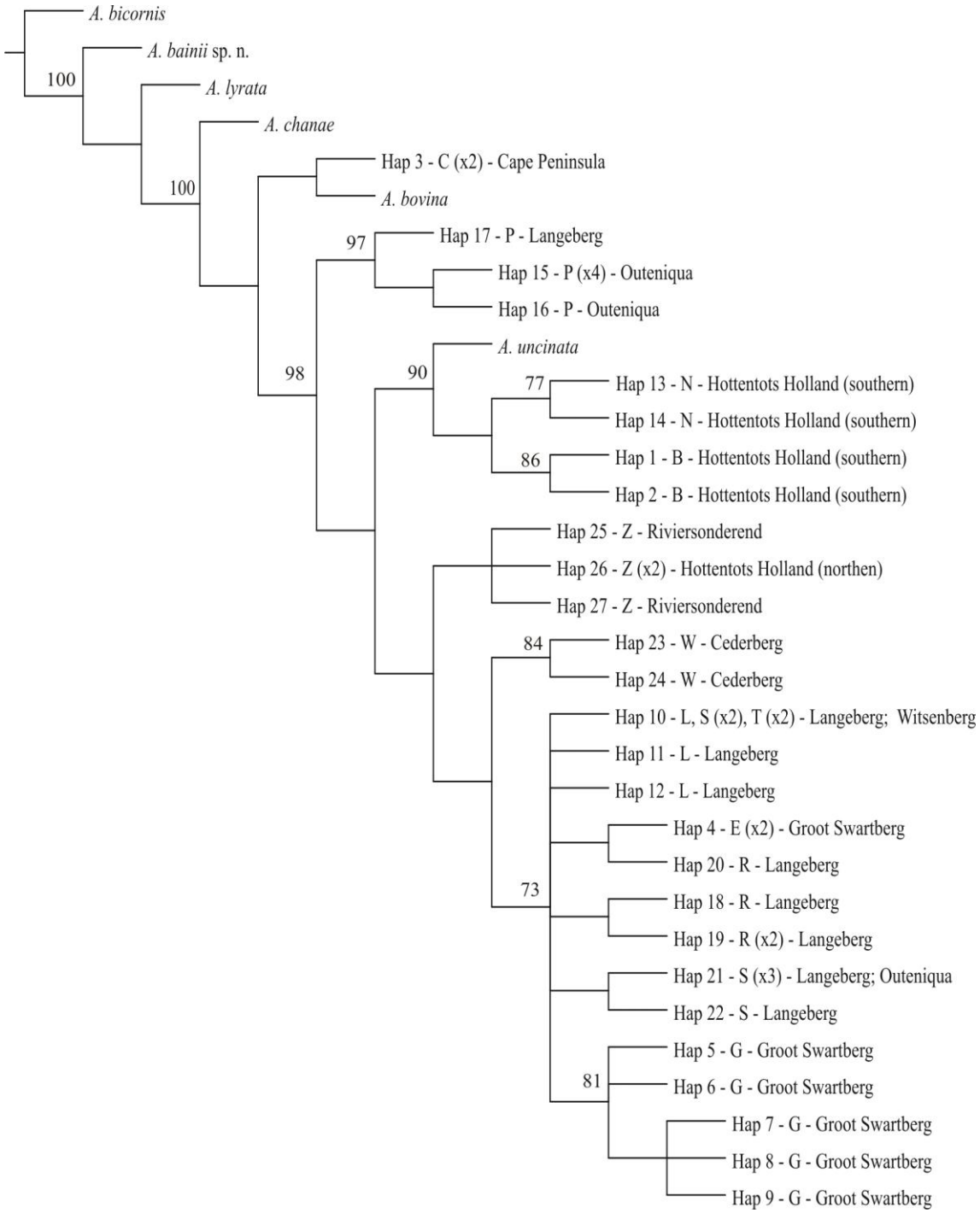


Fig. 3.11. One of two most parsimonious cladograms of 27 mtDNA COI haplotypes of the *Aphanicerca capensis* species complex, with six congeneric outgroup species of which one was used as the root. The other MP cladogram has an identical topology to the ML tree. Bootstrap values < 70% are not shown. Where more than one individual share a haplotype, this in indicated in parentheses. The morphogroup and mountain range locality are given to the right of the haplotype number.

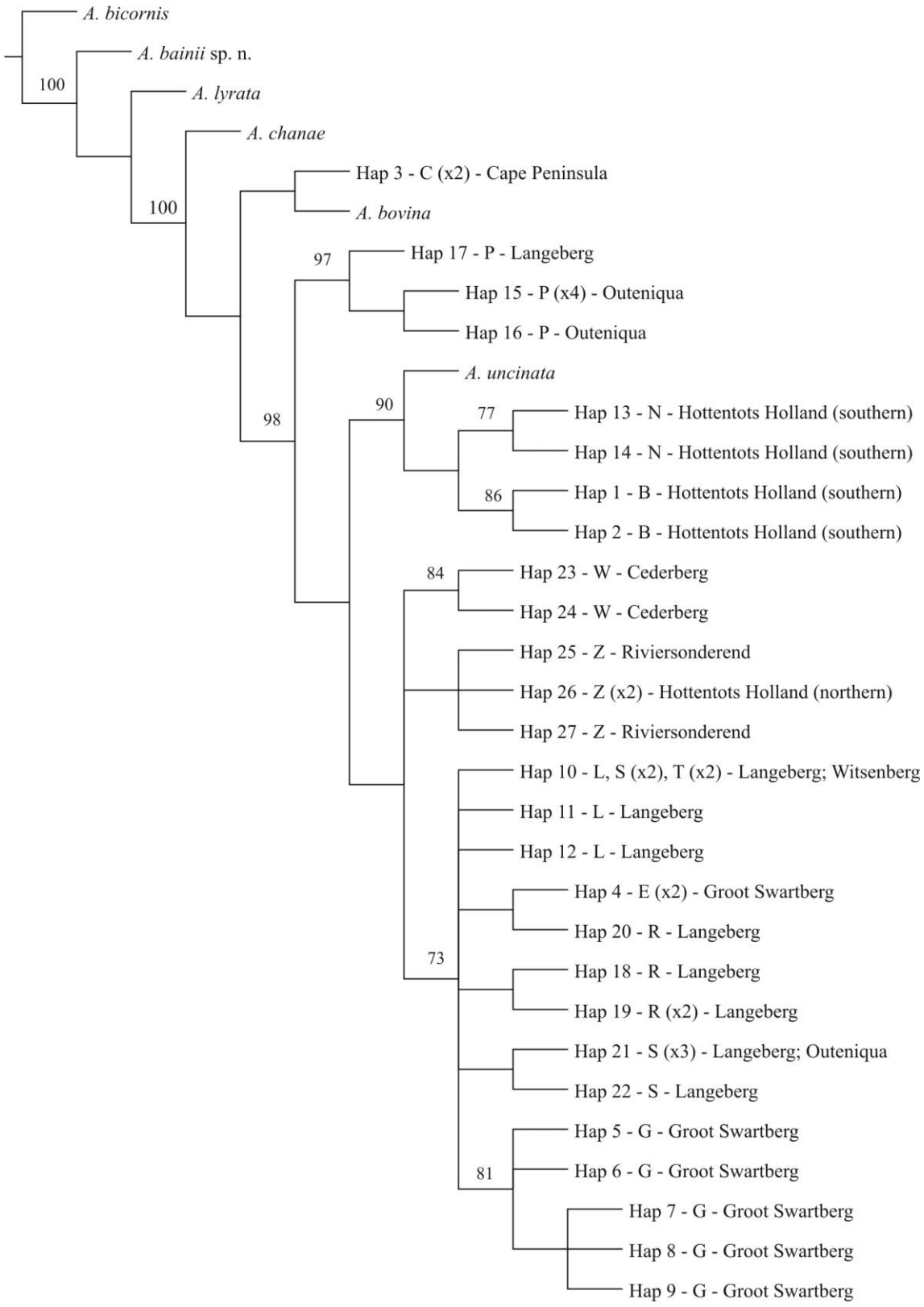


Fig. 3.12. Strict consensus tree of the two most parsimonious cladograms of 27 mtDNA COI haplotypes of the *Aphanicerca capensis* species complex, with six congeneric outgroup species of which one was used as the root. Bootstrap values < 70% are not shown. Where more than one individual share a haplotype, this in indicated in parentheses. The morphogroup and mountain range locality are given to the right of the haplotype number.

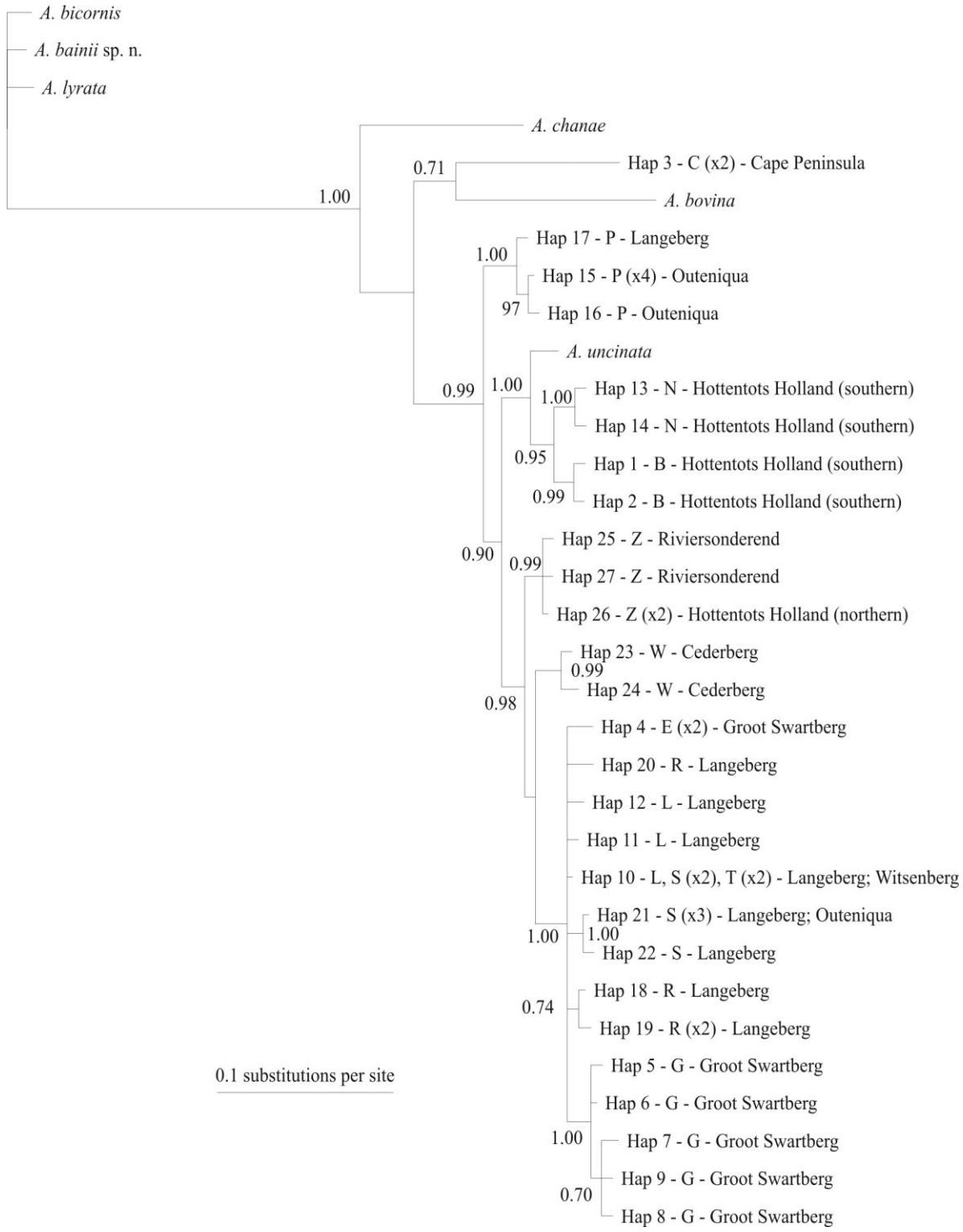


Fig. 3.13. Bayesian Inference majority rule consensus phylogram of 27 mtDNA COI haplotypes of the *Aphanicerca capensis* species complex, with six congeneric outgroup species of which one was used as the root. The model of nucleotide substitution used was GTR + I + gamma. Posterior probabilities are given. Where more than one individual share a haplotype, this in indicated in parentheses. The morphogroup and mountain range locality are given to the right of the haplotype number.

Population level analysis

Haplotypes and diversity

Parsimony informative sites numbered 63, and there were 74 variable sites (Table 3.11). There were 77 mutations of which 76 were synonymous (see above). Mutations did not deviate from selective neutrality (Fu & Li, 1993. D* = 0.70273, $P > 0.1$; F* = 0.11590, $P > 0.1$). The transition to transversion ratio was nine to one. The 40 *A. capensis* species complex individuals sampled comprised 27 haplotypes, of which 20 were unique, and seven were shared (Tables 3.12-3.14). Haplotypes 3, 4, 19 and 26 were shared by two individuals, haplotype 21 by three, haplotype 15 by four, and haplotype 10 by five. All haplotypes were unique to their morphogroups except for haplotype 10 which was shared by three morphogroups, namely ***L***, ***S*** and ***T***, of which the first two are syntopic at Kristalkloof in the Langeberg range. Three haplotypes were found in more than one mountain range each. Haplotype 10 (two individuals of morphogroup ***S***, one of ***L*** and two of ***T***) was found in the Langeberg and Witsenberg ranges which are geographically close, separated only by the narrow aspect of the Hex River Mountains. Haplotype 21 (both individuals of morphogroup ***S***) was found in the Langeberg and Outeniqua ranges which are contiguous. Haplotype 26 (both individuals of morphogroup ***Z***) was found in the Groot Drakenstein Mountains (Stellenbosch) and the Limietberge (Bain's Kloof) which are geographically close with only mountainous terrain separating the two localities and were grouped together for the analysis as the northern Hottentots Holland Mountains.

Table 3.14. Frequency distribution of the 27 COI haplotypes amongst the 12 sampled morphogroups (MG).

	Haplotypes																											
MG	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
B	1	1																									
C			2																								
E				2																							
G					1	1	1	1	1																		
L										1	1	1															
N													1	1													
P															4	1	1										
R																		1	2	1							
S										2											3	1					
T										2																	
W																							1	1			
Z																									1	2	1

Genetic diversity indices are provided in Table 3.15. Nucleotide diversity was zero for three morphogroups, but the sample size in each was low (two). In general, haplotype diversity was

high, with only three morphogroups having a single haplotype, and all of those had a sample size of only two. Nucleotide diversity among the other nine morphogroups ranged from 0.00185 ± 0.00164 (SD) to 0.00626 ± 0.00479, with few obvious trends identified. Morphogroups ***P***, ***S*** and ***Z*** had the widest geographical distributions and the lowest nucleotide diversities (not including the morphogroups of sample size two where the diversity is zero).

Genetic distance

Analysis of genetic structure showed that 91% of genetic variation was due to among population (morphogroup) haplotype differences, and 9% within population (AMOVA, F_{ST} = 0.91035, P = 0.0000 ± 0.0000). Population pairwise F_{ST} values showed significant differentiation for 29 out of 66 morphogroup pairs (Table 3.16) (Pairwise differences are given in Tables 3.16 and 3.17). Greater sample numbers would have probably increased the number of significant differences. Of the syntopic morphogroups, the pairs ***S*-*P*** (Langeberg) (F_{ST} and exact test), and ***L*-*P*** (Langeberg) (F_{ST} only) were genetically significantly different ($P < 0.05$), but not surprisingly with a shared haplotype, ***S*-*L*** (Langeberg) was not significantly different ($P > 0.05$). Morphogroup ***Z*** was syntopic with ***B*** at Betty's Bay (Table 3.9), but was not sampled for mtDNA. Comparing ***B*** with the other localities of ***Z***, there was no significant difference. Of the sympatric morphogroups, the pairs ***E*-*G*** (Groot Swartberg) (F_{ST}), ***S*-*P*** (Outeniqua) (F_{ST} and exact test), ***S*-*R*** (Langeberg) (F_{ST} and exact test) and ***P*-*R*** (F_{ST} and exact test) were significantly different, but ***B*-*N*** (Hottentots Holland southern) and ***L*-*R*** (Langeberg) were not.

Genetic distance was not correlated to morphology (matrix correlation analysis Mantel test, uncorrected distance: P = 0.474, r = 0.036, NS; corrected distance: P = 0.221, r = 0.034, NS). However, it showed a significant positive correlation to geographic distance (Mantel test, P = 0.000, r = 0.355 for both corrected and uncorrected distances).

Statistical parsimony phylogeographic structure

The statistical parsimony network (Fig. 3.14a) excluded morphogroups ***C*** (Cape Peninsula), ***B*** (Betty's Bay, southern Hottentots Holland), and ***N*** (Hermanus, southern Hottentots Holland) at the 95% confidence level. These groups required more than 10 mutational steps to join to the main network. Morphogroup ***C*** formed its own network (Fig. 3.14a), while ***B*** and ***N*** were separated from each other by seven mutational steps in a third network. In terms of the number of mutational steps in the main haplotype network diagram (Fig. 3.14a), there were four main groups of closely related haplotypes. Three of these were reciprocally monophyletic, namely ***Z*** (haplotypes 25, 26, 27)), ***P*** (15, 16, 17), and ***W*** (23, 24). The fourth group showed closer affinities between morphogroups ***G*** (5, 6, 7, 8, 9), ***L*** (10, 11, 12), ***S*** (10, 21, 22), ***T*** (10), ***R*** (18, 19, 20) and ***E*** (4). Within that fourth group, ***G*** formed a monophyletic clade three mutational

Table 3.15. Genetic diversity indices (COI mtDNA) for the 12 morphogroups of the *A. capensis* species complex sampled, using Tamura-Nei distance with a gamma distribution shape parameter α = 0.145. MG = morphogroup.

MG	Localities	Mountain range/s	*n*	No. of haplo-types	Haplotype diversity ± 1 S.D.	Mean number of nucleotide pairwise differences ± 1 S.D.	Nucleotide diversity x 10-3 ± 1 S.D.
B	1. Betty's Bay	Hottentots Holland Mts (southern)	2	2	1.0 ± 0.5	2.12 ± 1.82	3.80 ± 4.61
C	1. Slangolie Ravine 2. Boschenheuwel Arboretum (Kirstenbosch)	Cape Peninsula Mountain Chain	2	1	0	0	0
E	1. Boegoekloof (Swartberg Pass)	Groot Swartberg	2	1	0	0	0
G	1. Malvadraai (Swartberg Pass) 2. Oudemuragie 3. Seweweekspoort	Groot Swartberg	5	5	1.0 ± 0.13	2.73 ± 1.73	4.91 ± 3.64
L	1. Kristalkloof (Riversdale) 2. Tradouw Pass	Langeberg	3	3	1.0 ± 0.27	2.06 ± 1.55	3.70 ± 3.47
N	1. Fernkloof (Hermanus)	Hottentots Holland Mts (southern)	2	2	1.0 ± 0.5	2.06 ± 1.78	3.70 ± 4.51
P	1. Bergplaas (Knysna) 2. Montagu Pass (George) 3. Kristalkloof (Riversdale) 4. Prince Alfred's Pass (Knysna)	Outeniqua Mts; Langeberg	6	3	0.60 ± 0.22	1.03 ± 0.79	1.85 ± 1.64
R	1. Bergheim (Montagu-Barrydale) 2. Ravenna (Montagu-Barrydale)	Langeberg	4	3	0.83 ± 0.22	3.49 ± 2.23	6.26 ± 4.79
S	1. Gouna (Knysna) 2. Montagu Pass (George) 3. Kristalkloof (Riversdale) 4. Cloete's Pass	Outeniqua Mts; Langeberg	6	3	0.73 ± 0.16	1.44 ± 1.01	2.59 ± 2.10
T	1. Witsenberg Game Farm	Witsenberg	2	1	0	0	0
W	1. Sneeuberg 2. Algeria south road	Cederberg	2	2	1.0 ± 0.5	3.15 ± 2.56	5.65 ± 6.49
Z	1. Jonkershoek (Stellenbosch) 2. Bain's Kloof Pass 3. Oubos (Riviersonderend)	Hottentots Holland Mts (northern); Riviersonderend Mts	4	3	0.83 ± 0.22	1.04 ± 0.85	1.86 ± 1.83

Table 3.16. Above diagonal: Population pairwise F_{ST} values. * indicates significance at $P < 0.05$. Shaded blocks indicate significant pairwise differences according to the exact test of differentiation (null hypothesis is panmixia). Below diagonal: corrected average pairwise difference (PiXY-(PiX+PiY)/2). Distance measure: Tamura-Nei with α = 0.145.

	B	***C***	***E***	***G***	***L***	***N***	***P***	***R***	***S***	***T***	***W***	***Z***
B		0.99	0.96	0.91*	0.91	0.73	0.94*	0.87	0.93*	0.95	0.87	0.93
C	79.86		1.00	0.97*	0.98	0.99	0.99*	0.96	0.98*	1.00	0.98	0.99
E	27.21	63.97		0.75*	0.73	0.95	0.96*	0.61	0.80*	1.00	0.88	0.94
G	27.08	64.53	6.92		0.59*	0.90*	0.93*	0.6*	0.7*	0.59	0.82*	0.86*
L	21.23	63.19	3.94	3.59		0.90	0.93*	0.18	0.36	-0.21	0.75	0.84*
N	5.62	78.70	21.79	23.66	18.04		0.94*	0.85	0.93*	0.94	0.84	0.93
P	18.61	65.58	23.73	24.35	18.25	18.42		0.91*	0.93*	0.95*	0.93*	0.91*
R	21.89	63.77	4.65	4.47	0.71	18.03	18.69		0.43*	0.04	0.71	0.78*
S	20.14	60.16	5.22	4.65	0.86	18.97	17.39	1.59		0.31	0.84*	0.85*
T	20.68	61.52	4.23	3.69	0.00	17.60	17.83	0.65	0.83		0.83	0.91
W	17.28	61.56	11.68	12.37	7.30	14.16	17.69	8.43	9.02	7.89		0.82
Z	16.87	60.05	12.64	12.33	7.80	16.66	10.59	8.09	7.09	7.62	6.89	

Table 3.17. Above diagonal: Average number of pairwise differences between populations (PiXY). Diagonal: Average number of pairwise differences within populations (PiX). Below diagonal: Corrected average pairwise difference (PiXY-(PiX+PiY)/2). Distance measure: Pairwise difference.

	B	***C***	***E***	***G***	***L***	***N***	***P***	***R***	***S***	***T***	***W***	***Z***
B	2.00	41.00	21.00	21.20	18.00	7.00	16.17	18.75	17.17	17.00	15.50	14.50
C	40.00	0.00	37.00	37.80	37.00	41.00	37.17	37.25	36.17	36.00	35.50	35.50
E	20.00	37.00	0.00	7.40	4.67	18.00	19.17	5.75	5.50	4.00	11.50	11.50
G	18.90	36.50	6.10	2.60	5.40	19.20	19.77	6.75	6.10	4.60	12.70	12.10
L	16.00	36.00	3.67	3.10	2.00	16.00	16.17	3.25	2.50	1.00	8.83	8.50
N	5.00	40.00	17.00	16.90	14.00	2.00	16.17	16.50	16.50	15.00	13.50	14.50
P	14.67	36.67	18.67	17.97	14.67	14.67	1.00	16.92	15.33	15.17	15.67	10.17
R	16.17	35.67	4.17	3.87	0.67	13.92	14.83	3.17	3.75	2.25	10.25	9.25
S	15.47	35.47	4.80	4.10	0.80	14.80	14.13	1.47	1.40	1.50	10.00	7.67
T	16.00	36.00	4.00	3.30	0.00	14.00	14.67	0.67	0.80	0.00	8.50	7.50
W	13.00	34.00	10.00	9.90	6.33	11.00	13.67	7.17	7.80	7.00	3.00	8.00
Z	13.00	35.00	11.00	10.30	7.00	13.00	9.17	7.17	6.47	7.00	6.00	1.00

steps from haplotype 10. Not included in the main network, morphogroup ***C***, the type species *Aphanicerca capensis* from the Cape Peninsula, was 30 mutational steps from the main network. Also forming a separate haplotype network, morphogroups ***B*** and ***N*** were separated from each other by seven steps and from the main network by 11 steps, forming two reciprocally monophyletic clades.

Nested clade analysis

Contrary to the simple reading of the haplotype network (Fig. 3.14a) which suggests that haplotypes 21 and 22 were more closely related to haplotype 10 than to 26, the NCA included clade 2-5 (haplotypes 21 and 22) in clade 3-3 (and hence 4-2) and not in clade 3-2 (4-1) (Fig. 3.14a,b). This occurred because clade 2-5 was symmetrically stranded during the nesting procedure and was therefore nested with the clade (2-6) with the smaller sample size (see rules in Templeton & Sing 1993). The reason for this (Crandall 1996) is to provide greater sample numbers within and among clades for hypothesis testing, and therefore not because of a closer relationship to clade 3-3 than to clade 3-2. Clade 2-5 was in fact only two steps from clade 3-2, and seven steps from clade 2-8 in clade 3-3. Including clade 2-5 within clade 3-2 instead of within clade 3-3 is more intuitive, and would provide a more harmonious result, as all the members of the ***S*** morphogroup would be nested within the same clade, namely 3-2. This latter approach is in agreement with the BI phylogram (Fig. 3.13), the second MP cladogram (Fig. 3.11) and the strict consensus MP cladogram (Fig. 3.12) which all included clade 2-5 as part of the clade 4-1 polytomy. However, following the rules of Templeton & Sing (1993), the nesting of these two haplotypes (21 and 22) within clade 3-3 is in agreement with the ML and one MP tree (Fig. 3.10). It is clear from all the trees and the network that most of the morphogroups formed monophyletic clades, albeit with short branch lengths, with the exception of ***R***, ***S***, and ***L***. ***T*** was represented by only one haplotype which was shared with ***S*** and ***L***. All the phylogenetic trees showed a sister group relationship between morphogroup ***C*** (*A. capensis sensu strictu*) and *A. bovina*. Also, *A. uncinata* from the Hottentots Holland Mountains was the sympatric sister group to the morphogroups ***B*** and ***N***. Both of these groups (***C*** and ***B*** / ***N***) that were sister to previously recognized and morphologically divergent species, were not part of the main network at the 95% confidence level.

Permutation contingency analyses showed that in only three instances could the null hypothesis of no association between the nested clades and geographic location be rejected, namely in clades 2-3, 4-1, and the total network (Table 3.18).

Table 3.18. Nested clade permutation contingency test, and inference key steps and conclusions from geographic distance analysis. Only clades with significant ($P < 0.05$) geographic structure are shown. Clades 3-3 and 4-2 contingency analysis chi-square statistics were not significant.

Clade	Chi-square	Probability	Inference key steps	Inferred historical pattern
2-3	10.00	0.034	1-19-no	Allopatric fragmentation
3-3	24.00	0.090	1-19-20-2-11-12-13-yes	Past gradual range expansion followed by fragmentation
4-1	18.00	0.008	1-19-no	Allopatric fragmentation
4-2	13.16	0.213	1-2-3-4-no	Restricted gene flow with isolation by distance
Total cladogram	30.79	0.001	1-2	Inconclusive outcome

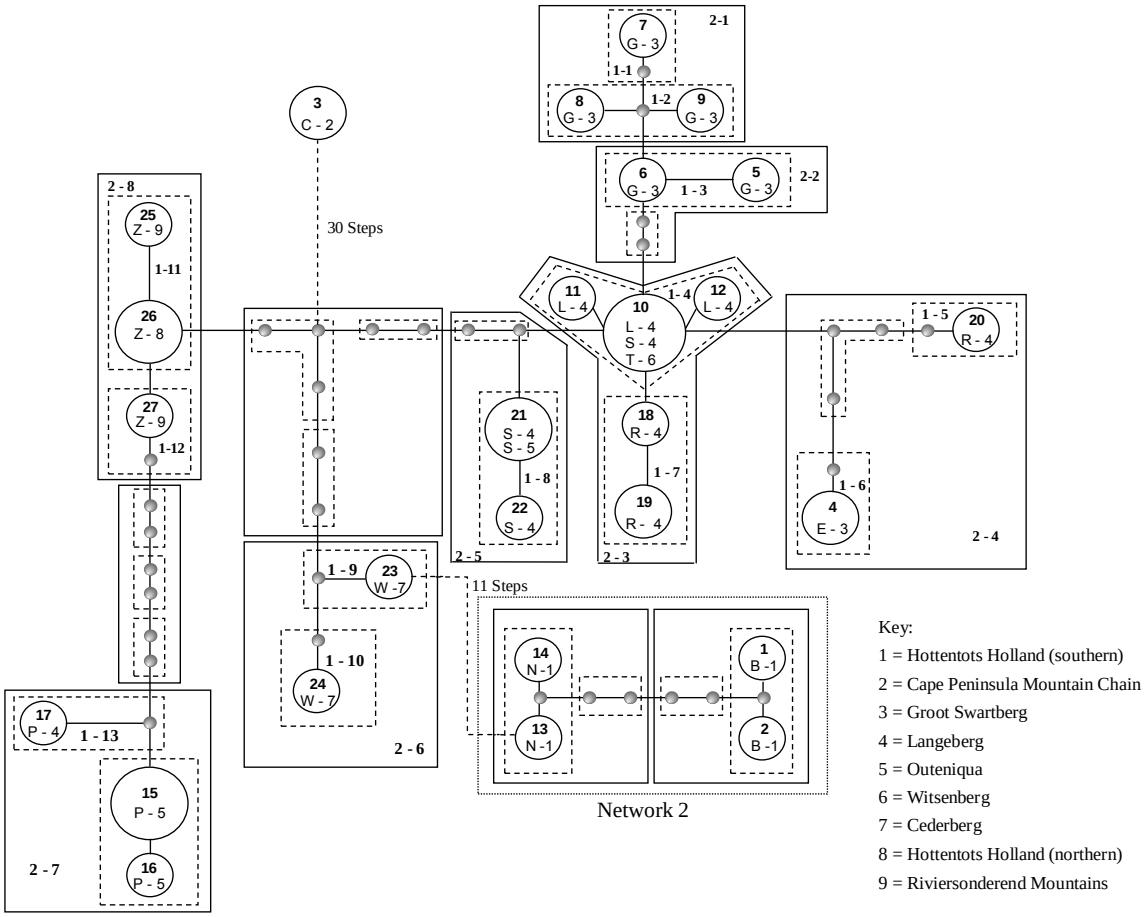
Table 3.19. Results of the Geodis cladistic nested analysis of the geographical distribution of *A. capensis* species complex haplotypes. Clade distance (D_C) which measures the geographical range of a clade, and nested clade distance (D_N) which measures the average distance of a clade from the average geographic centre of all clades nested within the immediate higher clade, are given. The interior-tip statistic (I-T) which represents the average distance between interior and tip clades, is given where appropriate. This mainly corresponds to a young-old contrast. Significantly large or small values ($P < 0.05$) are indicated by an **L** or **S** respectively.

Nesting clades	Clade number	Clade location	D_C	D_N	Nesting clades	Clade number	Clade location	D_C	D_N
1-2	8	Tip	0.0	20.1	2-4	1-5	Tip	0.0	111.8
	9	Tip	0.0	10.1		1-6	Tip	0.0	56.0
1-3	5	Tip	0.0	1.1	2-6	1-9	Interior	0.0	2.4
	6	Interior	0.0	1.1		1-10	Tip	0.0	2.4
	I-T		0.0	0.0		I-T		0.0	0.0
1-4	10	Interior	92.4	101.8	2-7	1-13	Interior	0.0	135.1
	11	Tip	0.0	43.2		1-14	Tip	25.7	23.2
	12	Tip	0.0	86.5		I-T		-25.7	111.9
	I-T		92.4	37.0	2-8	1-11	Interior	33.1	37.2
1-7	18	Interior	0.0	1.1		1-12	Interior	0.0	53.4
	19	Tip	0.0	0.5	3-1	2-1	Tip	15.1	37.8
	I-T		0.0	0.5		2-2	Interior	1.1	37.2
1-8	21	Interior	51.6	53.4		I-T		-14.0	-0.6
	22	Tip	0.0	64.2	3-2	2-3	Interior	55.4	60.2
	I-T		51.6	-10.8		2-4	Tip	74.6	102.6
1-11	25	Tip	0.0	64.1		I-T		-19.2	-42.4
	26	Interior	19.7	25.3	3-3	2-5	Interior	55.7 **S**	207.5 **L**
	I-T		19.7	-38.8		2-6	Tip	2.4	157.4
1-14	15	Interior	24.9	24.2		2-8	Interior	39.9 **S**	105.0 **S**
	16	Tip	0.0	35.6		I-T		45.4	-1.2
	I-T		25.0	-11.4	4-1	3-1	Tip	37.5 **S**	69.6
2-1	1-1	Tip	0.0	15.1		3-2	Interior	71.2	88.7
	1-2	Interior	13.4	15.1		I-T		33.6	19.1
	I-T		13.4	0.0	4-2	3-3	Interior	151.9	168.0
2-3	1-4	Interior	79.8 **L**	78.6 **L**		3-4	Tip	31.0 **S**	165.8
	1-7	Tip	0.7 **S**	12.2 **S**		I-T		120.9 **L**	2.3
	I-T		79.1 **L**	66.5 **L**	Total	4-1	Tip	80.8 **S**	82.0 **S**
						4-2	Tip	167.4 **L**	167.2 **L**

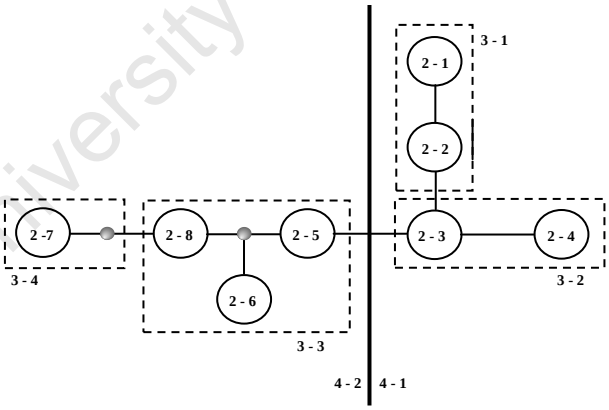
Distribution trends – clades and ranges

The two penultimate level clades of the NCA main network, clades 4-1 and 4-2, did not define mutually exclusive geographical areas. Clade 4-1 had a more northern biased distribution, uniting the Groot Swartberg, the Langeberg and the Witsenberg Mountains. The latter two are connected by mountainous terrain, but the Groot Swartberg is separated from the Langeberg by the inhospitable Little Karoo. There are however, higher lying areas at the eastern extreme of the Langeberg, providing a plausible route for gene flow between the two mountains. Clades 4-2 and 4-1 shared the Langeberg in common. Clade 4-2 had a more southern distribution in the Outeniqua, Langeberg, northern and southern Hottentots Holland, and Riviersonderend Mountains, but also a disjunct (relative to the rest of the clade) population in the Cederberg. The zero-step clade, haplotype 3, occurred only on the Cape Peninsula in the extreme south west, while network 2 (Fig. 3.14a) was confined to the southern Hottentots Holland.

Clade 1-4 had a significantly large range and clade 1-7 a significantly small range relative to each other within clade 2-3 (Table 3.19). Clade 1-4 included haplotypes from the Langeberg and Witsenberg Mountains (Fig. 3.1), and clade 1-7 had been collected from a small area in the Langeberg. The significant I-T value indicated that clade 1-7 is younger (and possibly contains rarer haplotypes) than clade 1-4. ***L*** and ***S*** are sympatric (probably parapatric), but not syntopic, with ***R*** in the Langeberg. These results suggest, concurring with conclusions from the inference key (Templeton 1998 and version of 11 November 2005 downloaded at http://darwin.uvigo.es) (Table 3.18), that allopatric fragmentation has occurred with haplotype 10 being ancestral. This putative ancestral or oldest of the recovered haplotypes lies geographically near the centre of the four montane regions defined earlier, which lends weight to the idea of ancestral status. Clades 2-5 and 2-8, nested within clade 3-3, had much smaller ranges, the former in the eastern Langeberg and the Outeniqua Mountains and the latter in the Riviersonderend and northern Hottentots Holland Mountains. Clade 2-6, also within clade 3-3, also had a narrow distribution in the Cederberg. These three clades had non-overlapping ranges and represented three distinct morphogroups. Clade 3-1 nested within clade 4-1 had a significantly narrow geographical distribution, with all individuals occurring in the Groot Swartberg Mountains, and all representing the same morphogroup. The inference key attributed the structure of clade 4-1 to allopatric fragmentation (Table 3.18). Like clade 3-1 (4-1), clade 3-4 (4-2) had a significantly narrow range, with all individuals being from the same morphogroup, and occurring in the Langeberg and Outeniqua Mountains. The significant I-T value for clade 4-2 provided evidence that clade 3-4 is a more recent lineage than clade 3-3. The historical process that led to the population structure of clade 4-2 is hypothesized (using the inference key) to be restricted gene flow with isolation by distance.



A



B

Fig. 3.14. Statistical parsimony network for the 27 *Aphanicerca capensis* species complex mtDNA COI haplotypes sampled, with the superimposed nested design. **A**, One-step clades are enclosed by a dashed box, and two-step clades by a solid box. The small solid circles represent unsampled or extinct haplotypes. Each line represents one mutational step. There are five sizes of open circles, each size representing a frequency of one to five individuals for that haplotype. The haplotype number is at the top of the circle. Underneath that, are the morphogroup letter to the left of the dash, and the mountain range group to the right of the dash, the key to which is at the right of the diagram. Haplotype 3 (the type species *A. capensis*) and network 2 are joined to the main network by dashed lines as they are not connected by statistically significant parsimonious connections at the 95% level which required ten steps. They were separated from the main network by 30 and 11 mutational steps respectively. Network 2 unites ***B*** and ***N*** into a three-step clade (dotted box). **B**, Higher level clades. Three-step clades are enclosed by dashed boxes, and the two four-step clades are separated by the vertical solid line.

In recent months, the literature on statistical approaches in the field of phylogeography has seen a number of important commentaries criticizing the use of Templeton's NCA (Knowles 2008; Petit 2008). At the heart of this debate is the incorrect inference of historical processes that erroneously account for the phylogeographic patterns of a data set (Knowles and Maddison 2002; Panchal and Beaumont 2007). On the one hand Petit (2008) advocates abandoning the approach until further evaluation, while Garrick *et al*. (2008) argue that NCA is a unique approach to studying evolutionary scenarios with no substitute, and should instead inspire practical approaches to validate or strengthen its inferences. Nonetheless, using data sets that simulate simple evolutionary scenarios, Panchal and Beaumont (2007) have clearly demonstrated the high failure rate of NCA to correctly infer a number of these evolutionary processes (see Templeton 2004 and 2008 for opposing viewpoint). The reality is that species have complicated histories. But methods that can effectively accommodate biological reality are currently thin on the ground; 'achieving a joint estimate of the multiple processes that characterize a species' history is difficult' (Knowles 2008). In this study I have used a total-evidence based approach to elucidate the evolutionary history and cryptic diversity of stonefly lineages within *Aphanicerca capensis*. The conclusions presented here are not drawn from the results of NCA alone; instead they are based on morphology, mate choice behaviour, the relationship between morphology and distribution, phylogeny and finally phylogeography. To quote Richard Zander (2007), a champion of the need for comparative evolutionary ecology in taxonomy and biodiversity discovery, 'Biodiversity investigated with molecular analysis alone is rendered a mere game by excessive atomization due to focus on neutral evolution and forced monophyly.'

DISCUSSION

Lines of evidence used to infer separately evolving metapopulation lineages of notonemourids in the *Aphanicerca capensis* species complex in this study, and expanded on below, include: allopatric fragmentation, genetic structure, intrinsic reproductive isolation in syntopic morphogroups, intrinsic reproductive isolation in sympatric morphogroups, intrinsic reproductive isolation inferred by complete premating isolation in experimental trials, intrinsic reproductive isolation inferred by unidirectional or incomplete premating isolation in experimental trials, phenetic distinctiveness, morphological diagnosability, reciprocal monophyly, and monophyly. These ten criteria as applied relationally to the *A. capensis* morphogroups are summarized in Table 3.20.

Allopatric fragmentation and speciation

Allopatric fragmentation (Templeton 1989, 2001) as inferred from the nested clade analysis (NCA) was likely to have promoted speciation between 11 of the possible 66 pairwise

comparisons of morphogroups (Table 3.20). The lack of correlation (Mantel tests) between genetic divergence and morphology, and between geographic distance and morphology, would suggest that isolated populations of the ancestral species radiated from the centre of origin with random mutations resulting in morphological divergence rather than selective changes, across temporal spatial clines from a centre of origin. Small population size would have resulted in rapid morphological and genetic divergence and hence diversification via genetic drift, and even more so in cases where selection plays a role. Genetic diversity however is correlated with geographic distance, suggesting restricted gene flow with isolation by distance (Finn & Adler 2006); this supports the similar conclusion obtained from the NCA with respect to clades nested within clade 4-2 (Fig. 3.14). This weakly positive Mantel test correlation then, is probably due to clade 4-2, as this was the only such result from the NCA. IBD within this clade means restricted gene flow between morphogroup ***P*** (Langeberg and Outeniqua Mountains) and morphogroups ***Z*** (northern Hottentots Holland and Riviersonderend Mountains), ***W*** (Cederberg) and ***S*** (Langeberg and Outeniqua Mountains). Because the known distributions of ***P*** and ***S*** are completely sympatric, some degree of ongoing gene flow may occur, but population-specific morphological characteristics could still be maintained if gene flow is negligible relative to the population size. The extent of inhospitable terrain between the Langeberg and the other mountain ranges involved is not great, such that periodic gene flow is possible although unlikely given the habitat specificity and low vagility of the group. Because there is support for both allopatric fragmentation and isolation by distance (with the positive correlation in the latter being weak), more sampling would aid in further elucidating historical processes that have influenced these lineages.

The inference key conclusion for clade 3-3 was that of a scenario of gradual historic range expansion followed by fragmentation. Because it is hypothesized that the Langeberg region is the centre of origin of the species complex, this range expansion probably occurred south-westwards from the Langeberg into the Riviersonderend and then into the northern Hottentots Holland Mountains, and north-westwards into the Cederberg. The inferred historical pattern for clades 2-3 and 4-1 was allopatric fragmentation. Fragmentation, with limited gene flow, is the NCA inference from which deductions of speciation can be made (Templeton 2001). The morphogroups in clade 3-3, ***W***, ***Z*** and ***S***, can therefore be given species status according to the unified concept based on the criterion of allopatric fragmentation. Similarly, morphogroup ***R*** in clade 2-3 is a species relative to ***L***, ***S*** and ***T***. The reason for allopatric fragmentation in clade 2-3, even though the two sub-clades 1-4 and 1-7 were both found in the Langeberg, is the very restricted distribution of ***R***. It is always possible that further sampling may change this inference. In clade 4-1, ***G*** is a species relative to ***L***, ***S***, ***T***, ***R*** and ***E*** (Table 3.20). Again, morphogroup ***E*** in the Groot Swartberg has a very restricted distribution (a single stream thus far) and was not

Table 3.20. A unified (general lineage) species concept approach to species delimitation in the *Aphanicerca capensis* species complex. Lines of evidence (criteria) are given (grey blocks) for each pairwise comparison between morphogroups listed in the first column. Symbols: ■ = allopatric fragmentation as inferred from nested clade analysis; □ = genetic differentiation as indicated by significant F_{ST} values; ● = intrinsic reproductive isolation (IRI) (syntopic); ○ = IRI (sympatric); ▲ = IRI (complete pre-mating isolation during mate choice trials); △ = IRI (incomplete pre-mating isolation during mate choice trials); ✚ = morphometric distinguishability (total number of pairwise significant variables between males given); ◆ = morphological diagnosability (MD): differences between males are obvious; ⦿ = MD: differences between females are obvious; ◇ = MD: differences between males are subtle; ⊡ = MD: differences between females are subtle; ✱ = reciprocal monophyly or monophyly in one morphogroup, in one or more cladograms. The table is ordered from top to bottom firstly by the total number of criteria (i.e. the number of grey blocks per pair), except for CW and CZ where criteria ▲ and △ were counted together as one criterion, and then by the number of morphometric variable differences (given in column ✚).

	■	□	●	○	▲	△	✚	◆	◇	⦿	⊡	✱
EG	■	■		■			8	■				■
SZ	■	■					7	■		■		■
PR		■		■			7	■		■		■
GS	■	■					7	■		■		■
GR	■	■					7	■		■		■
BZ			■		■		7	■			■	■
RS	■	■		■			6	■		■		
SW	■	■					5	■		■		■
PS		■	■				5	■		■		■
LP		■	■				4		■		■	■
LR	■			■			9	■		■		
CS		■					9	■		■		■
PZ		■					7	■			■	■
PW		■					7	■			■	■
GZ		■					7	■			■	■
EP		■					7	■			■	■
CW					■	■	7	■			■	■
BG		■					7	■			■	■
WZ	■						6		■		■	■
RZ		■					6	■		■		■
NP		■					6	■			■	■
ES		■					6	■		■		■
BP		■					6	■			■	■
BN				■			6		■		■	■
LZ		■					5	■			■	■
CZ					■	■	5	■			■	■
GL	■	■					4		■			■
CP		■					4		■	■		■
CG		■					4		■		■	■
NZ				■			3	■		■		■
NS		■					3	■		■		■
BS		■					3	■		■		■
CR							9	■		■		■
RT	■						8	■		■		
NR							8	■		■		■
EZ							8	■		■		■
ET							8	■			■	■
BT							8	■			■	■
BC							8	■			■	■
TZ							7	■			■	■
TW							7	■			■	■
BR							7	■		■		■
BL							7	■			■	■
NT							6	■		■		■
CT							6	■		■		■
CN							6	■			■	■
BW							6	■			■	■
LS			■				5	■		■		
GW		■					5		■			■
GN		■					5	■				■
BE							5	■			■	■
RW							4	■		■		■
ER							4	■		■		■
CL							4		■		■	■
PT		■					3		■			■
GP		■					3		■			■
GT	■						2		■			■
ST							8	■		■		
EL							8	■				■
CE							8	■				■
EW							7	■				■
EN							7	■				■
LW							6	■				■
LT							3	■		■		
LN							3	■				■
NW							2	■				■

found to be syntopic with morphogroup ***G***. Where other methods may indicate the likelihood of other morphogroups being good species, this would not necessarily contradict the findings of the NCA, as failure to reject the null hypothesis of all morphogroups being part of the same lineage does not mean that they are not separate lineages, as this result could be due to a lack of statistical power (Templeton 2001) via insufficient sampling. Morphogroup ***C*** (clade 0-3) was not connected to the main network (separated by 30 steps; exceeds the 95% confidence limit); because of its molecular divergence from the other morphogroups and its allopatric distribution it can be surmised that allopatric fragmentation was the process driving this structure. Morphogroups ***B*** and ***N*** were also found to exceed the 95% confidence limits (11 steps) and were also isolated from the main network.

Genetic structure across the Cape Folded Mountains

Genetic structure, in this case referring to differentiation between populations, was inferred from the statistical significance of population pairwise F_{ST} estimates (Tables 3.16, 3.20). Large pairwise values of F_{ST} suggest isolation between lineages and hence very little dispersal among streams (Hughes *et al*. 1999). Two of the three syntopic pairs for which data were available were significantly differentiated, along with four of the six sympatric morphogroup pairs. The genetic distances separating the syntopic morphogroups at Kristalkloof (Langeberg) were: ***S*** and ***P*** 2.69 / 3.25; ***L*** and ***P*** 2.69-3.05 / 3.25-3.80; and ***S*** and ***L*** 0.00-0.36 / 0.00-0.37 (Appendices 3.4 and 3.5; percentages given as uncorrected distance / corrected distance; a range is given where more than one haplotype was present). The shared haplotype of ***S*** and ***L*** at Kristalkloof suggests that differential rates of mutation may occur within the *Aphanicerca* genome, as there must be sufficient genetic differentiation to maintain reproductive isolation, i.e. the suite of nuclear genes responsible for reproductive isolation between ***S*** and ***L*** have diverged more rapidly than COI. The COI gene tree in this case does not mirror the species tree. Although in general terms the incidence of reproductive isolation corresponds with genetic divergence, the numerous contraventions of this trend negate sole use of genetic divergence in species delimitation (Ferguson 2002). The fact that morphogroups ***P***, ***S*** and ***Z*** have the widest geographical distributions together with the lowest nucleotide diversities (not including the morphogroups of sample size two where the diversity is zero) may indicate that a widely dispersed morphogroup has maintained its status over time through genetic stability, while less stable forms have diversified more rapidly via dispersal. This scenario can be tested with further detailed sampling. The *a priori* sorting of specimens into groups based on morphology is generally supported by the mtDNA data; principally the results from the global AMOVA where 91% and 9% of genetic variation was due to the distribution of between and within morphogroup variation respectively.

The evolution of reproductive isolation within *Aphanicerca capensis*

Reproductive isolation is the cornerstone of the Biological Species Concept (Mayr 1942, 1970), and generally contributed to 'good species' (Orr 2005). Any secondary criteria that demonstrate this condition between two morphogroups will further substantiate recognition of species distinction. Reproductively isolated lineages (Table 3.20) are inferred from the maintenance of morphological distinctiveness under syntopic (criterion of intrinsic syntopic reproductive isolation) and sympatric (criterion of intrinsic sympatric reproductive isolation) distributions of morphogroups, and from positive assortative mating (either complete, i.e. in both directions, or incomplete, i.e. unidirectional) in mate choice trials.

Mate choice trials are used to test for premating isolation (Mayr 1970), and in particular, isolation via ethological and/or mechanical mechanisms. In ethological isolation, potential mates meet but mating does not occur, whilst in mechanical isolation, mating is attempted but sperm is not transferred (Mayr 1970). The occurrence of postmating isolation was not tested for in this study and whilst it may provide further evidence for species status in future studies of this complex, it seems unlikely because postmating isolation mechanisms generally evolve at a slower rate than premating mechanisms (Coyne & Orr 1997). Mate choice trials, including assessments of fertility in subsequent generations, are the only way to verify the existence of allopatric sibling species in terms of reproductive isolation (Mayr 1970). The reproductive signal modalities employed by *Aphanicerca* are unknown, but drumming has been observed in *Aphanicerca* species (pers. obs.) in the laboratory; drumming was not, however, routinely observed during mate choice trials. Drumming, or vibrational intersexual communication, is a common mate locating behaviour in arctoperlarian stoneflies, and takes the form of rubbing, tapping, or tremulating the abdomen against wooded or leafy substrates (Abbott & Stewart 1997). Mating on rock surfaces is extremely common (pers. obs.) and therefore drumming cannot be the only form of communication, a conclusion which is also intuitive given that this behaviour is not found in all stonefly taxa. The mate communication system of *Aphanicerca* needs to be investigated thoroughly, as it may provide characters that are phylogenetically informative (Stewart & Zeigler 1984). What was unexpected in this study were the incorrect pairings in control group 1 (one mating of *A. capensis* complex ***C*** male with an *A. bicornis* female) and trial 4 (one mating of *A. capensis* complex ***B*** male with an *A. capensis* complex ***Z*** female, two morphogroups that are syntopic). It may be that the artificial conditions of the laboratory and the lack of escape routes for females may account for these errors. It may be that occasional inapt matings occur in nature, but postmating isolation mechanisms are sufficiently developed. It is reasonable to attribute some of the cases of negative assortative mating in trials 1, 2 and 3 to the same causes as in control 1 and trial 4.

Intrinsic reproductive isolation (syntopic morphogroups)

Of the 12 morphogroups, those that are syntopic can be designated as separate biological species relative to each other at each respective locality (Table 3.20). It will be instructive to examine the differences between these reproductively isolated syntopic groups (this is done in Appendix 3.6). Because they occur in the same stream and the defining characters remained constant within each morphogroup (lack of intermediates), ***P***, ***S***, and ***L*** (Kristalkloof, Langeberg) are all unique species relative to each other as are ***B*** and ***Z*** (Betty's Bay, southern Hottentots Holland). ***P*** and ***S*** are also syntopic at Bergplaas north of Knysna (Outeniqua Mountains). It is possible that morphogroup ***P*** from the Langeberg may be a different species from the Outeniqua population, but further molecular, morphological and mate choice data are needed to fully address this question.

Intrinsic reproductive isolation (sympatric morphogroups)

The three species ***S***, ***P*** and ***L*** are sympatric in the Langeberg together with morphogroup ***R*** (Fig. 3.1B-D; Table 3.9). In addition, ***N*** is sympatric with ***B*** and ***Z*** in the southern Hottentots Holland Mountains, and ***E*** and ***G*** are sympatric in the Groot Swartberg (Fig. 3.1B-D; Table 3.9). While syntopic species pose no problem in their delimitation, sympatric (same mountain but different stream) groups pose a greater challenge. Data on additional sympatric species and levels of vagility will also be very useful in dissecting this challenge. Although notonemourids are not very efficient fliers and consequently long distance dispersal is considered rare (e.g. Schultheis *et al*. 2002), they may occasionally move between nearby catchments (Macneale *et al*. 2005), given suitable habitat. Dispersal, however, would be limited by their susceptibility to desiccation. A number of the South African notonemourids have very restricted distributions, being found in single streams, while others occur on multiple mountain ranges. For example, the notonemourids *Aphanicercella flabellata* Stevens & Picker (Stevens & Picker 1999) and *Aphanicercella clavata* Stevens & Picker (a new locality record in Bain's Kloof is yet to be critically examined) occur on the Cape Peninsula and in the northern Hottentots Holland Mountains, while *Aphanicercopsis denticulata* (Barnard 1934) has a wide distribution including the Cape Peninsula, southern and northern Hottentots Holland and Groot Swartberg Mountains. Within the *A. capensis* species complex, a number of morphogroups such as ***S***, ***P*** and ***Z*** have wide distributions, indicating some degree of dispersal ability. Additionally, *Aphanicercella cassida* occurs in the Mpumalanga Drakensberg range in the north of the country, as well as in the Eastern and Western Cape Provinces, although a detailed morphological study is still required to determine if these populations are conspecific. Where sympatric or parapatric populations showed both little morphological and genetic differentiation, they are likely to be geographical variants. Similarly, where sympatric or parapatric populations showed consistent and appreciable morphological and/or genetic variation, it is likely that they are separate species.

Difficulties arise because morphological similarity in the face of genetic divergence may indicate sibling (cryptic) species, while the converse may indicate incomplete lineage sorting and/or mitochondrial introgression. The subjective assessment of what is "appreciable" in terms of morphological and genetic difference may also be aided by comparison with congeners or other species within the family of interest.

Intrinsic reproductive isolation (complete premating isolation in experimental trials)

Complete premating isolation (Table 3.20) in the mate choice trials occurred only in trial 4 between the syntopic pair ***B*** and ***Z*** from Betty's Bay, and in the two controls (Table 3.10). Because the two syntopic populations are clearly distinct in nature and therefore do not interbreed to an appreciable extent, this mate choice trial also served as a control, and highlighted that the stress and artificial conditions of the experiment did not result in random mating. Complete premating isolation is clearly indicative of separately evolving lineages. It can be hypothesized, and remains to be tested, that because ***B*** was morphologically and genetically most closely related to sympatric morphogroup ***N***, their most recent common ancestor was widespread in the southern Hottentots Holland area, with allopatric fragmentation leading to a scenario of vicariant speciation. Similarly, the southern Hottentots Holland ***Z*** may have spread to Betty's Bay via range expansion from the northern Hottentots Holland. In that way, ***B*** and ***Z***, already reproductively isolated, became sympatric.

The molecular results provided additional evidence that the characters used to define the species complex did indeed delimit a compact group within the genus, with two exceptions which are at odds with morphological relationships, namely *A. bovina* and *A. uncinata*. The type species, *A. capensis* (morphogroup ***C***), was the sister group to *A. bovina* in all the cladistic analyses (Figs 3.10-3.13) rather than to the rest of the morphologically defined species complex. ***C*** and *A. bovina* are allopatric and morphologically highly divergent; the former on the Cape Peninsula and the latter in the northern Hottentots Holland region. Recurrent gene flow is therefore extremely unlikely. A possibility is that the anomaly was due to relatively (relative to nuclear genes) high levels of homoplasy found in mtDNA, largely due to the high A/T bias in third codon positions (Lin & Danforth 2004), as was found in this study. The mate choice trials (Table 3.10) between *A. capensis* morphogroup ***C*** and *A. bovina* which according to the phylogenetic analyses (Figs 3.10-3.13) are more closely related than to any other morphogroup, showed positive assortative mating when *A. bovina* males were faced with a choice of ***C*** and *A. bovina* females; the result was no inappropriate pairings. But, morphogroups ***Z*** and ***C*** showed random mating between ***Z*** males and ***Z*** and ***C*** females. Likewise, morphogroups ***W*** and ***C*** showed random mating between ***W*** males and ***W*** and ***C*** females. These findings show that ***C*** is more closely related to ***Z*** and ***W*** than to *A. bovina* and hence are contradictory findings to the

COI relationships. Therefore, at least in the case of the relationship between morphogroup ***C*** and *A. bovina* and based on morphological and reproductive data, the COI gene tree and the species tree were again incongruent.

Intrinsic reproductive isolation (unidirectional or incomplete premating isolation in experimental trials)

The incomplete premating isolation results from the mate choice trials (Table 3.10), in which morphogroup ***C*** males mated preferentially with ***C*** females versus non-***C*** females while non-***C*** males mated randomly, suggest that either ***C*** males or non-***C*** females are responsible for mate discrimination. Because this result occurred in three different populations, namely ***Z*** (Bain's Kloof), ***Z*** (Stellenbosch) and ***W***, it is more likely that it is the ***C*** male that is selecting correctly. Further research, however, is needed to determine if indeed it is the male who is responsible for mate selection in *A. capensis*, as the trials carried out in this study were not designed for that purpose. It is also likely that a degree of interplay between the sexes may occur during mating encounters.

The experimental set-up used in this study was somewhat unnatural, testing for isolation among two allopatric populations between which there is presumably no recurrent gene flow in their natural environment. ***Z*** males (or an interplay between the sexes) cannot differentiate between their own and an allopatric morphogroup, but under natural conditions could presumably differentiate between themselves and two other sympatric morphogroups, namely ***B*** and ***N*** in the southern Hottentots Holland Mountains (although ***Z***-***N*** trials were not conducted, and they have not been found syntopically yet). Moreover, there was greater genetic distance between ***Z*** and ***C*** than there was between ***Z*** and ***B***, and ***Z*** and ***N*** and suggests that COI genetic distances are unlikely to be correlated with reproductive isolation. This conclusion is corroborated by additional evidence from Kristalkloof in the Langeberg where syntopic ***S*** and ***L*** shared a haplotype (probably due to retention of an ancestral polymorphism, but mitochondrial introgression is possible, if not likely, in syntopic species). The results from this study suggest that mating trials between additional morphogroups within this species complex may also result in incomplete or lack of isolation; the most likely cause of this is insufficient time for the full development of isolating mechanisms. It is interesting that incomplete premating isolation was observed between morphogroups with a 6.37% genetic divergence i.e. ***C*** and ***W*** or ***Z***, whilst presumed complete reproductive isolation (whether pre- or post-zygotic is unknown) occurred between syntopic morphogroups with 0% divergence (***L*** and ***S***). This presumed complete reproductive isolation may of course not be complete, but any ongoing gene flow most likely occurs at low enough levels to maintain morphological distinctiveness of the respective morphogroups.

It is likely that a degree of neutral drift characterizes the ***C***, ***W*** and ***Z*** populations. Similarly in *Drosophila*, high levels of prezygotic isolation were found in sympatric species where genetic differentiation approached near to zero (Coyne & Orr 1997). In that study, prezygotic isolation was high between sympatric populations, but not significantly different from postzygotic isolation between allopatric populations (Coyne & Orr 1997). In this study, premating isolation in experimental trials was effective in naturally sympatric populations, and incomplete in naturally allopatric populations; these results are similar to those reported in carabid beetles (Usami *et al*. 2006) where it is suggested to be due to either reinforcement or, more likely, to reproductive character displacement after sympatric contact. As with the beetle study (Usami *et al*. 2006), no relationship was found between genetic distance and premating isolation. Wishart (2002), in a study incorporating *A. capensis* morphological variants, concluded that although morphological and genetic data supported the delimitation of separate species, mate choice results of incomplete premating isolation (Roos, unpubl.; Stevens – this study) necessitated that the three varieties (Table Mountain (***C*** in the present study), Jonkershoek and Bain's Kloof (***Z***), and Garcia's Pass and Swellendam (***L***)) should not be considered as separate species, but rather as evolutionary significant units. Incomplete isolating mechanisms are, however, a common occurrence in incipient species, and from the viewpoint of the Biological Species Concept, speciation can be achieved even if these mechanisms are not yet complete (Mayr 1970). Hybridisation is common in closely related species and can obscure the discovery of evolutionary relationships (Wheeler & Platnick 2000; Usami *et al*. 2006; Nagata *et al*. 2007). For this reason, incomplete premating isolation in this study was regarded as a line of evidence for incipient or recent speciation. Limited introgression may occur in the syntopic and sympatric populations, but likely at low enough levels not to impact on morphological and organismal divergence.

Phenetic distinctiveness

Morphological comparisons usually comprise one or more presence / absence data descriptors encompassing shape, colour, sclerotization patterns, meristic data, linear morphometrics and landmark methods; the use of these descriptors depends on the applicability of the method to the group under study. Morphological lineages in this study were inferred using a morphological phenetic distinguishability criterion following Sneath & Sokal (1973) and de Queiroz (2007) using significant linear morphometric variation (from the MANOVA analysis, Table 3.3) (Table 3.20). Geographic morphological variation of spatially disjunct populations within a species is a well known phenomenon, but does not necessarily confer species status as strict adherence to a typological species concept would demand. Morphological approaches, on the other hand, do take morphological variation into account. Morphologists define their taxa taking into account individual variation, even if single specimens are available (Cracraft 2000).

Indeed, morphological variation between populations is the norm. Mayr (1970), states that no demes can ever be identical. Naturally, the question becomes one of how much variation is permissible before the species boundary is crossed? That is not a question that can be answered objectively in isolation, and would be decided on a case by case basis by examining the group as a whole. Deciding species boundaries is aided then by other lines of evidence, such as DNA lineage sorting, behavioural diversification, assortative mating, and ecological differentiation. Although a morphological concept of species has recently taken a back seat with the rise of molecular methodology, morphology will always retain a vital role in the philosophy of species concepts and in their practical applications. Using morphology to establish evolutionary relationships requires the use of synapomorphies to determine sister group relationships, but for taxonomic purposes similarity and dissimilarity of morphological structures is usually sufficient for species delineation. Morphology and character analysis, as integral and indispensable parts of species delimitation, are set to regain their rightful place in systematics (Wheeler 2007). Indeed, most molecular phylogenetic studies commence with a morphologically defined taxon. Like other criteria, morphology on its own is inefficient for species definition, but will always be an indispensable component of any operational approach to delimiting species. As in molecular systematics, morphological systematics is beset with the problems of disagreements about criteria and classification (subjectivity), along with the difficulty of passing intuitive expertise on to subsequent generations (Hull 1997).

The morphogroups in this study were primarily distinguishable when using the chosen variables because among-population variation was greater than within-population variation. The variables proved to be successful, in that they all were able to discriminate between some morphogroups, although no variable could distinguish between all morphogroups (Tables 3.2, 3.3). Examining the number of times a variable distinguished between two groups in the MANOVA, the PC1 and PC2 component loadings and the DFA partial Wilks' lambdas, the variables with the greatest discriminating power among the methods were dp, sp, and adp; these are all characters of the tergite 9 dorsal process which was therefore the most useful single distinguishing structure. This process, however, was not useful in discriminating between morphogroups ***G***, ***C***, and ***P***, between ***T*** and ***P***, between ***W*** and ***R***, and between ***Z*** and ***R*** (Table 3.3). Because the morphogroups within each of these four groups are allopatric with respect to each other, the dorsal process alone could be used to distinguish morphogroups within each mountain range for all known morphogroups and their distributions. This of course applies based on current taxonomic sampling. The general trend of syntopy or sympatry among the least similar morphogroups (Fig. 3.9) further supports the idea of morphological unity within morphogroups and therefore little or no interbreeding, i.e. the occurrence of reproductive isolation that is required for 'good species'.

Although easily distinguishable, ***P*** and ***L*** males only differed in four of the nine variables (Table 3.3), and yet were reproductively isolated. Minor morphological differences between two or more morphogroups do not necessarily imply conspecific status; this was also found to apply in morphological and mate choice analyses of the *Aphanicercella barnardi* species complex (Stevens & Picker 1999). In general terms, concurrent differences in both males and females between two or more populations provide added support in delimiting them as separate species.

The ubiquitous co-occurrence of sibling species across a variety of taxa, together with a spectrum of degrees of divergence, suggests that there can be no general rules that govern the degree of morphological distinctiveness required for species delimitation. Morphological studies within a narrow group of taxa is the most useful way to get as close as possible to rules that may be applicable to species delimitation problems within the same group. Similarly, the degree of genetic dissimilarity alone cannot be used in isolation for species delimitation (Ferguson 2002). Disjunct distributions of populations also cannot be used in isolation (Avise *et al.* 1987). Mayr (1970) points out that morphological difference has little value unless evaluated together with other criteria such as age, life stage and reproductive isolation. Interpreting morphological difference, genetic divergence, geographical distribution, reproductive isolation (estimated gene flow or even experimental trials), ecological specializations, and other criteria depending on the type of organism, within a combined framework will always provide the best estimate of species limits. It would be usual for the most morphologically similar populations of an organism to be the most closely related and hence to be the most similar genetically (Mayr 1970), but in this study it is clear that genetic and morphological divergence were not correlated. This discord between relative rates of morphological and genetic divergence is almost definitive for species complexes, with a classic example being the cichlids of the East African Great Lakes (Kornfield & Smith 2000). Another example is dwarf chameleons where younger lineages show incomplete lineage sorting although well defined morphologically, while other lineages may show little morphological but substantial genetic variability (Tolley & Burger 2004, Tolley *et al*. 2008).

Morphological diagnosability within *Aphanicerca capensis*

A more subjective criterion, morphological diagnosability (Cronquist 1978), was also used in this study. It is not explicitly morphometric but takes into account the investigator's analysis of morphological characteristics (e.g. male and female genitalia together and sclerotization patterns). This criterion was divided into two subcriteria (Table 3.20) to signify whether differences between some features are large or obvious, such that distinguishing between morphogroups is quick and easy, or whether differences are minor resulting in identification that may require greater taxonomic expertise but still remain clear cut and consistent. These

subcriteria indicated that morphological divergence was considered, in the context of the southern African Notonemouridae as a whole, significant enough to warrant species status.

Reciprocal monophyly and monophyly

Unique lineages were also identified through the criteria of reciprocal monophyly (Avise 2000; de Queiroz 2007) and monophyly (Donoghue 1985; Mishler 1985; Mishler & Theriot 2000), although some are of the opinion that monophyly is inapplicable at species level (Wheeler & Nixon 1990). Reciprocal monophyly as a criterion has also been criticized on the grounds that the species status of an entity is contingent on a property (monophyly) of another entity (Kizirian & Donnelly 2004). Therefore the lack of reciprocal monophyly does not negate species status in a unified species concept. The phylogenetic lineages inferred in this study from reciprocal monophyly or monophyly by one or more methods (ML, MP, BI) are indicated in Table 3.20. Morphogroups ***L***, ***R***, ***S*** and ***T*** were not monophyletic, and therefore any pairwise delimitation comparison including any of these, would not have met the criterion for reciprocal monophyly. The first three, of which ***L*** and ***S*** are syntopic and share the putative ancestral haplotype 10 with ***T***, are found in the Langeberg, and the last one, ***T***, in the nearby Witsenberg. Therefore, mitochondrial introgression and gene flow must be considered (recurrent or historical) in spite of highly divergent morphologies in sympatry. It is difficult to distinguish mitochondrial introgression from incomplete lineage sorting from gene trees alone, and the geographical evidence of sympatry makes introgression more likely (Ballard & Whitlock 2004). This is an interesting situation where reciprocal monophyly was present between eight morphogroups, which by some definitions would be sufficient for species status (or at least ESU's), but was absent in four sympatric (or possibly parapatric within the same mountain range) morphogroups which showed very distinct morphology even in syntopy, where no intermediates have yet to be discovered.

The ML phylogram indicated reciprocal monophyly between morphogroups ***C***, ***P***, ***N***, ***B***, ***Z***, ***W***, ***E*** and ***G***. It is possible that mitochondrial introgression could account for the shared haplotype 10 between ***S***, ***L***, and ***T***, because of their syntopy in the case of the first two, and the proximity of the Langeberg and Witsenberg ranges. This may also be the reason for the polyphyly of ***S*** and ***R***, and the paraphyly of ***L***. Besides gene flow, incomplete lineage sorting (retention of ancestral polymorphism) may also account for these scenarios. Paraphyly may occur in the early stages of speciation, with reciprocal monophyly occurring only after sufficient haplotype extinction has occurred (Kizirian & Donnelly 2004). Gene flow is perhaps a more likely scenario because all morphogroups off the Langeberg and Witsenberg were reciprocally monophyletic. In the case of incomplete lineage sorting one would expect paraphyly and polyphyly to be more randomly dispersed across the morphogroups and localities. Nevertheless,

one cannot discount the possibility that COI lineage sorting was completed in all morphogroups except for ***S***, ***L***, and ***T***. There appeared, however, to be no gene flow between ***P*** and the other morphogroups at that locality. ***P*** may have sufficiently diverged from ***S*** and ***L*** such that no gene flow is occurring.

As discussed above, it is clear that species status can be conferred on ***P***, ***S*** and ***L***, in spite of possible ongoing gene flow between the latter two, and the sharing of a haplotype in the segment of COI examined between ***S*** and ***L***. No apparent intermediate forms have so far been found at that locality in spite of potential gene flow, and the morphogroups at that locality were also found at other localities with minor within-morphogroup variation. This suggests that the gene flow is limited relative to the population size, or may be historical. The implication therefore is that lack of nucleotide divergence in this species complex cannot be used as evidence for conspecific status. Because two of these three syntopic morphogroups at Kristalkloof, which represent separately evolving reproductively lineages, share a haplotype, no operational species criteria based on COI molecular data, such as DNA barcoding, would be useful in species delimitation. These morphogroups, although syntopic, have been included as part of the species complex because of their morphological similarity with other morphogroups; thus, there remains a need to determine operationally the delimitation of species boundaries within the genus. Limited gene flow between ***S*** and ***L*** at Kristalkloof may be suspected because the ***S*** individuals from other localities had different haplotypes (Tables 3.13-3.15), but further sampling is required to fully investigate this.

In the phylogenetic analysis, *A. uncinata* was nested within the *A. capensis* species complex (Figs 3.9-3.12), as the sister group to the sympatric ***B*** and ***N***. Although it was markedly different morphologically from the species complex, it lay nested within the morphologically unified group, irrespective of what taxon status the members are accorded. *A. uncinata* is sympatric and probably syntopic with ***B*** in the Harold Porter Botanic Gardens in Betty's Bay, and sympatric with ***N*** in the southern Hottentots Holland Mountains. Because of sympatry, mitochondrial introgression cannot be ruled out without analysis of nuclear markers. Such anomalous relationships can also be accounted for by incomplete lineage sorting (although judging by the morphological disparity this is not likely) and third codon position homoplasy. The suspicion for the occurrence of one or more of these processes has its origin in a prior belief, which may be morphology, comparison with other genetic markers, or other criteria. Because *A. uncinata*, *A. bovina*, and the *A. capensis* species complex are morphologically divergent, it is clear that their apparent close relatedness in the COI trees renders that marker unsuitable as a DNA barcode at the species level. Incongruency between the COI gene tree and the species tree occurred in this respect. Although the species complex was paraphyletic in the cladograms presented (due to *A.*

bovina and *A. uncinata*), there is however relatively good phylogenetic signal in the data as a whole.

Centre of origin and dispersal

The haplotype with the highest frequency comprised five individuals that represented three morphogroups (two of morphogroup ***S***, two of ***T***, and one of ***L***). Two of these morphologically distinguishable groups, ***S*** and ***L***, are syntopic at Kristalkloof in the Langeberg Mountains, while both also occur allopatrically in other mountain ranges. A prediction of coalescent theory is that the most common haplotype in a gene pool tends to be the oldest (Crandall & Templeton 1996). Also, the oldest haplotype will usually give rise to the greatest number of descendant haplotypes (Posada & Crandall 2001). This node was indeed both the largest (highest frequency) and linked to the most haplotypes. The shared haplotype at this node may then be hypothesized to be the oldest of the current sample, suggesting a centre of origin of the *A. capensis* complex in the central south-western Cape (i.e. in the centre of an area corresponding to the Cape Floristic Region (Goldblatt & Manning 2002; Linder 2005)). This centre of origin hypothesis is in conflict with the conventional cladograms where haplotype 10 shared a common ancestor with other morphogroups (Figs 3.10-3.13), but is supported by the observation that six mutational branches arose from this node (*sensu* Cassens *et al*. 2005), more than from any other (Fig. 3.14). In this respect, the branching network pattern provides greater relational information than a standard cladogram or phylogram. This hypothesized centre of origin is the western-central area of the range of the species complex, corresponding to the Langeberg and Witsenberg ranges, and areas in close proximity. Four morphogroups occur within the Langeberg range, more than in any other mountain group, providing further support for the Langeberg area as the centre of origin. The trend of fewer morphogroups per montane region with increasing geographic distance from the Langeberg also suggests this area as the central point from which dispersal occurred.

Although evidence for sympatric speciation in general has been mounting, allopatric speciation remains the most accepted view by which speciation can occur (Bagnoli & Guardiani 2005); this is because reproductive isolation usually evolves in allopatry (Orr 2005). Following this argument, the most reasonable explanation then for the occurrence of syntopic and sympatric morphogroups is secondary contact via range expansion following vicariant allopatric speciation. It may be that vicariant allopatric speciation may not require a specific vicariant event in complex montane landscapes with steep valleys and inhospitable intervening terrain between streams, as deep valleys with high mountainsides may provide sufficient physical barriers to dispersal in some taxa (Hughes *et al*. 1999; Wishart & Hughes 2001). These physical barriers to dispersal within complex topographies may act as surrogates for major geological and

climatic events often cited as causes for vicariant allopatric speciation. Further isolation of populations may also have occurred through increased convolution of the montane landscape due to late Pliocene (*ca* 2.5 mya) neotectonic events; examples include the major uplift event of about 100 metres on the Cape west coast and 600-900 metres in the southern and eastern Cape (Artyushkov & Hofmann 1998, Partridge 1998). Periodic dispersals of stoneflies from catchments would have become less frequent with increased topographical complexity, thereby increasing the effects of isolation on populations. Together with small effective population sizes and founder events, genetic drift and bottlenecks would have contributed to genetic and morphological divergence between these isolated populations. The role of a number of genetic drift-based models in speciation though, is not supported empirically (Orr 2005). One recent vicariant event in the south-western Western Cape region is the erosion, completed by the end of the Pliocene, of the land bridge that once connected the Cape Peninsula with the Hottentots Holland Mountains (Walker 1952). Additionally, periodic flooding of the Cape Flats (the low-lying land separating Table Mountain from the Hottentots Holland Mountains) during the mid-Miocene and early Pleistocene (Hendey 1983b) resulted in isolation of Cape Peninsula species. Considering the average uncorrected genetic distance between ***C*** (the Cape Peninsula) and ***Z*** (Hottentots Holland Mountains) of about 6.4% (Appendix 3.4), the commonly used estimate of insect mtDNA divergence rates of 2.3% per million years (Brower 1994) suggests a divergence time of about 2.8 million years, which ties in well with the late Pliocene upliftment. It should be noted though, that the relationship between molecular divergence and time is not necessarily linear (Rambaut & Bromham 1998). A more detailed assessment of lineage diversification using a coalescent framework requires more detailed sampling within this group.

Future analyses

The choice of genetic markers will vary with the group of interest. There is no definitive answer on how many loci to use, but Knowles & Carstens (2007) recommend a "modest number" (e.g. five). It may not always be possible to use a modest number of loci on sufficient numbers of individuals (e.g. funding, time, or specimen collecting constraints) and this in turn can limit the utility of new coalescent-based methods (e.g. Knowles & Carstens 2007) in delimiting species in the face of incomplete lineage sorting; but its worth noting that these methods are ideal when incomplete lineage sorting is evident from conventional analyses. In addition to statistical methods, subjective assessments are important in interpretation of results. Even though application of a coalescent-based method may reveal patterns hidden by incomplete lineage sorting, it still requires a subjective judgment, made using patterns discovered in conjunction with other types of data (Knowles & Carstens 2007) and applied in a relational approach to species delimitation. It remains very useful for a researcher interested in phylogeography and speciation to first be a taxonomist. The trend of test tube taxonomy cannot

always be relied upon for accurate taxonomic decision making. Although incorrect decisions will always be made regardless of the method, intimate knowledge of the taxon will always improve accuracy when testing species status, but will also never eliminate differences of opinion among experts.

CONCLUSION

Assessment of the available lines of evidence corroborated the initial morphological hypothesis of species status for the 12 *Aphanicerca capensis* morphogroups. Two out of the ten lines of evidence (namely morphological phenetic distinguishability and male morphological diagnosability) provided simultaneous pairwise support for all 12 morphogroups as independently evolving metapopulation lineages. Sole reliance on any one of the remaining eight criteria failed to delimit all 12 morphogroups as species. The criterion of monophyly delineated eight of the 12 species. Morphology alone was sufficient to differentiate between these new species, but the additional lines of evidence afforded added support for species delimitation.

SUMMARY

The data from this study have provided:

- Lines of evidence used to infer separately evolving metapopulation lineages of notonemourids in the *Aphanicerca capensis* species complex. These included: allopatric fragmentation, genetic structure, intrinsic reproductive isolation in syntopic morphogroups, intrinsic reproductive isolation in sympatric morphogroups, intrinsic reproductive isolation inferred by complete premating isolation in experimental trials, intrinsic reproductive isolation inferred by unidirectional or incomplete premating isolation in experimental trials, phenetic distinctiveness, morphological diagnosability, reciprocal monophyly, and monophyly.
- Evidence to support recognition of 12 independently evolving species within the *Aphanicerca capensis* species complex (with additional species likely to be described following further collections of morphogroups too poorly represented to be of use in this analysis).
- Hypotheses for speciation processes in the species complex.
- Support for the controversial role of reinforcement as a force in speciation following secondary contact subsequent to allopatric speciation.
- Recognition of the non-congruency of the *Aphanicerca* COI gene tree and species tree.
- Further evidence of the inappropriate sole use of genetic distance in species delimitation (especially in recently diverged entities).

- Evidence that COI failed as a DNA barcode to unambiguously delimit all species within the genus *Aphanicerca* without reference to other lines of evidence, and therefore by inference failed in the southern African Notonemouridae as a whole.
- Evidence that reproductive cohesion appeared to be incomplete in the recently separated allopatric species of the *A. capensis* complex, but species unity was maintained in sympatric situations.
- Evidence that rates of change in mate recognition systems in the *A. capensis* complex may lag behind those of morphological and genetic divergence in vicariant speciation.
- Evidence of random spatial distribution of morphological types (i.e. not a cline) within this species complex across the CFM.
- Evidence of mitochondrial introgression (possibly historical) or incomplete lineage sorting (or both) within the species complex.
- Evidence of a centre of origin of the species complex in the central region of the Southern Folded Mountains (*sensu* Kleynhans *et al*. 2005) (possibly the Langeberg region).

Only one mitochondrial gene was examined in this study, and in relatively small numbers of individuals, limiting the degree to which a phylogeographic framework could be applied to the data set e.g. using the coalescent. Nevertheless, sufficient data were obtained through multiple data sources to delimit species and to indicate where future studies should concentrate, namely on the taxa where the COI data were inconclusive. In certain circumstances, even solely genetic distance of a few specimens can be of value in taxonomic decision making (Petersen *et al*. 2007). Data was insufficient to allow discrimination between incomplete lineage sorting and mitochondrial introgression, and to elucidate more than just a few speciation processes. Future studies can expand on the phylogeographic component of this study.

SPECIES DESCRIPTIONS

The 11 new species (morphogroup ***C*** retains the designation *Aphanicerca capensis*, being the population from which the type specimen of *A. capensis* was designated) will be formally described at a later date, following the same format as that of taxa described previously (Stevens & Picker 1995, 1999; Picker & Stevens 1997, 1999; Chapter 2 of this thesis). A list of tentative species names, which are used in Chapter 4, is given below:

B: *Aphanicerca brevispina* sp. n.
E: *Aphanicerca breviloba* sp. n.
G: *Aphanicerca swartbergensis* sp. n.
L: *Aphanicerca longiloba* sp. n.

N: *Aphanicerca pickeri* sp. n.

P: *Aphanicerca austrocapensis* sp. n.

R: *Aphanicerca incisura* sp. n.

S: *Aphanicerca mclellani* sp. n.

T: *Aphanicerca witsenbergensis* sp. n.

W: *Aphanicerca cederbergensis* sp. n.

Z: *Aphanicerca zwicki* sp. n.

Appendix 3.1. Morphometric raw data (mm) for nine variables of 215 individuals of 12 morphogroups of the *Aphanicerca capensis* species complex. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes. MG = morphogroup; loc = locality number (locality data available from author).

MG	loc	adp	dp	epd	epl	epw	hcw	pnw	ppw	sp
B	137	0.28	0.27	0.11	0.25	0.07	0.90	0.80	0.06	0.11
B	137	0.28	0.30	0.10	0.24	0.08	0.94	0.82	0.06	0.12
B	137	0.23	0.26	0.12	0.26	0.07	0.92	0.78	0.06	0.11
B	137	0.26	0.28	0.12	0.24	0.06	0.88	0.72	0.05	0.11
B	137	0.26	0.28	0.12	0.23	0.07	0.94	0.86	0.05	0.09
B	137	0.30	0.30	0.12	0.25	0.07	0.90	0.78	0.05	0.11
B	137	0.28	0.30	0.10	0.25	0.07	0.90	0.76	0.05	0.12
B	137	0.24	0.26	0.12	0.25	0.06	0.94	0.90	0.06	0.10
B	137	0.24	0.28	0.10	0.23	0.07	0.88	0.82	0.04	0.11
B	130	0.28	0.30	0.10	0.25	0.07	0.92	0.78	0.06	0.10
B	7	0.29	0.32	0.09	0.26	0.07	0.98	0.86	0.06	0.10
C	133	0.48	0.52	0.19	0.34	0.07	0.96	0.84	0.08	0.24
C	133	0.40	0.50	0.19	0.34	0.08	0.96	0.86	0.08	0.24
C	133	0.44	0.52	0.18	0.32	0.08	1.00	0.88	0.08	0.26
C	133	0.51	0.50	0.19	0.36	0.09	1.00	0.80	0.08	0.25
C	133	0.44	0.49	0.16	0.34	0.08	0.98	0.90	0.08	0.24
C	133	0.38	0.50	0.16	0.34	0.08	1.00	0.80	0.08	0.24
C	133	0.36	0.50	0.19	0.34	0.08	1.08	0.92	0.08	0.25
C	133	0.46	0.50	0.20	0.34	0.08	0.98	0.84	0.07	0.24
C	133	0.48	0.52	0.16	0.34	0.08	0.98	0.82	0.08	0.23
C	20	0.44	0.48	0.17	0.34	0.08	0.98	0.87	0.08	0.24
C	36	0.52	0.50	0.18	0.36	0.08	1.06	1.00	0.10	0.20
E	62	0.10	0.22	0.09	0.22	0.06	0.76	0.66	0.03	0.12
E	216	0.12	0.17	0.11	0.21	0.07	0.72	0.64	0.04	0.08
E	216	0.14	0.25	0.12	0.24	0.08	0.86	0.74	0.05	0.14
E	216	0.15	0.22	0.11	0.24	0.07	0.74	0.66	0.04	0.11
E	216	0.13	0.22	0.12	0.24	0.08	0.80	0.74	0.04	0.12
E	216	0.16	0.25	0.11	0.24	0.08	0.80	0.68	0.04	0.15
E	216	0.13	0.22	0.13	0.24	0.08	0.78	0.70	0.04	0.10
E	216	0.16	0.22	0.11	0.22	0.09	0.80	0.64	0.04	0.10
E	216	0.15	0.24	0.12	0.23	0.07	0.76	0.64	0.04	0.11
E	216	0.14	0.26	0.14	0.26	0.08	0.83	0.76	0.02	0.14
E	62	0.14	0.26	0.14	0.26	0.08	0.82	0.68	0.04	0.14
G	82	0.42	0.43	0.15	0.30	0.10	0.96	0.86	0.06	0.23
G	83	0.43	0.40	0.14	0.27	0.10	0.92	0.76	0.04	0.20
G	83	0.46	0.44	0.15	0.30	0.11	0.94	0.76	0.05	0.22
G	83	0.42	0.43	0.16	0.28	0.11	0.86	0.72	0.05	0.24
G	83	0.34	0.44	0.13	0.28	0.11	0.90	0.76	0.04	0.22
G	146	0.38	0.44	0.14	0.28	0.10	0.92	0.82	0.05	0.24
G	183	0.43	0.47	0.15	0.28	0.12	0.94	0.82	0.06	0.25
G	183	0.37	0.44	0.18	0.28	0.11	0.96	0.78	0.04	0.24
G	183	0.43	0.46	0.15	0.28	0.10	1.00	0.84	0.04	0.25
G	183	0.42	0.43	0.14	0.28	0.10	0.96	0.80	0.06	0.22
G	183	0.49	0.46	0.14	0.29	0.11	1.00	0.89	0.04	0.22
G	183	0.42	0.46	0.15	0.28	0.10	0.96	0.82	0.04	0.22

Appendix 3.1. Continued.

MG	site no.	adp	dp	epd	epl	epw	hcw	pnw	ppw	sp
G	183	0.46	0.44	0.15	0.28	0.12	0.94	0.78	0.04	0.23
G	183	0.44	0.44	0.13	0.26	0.10	0.93	0.74	0.04	0.22
G	183	0.38	0.42	0.15	0.26	0.10	0.92	0.74	0.04	0.22
G	183	0.41	0.46	0.15	0.28	0.10	0.93	0.75	0.05	0.26
G	150	0.45	0.49	0.15	0.31	0.10	1.02	0.87	0.04	0.27
G	150	0.42	0.51	0.14	0.28	0.12	1.02	0.84	0.04	0.28
G	150	0.48	0.51	0.13	0.29	0.11	1.02	0.88	0.04	0.26
G	150	0.52	0.48	0.14	0.28	0.10	0.98	0.80	0.04	0.24
G	150	0.45	0.48	0.13	0.28	0.10	1.00	0.86	0.04	0.26
G	150	0.49	0.52	0.13	0.28	0.12	1.04	0.90	0.04	0.30
G	150	0.49	0.50	0.13	0.28	0.12	1.02	0.88	0.04	0.25
G	150	0.53	0.52	0.13	0.28	0.12	1.08	0.88	0.04	0.24
G	150	0.49	0.48	0.14	0.30	0.12	1.04	0.88	0.04	0.22
G	150	0.49	0.45	0.15	0.28	0.10	0.98	0.84	0.04	0.23
G	150	0.54	0.54	0.15	0.31	0.11	1.04	0.90	0.05	0.26
G	150	0.44	0.49	0.15	0.29	0.10	1.00	0.90	0.05	0.24
G	150	0.48	0.54	0.16	0.31	0.12	1.04	0.90	0.04	0.28
G	150	0.52	0.48	0.17	0.30	0.12	1.02	0.85	0.04	0.25
G	210	0.50	0.50	0.16	0.32	0.10	1.00	0.88	0.06	0.28
G	217	0.50	0.52	0.15	0.29	0.12	1.02	0.90	0.05	0.26
L	118	0.46	0.52	0.16	0.30	0.08	1.06	0.90	0.06	0.28
L	118	0.43	0.50	0.16	0.29	0.08	0.98	0.80	0.06	0.26
L	118	0.50	0.52	0.17	0.30	0.07	0.98	0.80	0.06	0.26
L	118	0.46	0.55	0.16	0.31	0.09	1.06	0.96	0.06	0.30
L	78	0.44	0.50	0.12	0.26	0.08	0.95	0.84	0.06	0.28
L	78	0.42	0.46	0.12	0.27	0.08	0.94	0.84	0.07	0.27
L	78	0.46	0.48	0.12	0.27	0.08	0.96	0.78	0.06	0.24
L	78	0.46	0.54	0.16	0.30	0.08	0.98	0.88	0.06	0.28
L	78	0.48	0.50	0.15	0.31	0.08	0.96	0.78	0.06	0.28
L	78	0.44	0.48	0.14	0.29	0.08	0.95	0.84	0.06	0.24
L	80	0.44	0.52	0.16	0.30	0.08	0.96	0.80	0.07	0.24
L	80	0.45	0.54	0.15	0.29	0.08	0.98	0.82	0.07	0.28
L	80	0.54	0.52	0.14	0.29	0.08	1.00	0.82	0.05	0.24
L	80	0.42	0.52	0.13	0.28	0.08	0.96	0.82	0.06	0.26
L	80	0.45	0.54	0.14	0.28	0.08	0.96	0.78	0.06	0.30
N	117	0.38	0.40	0.15	0.31	0.08	0.96	0.84	0.07	0.13
N	117	0.36	0.40	0.16	0.32	0.06	0.96	0.84	0.06	0.17
N	117	0.44	0.42	0.16	0.34	0.07	1.04	0.93	0.08	0.18
N	117	0.41	0.40	0.14	0.29	0.07	0.94	0.80	0.07	0.13
N	180	0.34	0.37	0.17	0.30	0.07	0.88	0.78	0.06	0.14
N	180	0.34	0.37	0.13	0.28	0.07	0.96	0.80	0.06	0.14
N	180	0.33	0.37	0.12	0.28	0.07	0.90	0.74	0.06	0.12
N	180	0.36	0.41	0.13	0.27	0.07	0.96	0.85	0.06	0.16
N	180	0.40	0.40	0.14	0.30	0.06	0.98	0.88	0.06	0.14
N	180	0.41	0.44	0.15	0.32	0.08	1.00	0.84	0.07	0.16

Appendix 3.1. Continued.

MG	site no.	adp	dp	epd	epl	epw	hcw	pnw	ppw	sp
P	81b	0.51	0.48	0.14	0.28	0.14	0.96	0.80	0.06	0.26
P	191	0.40	0.41	0.12	0.22	0.13	0.88	0.80	0.04	0.22
P	194	0.50	0.41	0.12	0.22	0.12	0.84	0.70	0.04	0.22
P	152	0.52	0.50	0.12	0.30	0.14	0.96	0.83	0.04	0.26
P	159	0.48	0.45	0.13	0.28	0.12	0.94	0.76	0.04	0.24
P	159	0.47	0.50	0.12	0.28	0.14	0.98	0.86	0.04	0.28
P	159	0.51	0.50	0.13	0.28	0.12	0.94	0.82	0.04	0.28
P	159	0.46	0.50	0.12	0.28	0.12	1.02	0.86	0.06	0.28
P	151	0.47	0.44	0.13	0.28	0.12	1.02	0.86	0.04	0.22
P	151	0.46	0.48	0.13	0.28	0.10	0.96	0.80	0.04	0.27
P	154	0.46	0.52	0.17	0.31	0.14	1.06	0.90	0.04	0.32
P	154	0.46	0.52	0.15	0.24	0.14	1.04	0.86	0.06	0.27
P	154	0.45	0.50	0.13	0.29	0.14	1.06	0.88	0.04	0.28
P	154	0.50	0.50	0.14	0.30	0.14	1.06	0.88	0.04	0.28
P	154	0.49	0.54	0.14	0.29	0.14	1.04	0.88	0.05	0.31
P	197	0.52	0.50	0.15	0.26	0.13	0.94	0.84	0.04	0.26
P	197	0.44	0.46	0.14	0.28	0.14	0.99	0.88	0.05	0.26
P	197	0.52	0.48	0.13	0.26	0.12	0.96	0.88	0.05	0.26
P	197	0.44	0.44	0.12	0.25	0.13	0.90	0.80	0.04	0.25
P	197	0.44	0.44	0.13	0.28	0.14	0.94	0.82	0.04	0.25
P	197	0.54	0.50	0.12	0.26	0.14	0.98	0.85	0.05	0.26
P	197	0.44	0.44	0.13	0.26	0.14	0.92	0.82	0.05	0.26
P	197	0.52	0.48	0.12	0.25	0.13	0.98	0.80	0.04	0.26
P	197	0.44	0.44	0.12	0.26	0.13	0.90	0.82	0.05	0.24
P	197	0.45	0.48	0.14	0.28	0.14	0.98	0.90	0.04	0.26
R	170	0.39	0.34	0.13	0.24	0.10	0.84	0.74	0.06	0.22
R	170	0.33	0.34	0.13	0.24	0.10	0.82	0.70	0.05	0.20
R	170	0.32	0.33	0.11	0.25	0.10	0.80	0.72	0.04	0.17
R	170	0.28	0.34	0.11	0.24	0.08	0.82	0.74	0.04	0.20
R	170	0.34	0.34	0.11	0.24	0.10	0.82	0.71	0.04	0.20
R	170	0.33	0.33	0.10	0.24	0.10	0.84	0.72	0.04	0.22
R	171	0.32	0.32	0.13	0.25	0.10	0.82	0.70	0.04	0.20
R	171	0.37	0.36	0.12	0.26	0.10	0.82	0.74	0.04	0.21
R	171	0.36	0.35	0.12	0.25	0.10	0.86	0.78	0.04	0.22
R	171	0.38	0.35	0.12	0.26	0.10	0.88	0.78	0.04	0.22
S	70	0.18	0.32	0.15	0.29	0.07	0.94	0.82	0.06	0.16
S	70	0.18	0.30	0.14	0.30	0.07	0.98	0.74	0.07	0.16
S	70	0.20	0.32	0.14	0.30	0.07	1.00	0.80	0.06	0.14
S	70	0.26	0.30	0.13	0.28	0.07	0.90	0.76	0.05	0.16
S	70	0.24	0.34	0.13	0.28	0.07	0.94	0.84	0.05	0.16
S	70	0.26	0.32	0.12	0.27	0.07	0.90	0.78	0.06	0.15
S	68	0.09	0.27	0.13	0.28	0.06	0.92	0.78	0.06	0.16
S	68	0.08	0.32	0.14	0.28	0.06	0.94	0.80	0.06	0.16
S	153	0.11	0.30	0.14	0.28	0.08	0.98	0.84	0.06	0.19
S	153	0.04	0.32	0.13	0.28	0.06	0.98	0.80	0.06	0.21

Appendix 3.1. Continued.

MG	site no.	adp	dp	epd	epl	epw	hcw	pnw	ppw	sp
S	153	0.09	0.30	0.13	0.29	0.08	0.96	0.86	0.06	0.18
S	153	0.07	0.33	0.13	0.29	0.07	0.98	0.88	0.07	0.20
S	153	0.02	0.30	0.14	0.30	0.08	0.96	0.86	0.07	0.18
S	153	0.10	0.28	0.13	0.28	0.06	0.94	0.83	0.06	0.16
S	153	0.21	0.33	0.14	0.30	0.06	0.96	0.82	0.07	0.18
S	159	0.16	0.30	0.13	0.27	0.07	0.92	0.76	0.06	0.16
S	159	0.14	0.32	0.12	0.28	0.07	0.94	0.80	0.06	0.17
S	159	0.19	0.32	0.13	0.27	0.07	0.94	0.78	0.06	0.16
S	81a	0.28	0.33	0.12	0.26	0.07	0.92	0.76	0.06	0.18
S	194	0.30	0.35	0.11	0.25	0.08	0.86	0.72	0.06	0.17
S	194	0.24	0.29	0.10	0.25	0.06	0.86	0.76	0.06	0.13
S	196	0.28	0.34	0.12	0.28	0.07	0.90	0.78	0.07	0.16
S	196	0.29	0.34	0.10	0.26	0.06	0.92	0.80	0.07	0.13
S	191	0.28	0.33	0.10	0.26	0.06	0.89	0.76	0.06	0.16
T	16	0.48	0.44	0.14	0.30	0.14	1.04	0.84	0.06	0.26
T	16	0.36	0.44	0.14	0.30	0.12	1.02	0.90	0.04	0.28
T	16	0.51	0.50	0.16	0.30	0.12	1.08	0.92	0.04	0.32
T	16	0.44	0.44	0.16	0.28	0.12	1.00	0.80	0.04	0.26
T	16	0.47	0.48	0.16	0.28	0.12	1.02	0.86	0.04	0.28
T	16	0.45	0.44	0.15	0.28	0.12	1.04	0.86	0.04	0.26
T	16	0.41	0.46	0.15	0.27	0.12	1.00	0.84	0.04	0.28
T	16	0.44	0.44	0.16	0.30	0.12	1.02	0.86	0.04	0.28
T	16	0.38	0.46	0.16	0.28	0.12	1.02	0.84	0.04	0.30
T	16	0.54	0.50	0.16	0.29	0.12	1.02	0.88	0.04	0.28
T	16	0.52	0.48	0.17	0.30	0.12	1.02	0.90	0.05	0.30
T	16	0.40	0.44	0.16	0.28	0.12	1.00	0.92	0.05	0.25
T	16	0.50	0.47	0.15	0.28	0.12	1.02	0.84	0.04	0.27
T	16	0.40	0.43	0.14	0.28	0.12	1.02	0.89	0.04	0.26
T	16	0.48	0.44	0.14	0.29	0.12	1.02	0.84	0.05	0.28
T	16	0.46	0.45	0.18	0.30	0.12	1.02	0.86	0.05	0.27
W	120	0.34	0.37	0.14	0.30	0.08	0.94	0.74	0.04	0.24
W	120	0.32	0.36	0.14	0.30	0.08	0.92	0.76	0.06	0.22
W	120	0.36	0.36	0.12	0.30	0.08	0.86	0.72	0.04	0.21
W	120	0.28	0.36	0.14	0.29	0.08	0.86	0.76	0.04	0.21
W	120	0.37	0.37	0.13	0.28	0.08	0.90	0.76	0.05	0.22
W	120	0.38	0.36	0.14	0.30	0.08	0.86	0.76	0.04	0.23
W	120	0.32	0.37	0.14	0.28	0.08	0.92	0.76	0.05	0.20
W	121	0.40	0.42	0.14	0.31	0.08	0.94	0.81	0.04	0.24
W	121	0.40	0.40	0.15	0.32	0.08	0.96	0.86	0.05	0.25
W	125	0.40	0.38	0.16	0.29	0.07	0.88	0.70	0.04	0.22
W	125	0.34	0.37	0.13	0.29	0.08	0.96	0.80	0.05	0.22
W	126	0.32	0.34	0.14	0.29	0.08	0.86	0.74	0.04	0.21
W	126	0.38	0.40	0.14	0.29	0.08	0.92	0.76	0.06	0.24
W	126	0.34	0.33	0.12	0.27	0.09	0.94	0.78	0.05	0.22
Z	137	0.35	0.38	0.18	0.32	0.08	1.00	0.78	0.06	0.18

Appendix 3.1. Continued.

MG	site no.	adp	dp	epd	epl	epw	hcw	pnw	ppw	sp
Z	137	0.36	0.38	0.18	0.32	0.08	0.90	0.70	0.08	0.22
Z	137	0.36	0.38	0.18	0.32	0.09	0.96	0.80	0.06	0.22
Z	137	0.42	0.38	0.18	0.31	0.08	0.92	0.78	0.08	0.20
Z	137	0.36	0.36	0.18	0.32	0.08	0.96	0.84	0.06	0.20
Z	137	0.36	0.36	0.18	0.31	0.08	0.92	0.80	0.07	0.20
Z	137	0.35	0.33	0.19	0.32	0.08	0.94	0.82	0.07	0.14
Z	137	0.40	0.38	0.18	0.32	0.08	0.96	0.78	0.06	0.20
Z	137	0.37	0.38	0.19	0.33	0.08	0.94	0.77	0.07	0.22
Z	10	0.46	0.38	0.18	0.32	0.08	1.04	0.88	0.08	0.22
Z	10	0.40	0.38	0.17	0.32	0.08	1.00	0.86	0.07	0.21
Z	10	0.36	0.38	0.20	0.34	0.08	1.02	0.88	0.07	0.21
Z	119	0.32	0.34	0.17	0.31	0.07	0.96	0.82	0.05	0.20
Z	119	0.36	0.36	0.20	0.33	0.07	1.04	0.86	0.06	0.19
Z	119	0.32	0.34	0.15	0.30	0.07	0.98	0.84	0.05	0.20
Z	119	0.36	0.32	0.18	0.31	0.08	1.00	0.84	0.05	0.18
Z	119	0.38	0.36	0.18	0.30	0.06	0.96	0.80	0.06	0.19
Z	119	0.35	0.32	0.17	0.30	0.08	1.02	0.84	0.06	0.20
Z	119	0.32	0.36	0.19	0.32	0.08	1.02	0.84	0.05	0.22
Z	119	0.37	0.38	0.15	0.32	0.08	1.02	0.88	0.06	0.20
Z	119	0.32	0.33	0.15	0.28	0.08	0.98	0.82	0.06	0.20
Z	119	0.35	0.36	0.17	0.30	0.08	1.00	0.88	0.06	0.22
Z	94	0.35	0.37	0.18	0.34	0.09	0.98	0.83	0.08	0.20
Z	94	0.38	0.33	0.18	0.32	0.08	1.00	0.84	0.06	0.15
Z	94	0.41	0.37	0.18	0.32	0.08	0.98	0.86	0.06	0.17
Z	94	0.32	0.37	0.17	0.31	0.08	0.96	0.86	0.06	0.20
Z	94	0.44	0.36	0.20	0.34	0.10	1.06	0.88	0.07	0.16
Z	94	0.40	0.35	0.17	0.33	0.08	0.98	0.88	0.08	0.17
Z	94	0.42	0.36	0.18	0.33	0.08	0.98	0.86	0.06	0.18
Z	94	0.34	0.34	0.18	0.30	0.08	1.00	0.84	0.06	0.18
Z	94	0.38	0.35	0.16	0.32	0.08	0.94	0.86	0.06	0.20
Z	94	0.34	0.36	0.19	0.34	0.08	0.98	0.86	0.07	0.20
Z	113	0.38	0.36	0.18	0.34	0.09	0.98	0.82	0.06	0.18
Z	113	0.36	0.34	0.16	0.32	0.08	0.96	0.80	0.06	0.19
Z	113	0.39	0.38	0.17	0.32	0.07	0.96	0.78	0.07	0.20
Z	113	0.33	0.32	0.18	0.32	0.08	0.92	0.78	0.06	0.19

Appendix 3.2. Mean (mm), standard deviation and standard error of the mean of the nine variables for the 12 *A. capensis* morphogroups which are given in the first row. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes.

	B	***C***	***E***	***G***	***L***	***N***	***P***	***R***	***S***	***T***	***W***	***Z***
N	11	11	11	32	15	10	25	10	24	16	14	36
adp mean	0.267	0.446	0.138	0.453	0.457	0.377	0.476	0.342	0.179	0.453	0.354	0.368
adp Std.Dev.	0.023	0.051	0.018	0.049	0.031	0.037	0.035	0.033	0.087	0.052	0.037	0.034
adp Std.Err.	0.007	0.015	0.005	0.009	0.008	0.012	0.007	0.011	0.018	0.013	0.010	0.006
dp mean	0.286	0.503	0.230	0.471	0.513	0.398	0.476	0.340	0.315	0.457	0.371	0.358
dp Std.Dev.	0.019	0.013	0.026	0.037	0.026	0.023	0.035	0.012	0.020	0.023	0.024	0.020
dp Std.Err.	0.006	0.004	0.008	0.006	0.007	0.007	0.007	0.004	0.004	0.006	0.006	0.003
epd mean	0.109	0.179	0.118	0.146	0.145	0.145	0.132	0.118	0.127	0.155	0.138	0.177
epd Std.Dev.	0.011	0.014	0.015	0.012	0.017	0.016	0.012	0.010	0.014	0.012	0.011	0.013
epd Std.Err.	0.003	0.004	0.004	0.002	0.004	0.005	0.002	0.003	0.003	0.003	0.003	0.002
epl mean	0.246	0.342	0.236	0.286	0.289	0.301	0.271	0.247	0.278	0.288	0.294	0.319
epl Std.Dev.	0.010	0.011	0.016	0.014	0.015	0.022	0.023	0.008	0.015	0.010	0.013	0.014
epl Std.Err.	0.003	0.003	0.005	0.002	0.004	0.007	0.005	0.003	0.003	0.003	0.003	0.002
epw mean	0.069	0.080	0.076	0.108	0.080	0.070	0.132	0.098	0.068	0.122	0.080	0.080
epw Std.Dev.	0.005	0.004	0.008	0.009	0.004	0.007	0.011	0.006	0.007	0.005	0.004	0.007
epw Std.Err.	0.002	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.001	0.001	0.001	0.001
hcw mean	0.918	0.998	0.788	0.980	0.979	0.958	0.970	0.832	0.935	1.023	0.909	0.978
hcw Std.Dev.	0.030	0.038	0.041	0.050	0.036	0.046	0.058	0.023	0.037	0.019	0.038	0.037
hcw Std.Err.	0.009	0.012	0.012	0.009	0.009	0.014	0.012	0.007	0.008	0.005	0.010	0.006
pnw mean	0.807	0.866	0.685	0.831	0.831	0.830	0.836	0.733	0.797	0.866	0.765	0.829
pnw Std.Dev.	0.052	0.059	0.044	0.057	0.050	0.053	0.047	0.029	0.041	0.033	0.039	0.041
pnw Std.Err.	0.016	0.018	0.013	0.010	0.013	0.017	0.009	0.009	0.008	0.008	0.011	0.007
ppw mean	0.055	0.081	0.038	0.045	0.061	0.065	0.045	0.043	0.062	0.044	0.046	0.064
ppw Std.Dev.	0.007	0.007	0.008	0.007	0.005	0.007	0.007	0.007	0.006	0.006	0.007	0.009
ppw Std.Err.	0.002	0.002	0.002	0.001	0.001	0.002	0.001	0.002	0.001	0.002	0.002	0.001
sp mean	0.107	0.239	0.119	0.244	0.267	0.147	0.262	0.206	0.165	0.277	0.224	0.194
sp Std.Dev.	0.009	0.015	0.022	0.023	0.021	0.019	0.024	0.016	0.019	0.018	0.014	0.019
sp Std.Err.	0.003	0.005	0.007	0.004	0.005	0.006	0.005	0.005	0.004	0.005	0.004	0.003

Appendix 3.3. *Aphanicerca capensis* species complex mtDNA (COI) sequence alignment.

```
hap 1     ATTCGAGCAG AATTAGGCCA ACCTGGATCT TTAATTGGTG ATGATCAAAT TTACAATGTG
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     ---------- -------T-- ------G--- ---------- ---------- ----------
hap 4     ---------- -------T-- ---------- ---------- ---------- ----------
hap 5     ---------- -------T-- ---------- -----C---- ---------- ----------
hap 6     ---------- -------T-- ---------- -----C---- ---------- ----------
hap 7     ---------- -------T-- ---------- -----C---- ---------- ----------
hap 8     ---------- -------T-- ------G--- -----C---- ---------- ----------
hap 9     ---------- -------T-- ---------- -----C---- ---------- ----------
hap 10    ---------- -------T-- ---------- ---------- ---------- ----------
hap 11    ---------- -------T-- ---------- ---------- ---------- ----------
hap 12    ---------- -------T-- ---------- ---------- ---------- ----------
hap 13    ---------- ---------- ---------- ---------- ---------- ----------
hap 14    ---------- ---------- ---------- ---------- ---------- ----------
hap 15    ---------- ---------- ---------- ---------- ---------- ----------
hap 16    ---------- ---------- ---------- ---------- ---------- ----------
hap 17    ---------- ---------- ---------- ---------- ---------- ----------
hap 18    ---------- -------T-- ---------- ---------- ---------- ----------
hap 19    ---------- -------T-- ---------- ---------- ---------- ----------
hap 20    ---------- -------T-- ------G--- ---------- ---------- ----------
hap 21    ---------- -------T-- ---------- ---------- ---------- ----------
hap 22    ---------- -------T-- ---------- ---------- ---------- ----------
hap 23    ---------- -------T-- ---------- ---------- ---------- ----------
hap 24    ---------- -------T-- ---------- ---------- ---------- ----------
hap 25    ---------- -------T-- ---------- ---------- ---------- ----------
hap 26    ---------- -------T-- ---------- ---------- ---------- ----------
hap 27    ---------- -------T-- ---------- ---------- ---------- ----------60


hap 1     ATCGTTACTG CTCACGCTTT CGTAATGATT TTCTTCATAG TTATACCTAT TATAATTGGT
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     ---------- ----T----- ------A--- ---------- ---------- ----------
hap 4     ---------- ----T----- ---------- ---------- ---------- C-----C---
hap 5     ---------- ----T----- ---------- ---------- ---------- C---------
hap 6     ---------- ----T----- ---------- ---------- ---------- C---------
hap 7     ---------- ----T----- ---------- ---------- ---------- C---------
hap 8     ---------- ----T----- ---------- ---------- ---------- C---------
hap 9     ---------- ----T----- ---------- ---------- ---------- C---------
hap 10    ---------- ----T----- ---------- ---------- ---------- C---------
hap 11    ---------- ---T------ ---------- ---------- ---------- C---------
hap 12    ---------- ---T------ ---------- ---------- ---------- C---------
hap 13    ---------- ---T------ ---------- ---------- ---------- ------C---
hap 14    ---------- ---T------ ---------- ---------- ---------- ----------
hap 15    ---------- -C--T----- ---------- ---------- ---------- ----------
hap 16    ---------- -C--T----- ---------- ---------- ---------- ----------
hap 17    ---------- -C--T----- ---------- ---------- ---------- ----------
hap 18    ---------- ----T----- ---------- ---------- ---------- C---------
hap 19    ---------- ----T----- ---------- ---------- ---------- C---------
hap 20    ---------- ----T----- ---------- ---------- ---------- C-----C---
hap 21    ---------- ----T----- ---------- ---------- ---------- C--------A
hap 22    ---------- ----T----- ---------- ---------- ---------- C--------A
hap 23    ---------- ----T----- ---------- ---------- ---------- ----------
hap 24    ---------- ----T----- ---------- ---------- ---------- ----------
hap 25    ---------- ----T----- ---------- ---------- ---------- ----------
hap 26    ---------- ----T----- ---------- ---------- ---------- ----------
hap 27    ---------- -C--T----- ---------- ---------- ---------- ----------120
```

Appendix 3.3. Continued.

```
hap 1     GGGTTTGGAA ATTGGCTAGT TCCTTTAATG CTAGGAGCCC CAGATATGGC CTTCCCCCGA
hap 2     ---------- ---------- C--------- ---------- ---------- ----------
hap 3     --------G- ----AT---- C--------A T-G------- ----C--A-- ---------C
hap 4     ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 5     ---------- ----A----- C--------- T-------T- -------A-- ------A---
hap 6     ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 7     ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 8     ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 9     ---------- ----A----- C--------- ---------- -------A-- ------A---
hap 10    ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 11    ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 12    ---------- ----A----- C--------- T----G---- -------A-- ------A---
hap 13    ---------- ----A----- C--------- ---------- ---------- ---T------
hap 14    ---------- ----A----- C--------- ---------- ---------- ---T------
hap 15    ---------- ----A----- C--------- T--------- -------A-- ----------
hap 16    ---------- ----A----- C--------- T--------- -------A-- ----------
hap 17    ---------- ----A----- C--------- T--------- -------A-- ----------
hap 18    ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 19    ---------- ----A----- C--------- T--------- -------A-- ------G---
hap 20    ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 21    ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 22    ---------- ----A----- C--------- T--------- -------A-- ------A---
hap 23    ---------- ----A----- C--------- -----G---- -------A-- ----------
hap 24    ---------- ----A----- C--------- -----G---- -------A-- ----------
hap 25    ---------- ----A----- C--------- T--------- -------A-- ----------
hap 26    ---------- ----A----- C--------- T--------- -------A-- ----------
hap 27    ---------- ----A----- C--------- T---------------A-- ----------180


hap 1     ATGAATAATA TAAGATTTTG ATTACTACCA CCTTCCTTAA CTCTATTGTT AGCCAGTAGC
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     ---------- ---------- G--------- ---------- ---------- ----------
hap 4     ---------- ---------- ---------- ---------- ---------- ----------
hap 5     ---------- ---------- ---------- ---------- -C-------- ----------
hap 6     ---------- ---------- ---------- ---------- -C-------- ----------
hap 7     ---------- ---------- G--------- ---------- -C-------- ----------
hap 8     ---------- ---------- ---------- ---------- -C-------- ----------
hap 9     ---------- ---------- ---------- ---------- -C-------- ----------
hap 10    ---------- ---------- ---------- ---------- ---------- ----------
hap 11    ---------- ---------- ---------- ---------- ---------- ----------
hap 12    ---------- ---------- ----T----- ---------- ---------- ----------
hap 13    ---------- ---------- ---------- ---------- ---------- ----------
hap 14    ---------- ---------- ---------- ---------- ---------- ----------
hap 15    ---------- ---------- ---------- ---------- ----C----- ---T------
hap 16    ---------- ---------- ---------- ---------- ----C----- ---T------
hap 17    ---------- ---------- ---------- ---------- ---------- ---T------
hap 18    --A------- ---------- ---------- ---------- ---------- ----------
hap 19    --A------- ---------- ---------- ---------- ---------- ----------
hap 20    ---------- ---------- ---------- ---------- ---------- ----------
hap 21    ---------- ---------- ---------- ---------- ---------- ----------
hap 22    ---------- ---------- ---------- ---------- ---------- ----------
hap 23    ---------- ---------- ---------- ---------- ---------- ----------
hap 24    ---------- ---------- ---------- ---------- ---------- ----------
hap 25    ---------- ---------- ---------- ---------- ---------- ---T------
hap 26    ---------- ---------- ---------- ---------- ---------- ---T------
hap 27    ---------- ---------- ---------- ---------- ---------- ---T------240
```

Appendix 3.3. Continued.

```
hap 1     TTAGTTGAAA ATGGAGCGGG TACAGGGTGA ACTGTCTACC CACCTCTATC AGCAGGTATC
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     C--------- -------A-- ---T--A--G --A------- ---------- ---G------
hap 4     C--------- ---------- ---C--A--- ---------- -G-------- C---------
hap 5     C--------- ---------- ------A--- ---------- ---------- C---------
hap 6     C--------- ---------- ------A--- ---------- ---------- C---------
hap 7     C--------- ---------- ------A--- ---------- -G-------- C---------
hap 8     C--------- ---------- ------A--- ---------- -G-------- C---------
hap 9     C--------- ---------- ------A--- ---------- -G-------- C---------
hap 10    C--------- ---------- ------A--- ---------- ---------- C---------
hap 11    C--------- ---------- ------A--- ---------- -C-------- C---------
hap 12    C--------- ---------- ------A--- ---------- ---------- C---------
hap 13    ---------- ---------- ---------- ---------- ---------- ----------
hap 14    ---------- ---------- ---------- ---------- ---------- ----------
hap 15    C--------- ---------- ------A--- ---------- ---------- ----------
hap 16    C--------- ---------- ------A--- ---------- ---------- ----------
hap 17    C--------- ---------- ------A--- ---------- ---------- ----------
hap 18    C--------- ---------- ------A--- ---------- ---------- C---------
hap 19    C--------- ---------- ------A--- ---------- ---------- C---------
hap 20    C--------- ---------- ------A--- ---------- ---------- C---------
hap 21    C--------- ---------- ------A--- ---------- ---------- C---------
hap 22    C--------- ---------- ------A--- ---------- ---------- C---------
hap 23    C--------- ---------- ------A--- ---------- ---------- ----------
hap 24    C--------- ---------- ------A--- ---------- ---------- ----------
hap 25    C--------- ---------- ------A--- ---------- -----T---- ----------
hap 26    C--------- ---------- ------A--- ---------- ---------- ----------
hap 27    C--------- ---------- ------A--- ---------- ---------- ----------300


hap 1     GCCCATGCAG GTTCATCTGT AGATTTAGCA ATTTTTTCGT TGCATCTAGC TGGTGTATCT
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     ---------- -C-------- ---------- ---------- -----T---- ----------
hap 4     ---------- ---------- ---C------ --------AC ---------- ----------
hap 5     ---------- -C-------- ---C------ --------AC ---------- ----------
hap 6     ---------- -C-------- ---C------ --------AC ---------- ----------
hap 7     ---------- -C-------- ---C------ --------AC ----C----- ----------
hap 8     ---------- -C-------- ---C------ --------AC ---------- ----------
hap 9     ---------- -C-------- ---C------ --------AC ---------- ----------
hap 10    ---------- ---------- ---C------ --------AC ---------- ----------
hap 11    ---------- ---------- ---C------ --------AC ---------- ----------
hap 12    ---------- ---------- ---C------ --------AC ---------- ----------
hap 13    ---------- ---------- ---------- --------A- -------C-- ----------
hap 14    ---------- ---------- ---------- --------A- -------C-- ----------
hap 15    --T------- ---------- ---------- ---------- -A-------- ----------
hap 16    --T------- ---------- ---------- ---------- -A-------- ----------
hap 17    --T------- ---------- ---------- ---------- -A-------- ----------
hap 18    ---------- ---------- ---C------ --------AC ---------- ----------
hap 19    ---------- ---------- ---C------ --------AC ---------- ----------
hap 20    ---------- ---------- ---C------ --------AC ---------- ----------
hap 21    ---------- ---------- ---C------ ---------C ---------- ----------
hap 22    ---------- ---------- ---C------ ---------C ---------- ----------
hap 23    ---------- ---------- ---C------ --------AC ---------- ----------
hap 24    ---------- ---------- ---C------ --------AC ---------- ----------
hap 25    ---------- ---------- ---C-----C ---------C ---------- ----------
hap 26    ---------- ---------- ---C-----C ---------C ---------- ----------
hap 27    ---------- ---------- ---C-----C ---------C ---------- ----------360
```

Appendix 3.3. Continued.

```
hap 1     TCAATTTTAG GGGCAGTAAA TTTTATTACC ACTGTAATTA ACATACGTTC AAGAGGTATA
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     ---------- -A--C----- ---------A ---------- -T-------- ---------G
hap 4     ---------- -A-------- ---------- ---------- ---------- ----------
hap 5     ---------- ---------- ---------- ---------- ---------- ----------
hap 6     ---------- ---------- ---------- ---------- ---------- ----------
hap 7     ---------- ---------- ---------- ---------- ---------- ----------
hap 8     ---------- ---------- ---------- ---------- ---------- ----------
hap 9     ---------- ---------- ---------- ---------- ---------- ----------
hap 10    ---------- ---------- ---------- ---------- ---------- ----------
hap 11    ---------- ---------- ---------- ---------- ---------- ----------
hap 12    ---------- ---------- ---------- ---------- ---------- ----------
hap 13    ---------- ---------- ---------- ---------- ---------- ----------
hap 14    ---------- ---------- ---------- ---------- ---------- ----------
hap 15    ---------- ---------- ---------A ---------- ---------- ------G---
hap 16    ---------- ---------- ---------A ---------- ---------- ------G---
hap 17    ---------- ---------- ---------A ---------- ---------- ------GG--
hap 18    ---------- ---------- ---------- ---------- ---------- ----------
hap 19    ---------- ---------- ---------- ---------- ---------- ----------
hap 20    ---------- ---------- ---------- ---------- ---------- ----------
hap 21    ---------- ---------- ---------- ---------- ---------- ----------
hap 22    ---------- ---------- ---------- ---------- ---------- ----------
hap 23    ---------- ---------- ---------- ---------- ------C--- ----------
hap 24    ---------- -A-------- ---------- ---------- ------C--- ----------
hap 25    ---------- ---------- ---------- ---------- ---------- ----------
hap 26    ---------- ---------- ---------- ---------- ---------- ----------
hap 27    ---------- ---------- ---------- ---------- ---------- ----------420


hap 1     ACCTTGGATC GAATACCATT ATTTGTTTGA GCAGTTGTGA TTACAGCTCT ATTGCTCCTT
hap 2     ---------- ---------- ---------- ---------- ---------- ----------
hap 3     -----A--C- ---------- ---------- --------A- ---------- ---A--A---
hap 4     -----A--C- ---------- ---------- ---------- ---------- ----------
hap 5     -----A--C- ---------- ---------- ---------- ---------- ----------
hap 6     -----A--C- ---------- ---------- ---------- ---------- ----------
hap 7     -----A--C- ---------- ---------- ---------- ---------- ----------
hap 8     -----A--C- ---------- ---------- ---------- ---------- ----------
hap 9     -----A--C- ---------- ---------- ---------- ---------- ----------
hap 10    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 11    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 12    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 13    ---------- ---------- ---------- ---------- ---------- ----------
hap 14    ---------- ---------- ---------- ---------- ---------- ----------
hap 15    ---------C ---------- ---------- ---------- ---------- ----------
hap 16    --T-------C ---------- ---------- ---------- ---------- ----------
hap 17    ---------C ---------- ---------- ---------- ---------- ----------
hap 18    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 19    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 20    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 21    -----A--C- ---------- ---------- ---------- ---------- ----------
hap 22    -----A--C- --------C- ---------- ---------- ---------- ----------
hap 23    --------C- ---------- ---------- ---------- ---------- ----------
hap 24    --------C- ---------- ---------- ---------- ---------- ----------
hap 25    --------C- ---------- ---------- ---------- ---------- ----------
hap 26    --------C- ---------- ---------- ---------- ---------- ----------
hap 27    --------C- ---------- ---------- ---------- ---------- ----------480
```

Appendix 3.3. Continued.

```
hap 1     TTATCTTTAC CAGTATTAGC TGGAGCCATT ACTATACTCT TAACAGACCG GAACTTAAAT
hap 2     ---------- ---------- ---------- --C------- ---------- ----------
hap 3     ---------- ----G----- ---G--A--C ---------C ---------- A---------
hap 4     ---------- ---------- ---------- ---------- ---------- A---------
hap 5     ---------- ---------- ---------- ---------- ---------- A---------
hap 6     ---------- ---------- ---------- ---------- ---------- A---------
hap 7     ---------- ---------- ---------- ---------- ---------- A---------
hap 8     ---------- ---------- ---------- ---------- ---------- A---------
hap 9     ---------- ---------- ---------- ---------- ---------- A---------
hap 10    ---------- ---------- ---------- ---------- ---------- A---------
hap 11    ---------- ---------- ---------- ---------- ---------- A---------
hap 12    ---------- ---------- ---------- ---------- ---------- A---------
hap 13    ---------- ---------- ---------- ---------- ---------- ----------
hap 14    ---------- ---------- ---------- ---------- ---------- A---------
hap 15    ---------- ---------- ---------- ---------- ---------- A---------
hap 16    ---------- ---------- ---------- ---------- ---------- A---------
hap 17    ---------- ---------- ---------- ---------- ---------- A---------
hap 18    ---------- ---------- ---------- ---------- ---------- A---------
hap 19    ---------- ---------- ---------- ---------- ---------- A---------
hap 20    ---------- -------G-- ---------- --------T- ---------- A---------
hap 21    ---------- ---------- ---------- ---------- ---------- A---------
hap 22    ---------- ---------- ---------- ---------- ---------- A---------
hap 23    ---------- ---------- ---------- ---------C ---------- A---------
hap 24    ---------- ---------- ---------- ---------- ---------- A---------
hap 25    ---------- ---------- ---------- ---------- ---------- A---------
hap 26    ---------- ---------- ---------- ---------- ---------- A---------
hap 27    ---------- ---------- ---------- ---------- ---------- A---------540

hap 1     ACATCATTTT TCGATCC
hap 2     ---------- -------
hap 3     ---------- ----C--
hap 4     ---------- -------
hap 5     ---------- -------
hap 6     ---------- -------
hap 7     ---------- -------
hap 8     ---------- -------
hap 9     ---------- -------
hap 10    ---------- -------
hap 11    ---------- -------
hap 12    ---------- -------
hap 13    ---------- -------
hap 14    ---------- -------
hap 15    ---------- -------
hap 16    ---------- -------
hap 17    ---------- -------
hap 18    ---------- -------
hap 19    ---------- -------
hap 20    ---------- -------
hap 21    ---------- -------
hap 22    ---------- -------
hap 23    ---------- -------
hap 24    ---------- ----C--
hap 25    ---------- -------
hap 26    ---------- -------
hap 27    ---------- -------557
```

Appendix 3.4. *Aphanicerca capensis* species complex uncorrected p-distances between the 40 individuals and 6 outgroup taxa sampled. Row and column headers for the ingroup comprise the specimen field codes. The exact localities and morphogroups are provided in Table 3.12. To calculate percentage difference, multiply the value by 100.

Specimen code	D4	D3	A2	A1	C1	C2	J2	J1	O2	O1	DDD2
D4	-										
D3	0.0036	-									
A2	0.0736	0.0736	-								
A1	0.0736	0.0736	0.0000	-							
C1	0.0377	0.0377	0.0664	0.0664	-						
C2	0.0377	0.0377	0.0664	0.0664	0.0000	-					
J2	0.0377	0.0377	0.0682	0.0682	0.0144	0.0144	-				
J1	0.0359	0.0359	0.0664	0.0664	0.0126	0.0126	0.0018	-			
O2	0.0413	0.0413	0.0682	0.0682	0.0144	0.0144	0.0072	0.0054	-		
O1	0.0395	0.0395	0.0664	0.0664	0.0126	0.0126	0.0054	0.0036	0.0054	-	
DDD2	0.0359	0.0359	0.0700	0.0700	0.0126	0.0126	0.0054	0.0036	0.0054	0.0036	-
M2	0.0287	0.0287	0.0664	0.0664	0.0341	0.0341	0.0341	0.0323	0.0377	0.0359	0.0359
I3	0.0305	0.0305	0.0646	0.0646	0.0072	0.0072	0.0072	0.0054	0.0108	0.0090	0.0090
I2	0.0323	0.0323	0.0664	0.0664	0.0072	0.0072	0.0090	0.0072	0.0108	0.0090	0.0090
I1	0.0341	0.0341	0.0682	0.0682	0.0108	0.0108	0.0108	0.0090	0.0144	0.0126	0.0126
F2	0.0126	0.0126	0.0754	0.0754	0.0323	0.0323	0.0359	0.0341	0.0395	0.0377	0.0341
F4	0.0126	0.0126	0.0718	0.0718	0.0323	0.0323	0.0323	0.0305	0.0359	0.0341	0.0305
N2	0.0287	0.0287	0.0664	0.0664	0.0341	0.0341	0.0341	0.0323	0.0377	0.0359	0.0359
CCC3	0.0287	0.0287	0.0664	0.0664	0.0341	0.0341	0.0341	0.0323	0.0377	0.0359	0.0359
N3	0.0287	0.0287	0.0664	0.0664	0.0341	0.0341	0.0341	0.0323	0.0377	0.0359	0.0359
CCC1	0.0305	0.0305	0.0682	0.0682	0.0359	0.0359	0.0359	0.0341	0.0395	0.0377	0.0377
P5	0.0323	0.0323	0.0664	0.0664	0.0090	0.0090	0.0090	0.0072	0.0126	0.0108	0.0108
P1	0.0323	0.0323	0.0664	0.0664	0.0108	0.0108	0.0108	0.0090	0.0144	0.0126	0.0126
P3	0.0377	0.0377	0.0682	0.0682	0.0108	0.0108	0.0144	0.0126	0.0180	0.0126	0.0162
P4	0.0323	0.0323	0.0664	0.0664	0.0108	0.0108	0.0108	0.0090	0.0144	0.0126	0.0126
L4a	0.0305	0.0305	0.0646	0.0646	0.0072	0.0072	0.0072	0.0054	0.0108	0.0090	0.0090
L3	0.0305	0.0305	0.0646	0.0646	0.0072	0.0072	0.0072	0.0054	0.0108	0.0090	0.0090
L5	0.0305	0.0305	0.0646	0.0646	0.0108	0.0108	0.0108	0.0090	0.0144	0.0126	0.0126
L2	0.0323	0.0323	0.0664	0.0664	0.0126	0.0126	0.0126	0.0108	0.0162	0.0144	0.0144
E6	0.0305	0.0305	0.0646	0.0646	0.0108	0.0108	0.0108	0.0090	0.0144	0.0126	0.0126
E1	0.0305	0.0305	0.0646	0.0646	0.0108	0.0108	0.0108	0.0090	0.0144	0.0126	0.0126
N4	0.0287	0.0287	0.0664	0.0664	0.0341	0.0341	0.0341	0.0323	0.0377	0.0359	0.0359
G2	0.0305	0.0305	0.0646	0.0646	0.0072	0.0072	0.0072	0.0054	0.0108	0.0090	0.0090
G1	0.0305	0.0305	0.0646	0.0646	0.0072	0.0072	0.0072	0.0054	0.0108	0.0090	0.0090
H2	0.0269	0.0269	0.0646	0.0646	0.0215	0.0215	0.0215	0.0197	0.0251	0.0233	0.0197
H3	0.0287	0.0287	0.0628	0.0628	0.0197	0.0197	0.0233	0.0215	0.0269	0.0251	0.0215
B5	0.0269	0.0269	0.0646	0.0646	0.0215	0.0215	0.0215	0.0197	0.0251	0.0233	0.0233
B1	0.0251	0.0251	0.0628	0.0628	0.0197	0.0197	0.0197	0.0180	0.0233	0.0215	0.0215
B2	0.0251	0.0251	0.0628	0.0628	0.0197	0.0197	0.0197	0.0180	0.0233	0.0215	0.0215
B4	0.0269	0.0269	0.0646	0.0646	0.0215	0.0215	0.0215	0.0197	0.0251	0.0233	0.0233
A. bainii sp. n. AD1	0.0969	0.0934	0.0916	0.0916	0.0880	0.0880	0.0934	0.0916	0.0952	0.0934	0.0934
A. bicornis S3	0.0969	0.0934	0.0969	0.0969	0.0880	0.0880	0.0969	0.0952	0.0987	0.0969	0.0934
A. bovina T1	0.0790	0.0754	0.0718	0.0718	0.0736	0.0736	0.0736	0.0718	0.0772	0.0754	0.0754
A. chanae U3	0.0700	0.0700	0.0736	0.0736	0.0664	0.0664	0.0682	0.0664	0.0700	0.0682	0.0646
A. lyrata W2	0.0934	0.0898	0.0934	0.0934	0.0880	0.0880	0.0934	0.0916	0.0916	0.0934	0.0898
A. uncinata X3	0.0180	0.0180	0.0664	0.0664	0.0341	0.0341	0.0341	0.0323	0.0377	0.0359	0.0359

Appendix 3.4. Continued.

	M2	I3	I2	I1	F2	F4	N2	CCC3	N3	CCC1
M2	-									
I3	0.0269	-								
I2	0.0287	0.0018	-							
I1	0.0305	0.0036	0.0054	-						
F2	0.0305	0.0287	0.0305	0.0323	-					
F4	0.0269	0.0251	0.0269	0.0287	0.0036	-				
N2	0.0036	0.0269	0.0287	0.0305	0.0305	0.0269	-			
CCC3	0.0036	0.0269	0.0287	0.0305	0.0305	0.0269	0.0000	-		
N3	0.0036	0.0269	0.0287	0.0305	0.0305	0.0269	0.0000	0.0000	-	
CCC1	0.0054	0.0287	0.0305	0.0323	0.0323	0.0287	0.0018	0.0018	0.0018	-
P5	0.0287	0.0018	0.0036	0.0054	0.0305	0.0269	0.0287	0.0287	0.0287	0.0305
P1	0.0287	0.0036	0.0054	0.0072	0.0305	0.0269	0.0287	0.0287	0.0287	0.0305
P3	0.0341	0.0072	0.0090	0.0108	0.0323	0.0323	0.0341	0.0341	0.0341	0.0359
P4	0.0287	0.0036	0.0054	0.0072	0.0305	0.0269	0.0287	0.0287	0.0287	0.0305
L4a	0.0269	0.0000	0.0018	0.0036	0.0287	0.0251	0.0269	0.0269	0.0269	0.0287
L3	0.0269	0.0000	0.0018	0.0036	0.0287	0.0251	0.0269	0.0269	0.0269	0.0287
L5	0.0269	0.0036	0.0054	0.0072	0.0323	0.0287	0.0269	0.0269	0.0269	0.0287
L2	0.0287	0.0054	0.0072	0.0090	0.0341	0.0305	0.0287	0.0287	0.0287	0.0305
E6	0.0269	0.0036	0.0054	0.0072	0.0323	0.0287	0.0269	0.0269	0.0269	0.0287
E1	0.0269	0.0036	0.0054	0.0072	0.0323	0.0287	0.0269	0.0269	0.0269	0.0287
N4	0.0036	0.0269	0.0287	0.0305	0.0305	0.0269	0.0000	0.0000	0.0000	0.0018
G2	0.0269	0.0000	0.0018	0.0036	0.0287	0.0251	0.0269	0.0269	0.0269	0.0287
G1	0.0269	0.0000	0.0018	0.0036	0.0287	0.0251	0.0269	0.0269	0.0269	0.0287
H2	0.0269	0.0144	0.0162	0.0144	0.0251	0.0215	0.0269	0.0269	0.0269	0.0287
H3	0.0287	0.0162	0.0180	0.0162	0.0269	0.0233	0.0287	0.0287	0.0287	0.0305
B5	0.0197	0.0144	0.0162	0.0180	0.0287	0.0251	0.0197	0.0197	0.0197	0.0215
B1	0.0180	0.0126	0.0144	0.0162	0.0269	0.0233	0.0180	0.0180	0.0180	0.0197
B2	0.0180	0.0126	0.0144	0.0162	0.0269	0.0233	0.0180	0.0180	0.0180	0.0197
B4	0.0162	0.0144	0.0162	0.0180	0.0287	0.0251	0.0162	0.0162	0.0162	0.0180
A. bainii sp. n. AD1	0.0898	0.0862	0.0844	0.0862	0.0952	0.0916	0.0898	0.0898	0.0898	0.0880
A. bicornis S3	0.0934	0.0898	0.0880	0.0934	0.0952	0.0916	0.0934	0.0934	0.0934	0.0916
A. bovina T1	0.0628	0.0664	0.0682	0.0664	0.0772	0.0736	0.0628	0.0628	0.0628	0.0646
A. chanae U3	0.0610	0.0646	0.0628	0.0682	0.0682	0.0646	0.0610	0.0610	0.0610	0.0628
A. lyrata W2	0.0898	0.0862	0.0862	0.0898	0.0916	0.0880	0.0898	0.0898	0.0898	0.0880
A. uncinata X3	0.0251	0.0269	0.0287	0.0305	0.0162	0.0162	0.0251	0.0251	0.0251	0.0269

Appendix 3.4. Continued.

	P5	P1	P3	P4	L4a	L3	L5	L2	E6
P5	-								
P1	0.0018	-							
P3	0.0090	0.0108	-						
P4	0.0018	0.0000	0.0108	-					
L4a	0.0018	0.0036	0.0072	0.0036	-				
L3	0.0018	0.0036	0.0072	0.0036	0.0000	-			
L5	0.0054	0.0072	0.0108	0.0072	0.0036	0.0036	-		
L2	0.0072	0.0090	0.0126	0.0090	0.0054	0.0054	0.0018	-	
E6	0.0054	0.0072	0.0108	0.0072	0.0036	0.0036	0.0000	0.0018	-
E1	0.0054	0.0072	0.0108	0.0072	0.0036	0.0036	0.0000	0.0018	0.0000
N4	0.0287	0.0287	0.0341	0.0287	0.0269	0.0269	0.0269	0.0287	0.0269
G2	0.0018	0.0036	0.0072	0.0036	0.0000	0.0000	0.0036	0.0054	0.0036
G1	0.0018	0.0036	0.0072	0.0036	0.0000	0.0000	0.0036	0.0054	0.0036
H2	0.0162	0.0162	0.0215	0.0162	0.0144	0.0144	0.0180	0.0197	0.0180
H3	0.0180	0.0180	0.0233	0.0180	0.0162	0.0162	0.0197	0.0215	0.0197
B5	0.0162	0.0162	0.0215	0.0162	0.0144	0.0144	0.0144	0.0162	0.0144
B1	0.0144	0.0144	0.0197	0.0144	0.0126	0.0126	0.0126	0.0144	0.0126
B2	0.0144	0.0144	0.0197	0.0144	0.0126	0.0126	0.0126	0.0144	0.0126
B4	0.0162	0.0162	0.0215	0.0162	0.0144	0.0144	0.0144	0.0162	0.0144
A. bainii sp. n. AD1	0.0844	0.0844	0.0934	0.0844	0.0862	0.0862	0.0898	0.0916	0.0898
A. bicornis S3	0.0880	0.0880	0.0969	0.0880	0.0898	0.0898	0.0934	0.0952	0.0934
A. bovina T1	0.0646	0.0646	0.0736	0.0646	0.0664	0.0664	0.0700	0.0718	0.0700
A. chanae U3	0.0628	0.0628	0.0718	0.0628	0.0646	0.0646	0.0682	0.0700	0.0682
A. lyrata W2	0.0844	0.0844	0.0934	0.0844	0.0862	0.0862	0.0898	0.0916	0.0898
A. uncinata X3	0.0287	0.0287	0.0323	0.0287	0.0269	0.0269	0.0269	0.0287	0.0269

Appendix 3.4. Continued.

	E1	N4	G2	G1	H2	H3	B5	B1
E1	-							
N4	0.0269	-						
G2	0.0036	0.0269	-					
G1	0.0036	0.0269	0.0000	-				
H2	0.0180	0.0269	0.0144	0.0144	-			
H3	0.0197	0.0287	0.0162	0.0162	0.0054	-		
B5	0.0144	0.0197	0.0144	0.0144	0.0144	0.0162	-	
B1	0.0126	0.0180	0.0126	0.0126	0.0126	0.0144	0.0018	-
B2	0.0126	0.0180	0.0126	0.0126	0.0126	0.0144	0.0018	0.0000
B4	0.0144	0.0162	0.0144	0.0144	0.0144	0.0162	0.0036	0.0018
A. bainii sp. n. AD1	0.0898	0.0898	0.0862	0.0862	0.0880	0.0862	0.0898	0.0880
A. bicornis S3	0.0934	0.0934	0.0898	0.0898	0.0916	0.0898	0.0934	0.0916
A. bovina T1	0.0700	0.0628	0.0664	0.0664	0.0628	0.0646	0.0664	0.0682
A. chanae U3	0.0682	0.0610	0.0646	0.0646	0.0610	0.0592	0.0682	0.0664
A. lyrata W2	0.0898	0.0898	0.0862	0.0862	0.0880	0.0862	0.0898	0.0880
A. uncinata X3	0.0269	0.0251	0.0269	0.0269	0.0269	0.0287	0.0233	0.0215

Appendix 3.4. Continued.

	B2	B4	*A. bainii* sp.nov. AD1	*A. bicornis* S3	*A. bovina* T1	*A. chanae* U3	*A. lyrata* W2	*A. uncinata* X3
B2	-							
B4	0.0018	-						
A. bainii sp. n. AD1	0.0880	0.0898	-					
A. bicornis S3	0.0916	0.0934	0.0108	-				
A. bovina T1	0.0682	0.0700	0.0916	0.0952	-			
A. chanae U3	0.0664	0.0664	0.0826	0.0790	0.0754	-		
A. lyrata W2	0.0880	0.0898	0.0126	0.0126	0.0952	0.0808	-	
A. uncinata X3	0.0215	0.0233	0.0916	0.0952	0.0754	0.0664	0.0916	-

Appendix 3.5. *Aphanicerca capensis* species complex corrected distances (Tamura-Nei with gamma shape parameter α = 0.145) between the 40 individuals sampled. Row and column headers comprise the specimen field codes. The exact localities and morphogroups are provided in Table 3.12. To calculate percentage difference, multiply the value by 100.

	D4	D3	A2	A1	C1	C2	J2	J1	O2	O1	DDD2	M2	I3	I2
D4	-													
D3	0.0038	-												
A2	0.1453	0.1453	-											
A1	0.1453	0.1453	0.0000	-										
C1	0.0508	0.0508	0.1149	0.1149	-									
C2	0.0508	0.0508	0.1149	0.1149	0.0000	-								
J2	0.0530	0.0530	0.1186	0.1186	0.0162	0.0162	-							
J1	0.0492	0.0492	0.1144	0.1144	0.0139	0.0139	0.0018	-						
O2	0.0590	0.0590	0.1186	0.1186	0.0162	0.0162	0.0076	0.0057	-					
O1	0.0552	0.0552	0.1144	0.1144	0.0139	0.0139	0.0057	0.0038	0.0057	-				
DDD2	0.0485	0.0485	0.1255	0.1255	0.0142	0.0142	0.0057	0.0037	0.0057	0.0037	-			
M2	0.0360	0.0360	0.1208	0.1208	0.0433	0.0433	0.0453	0.0418	0.0507	0.0472	0.0479	-		
I3	0.0390	0.0390	0.1104	0.1104	0.0076	0.0076	0.0080	0.0059	0.0119	0.0097	0.0099	0.0325	-	
I2	0.0414	0.0414	0.1142	0.1142	0.0075	0.0075	0.0100	0.0078	0.0119	0.0096	0.0100	0.0349	0.0018	-
I1	0.0451	0.0451	0.1212	0.1212	0.0117	0.0117	0.0122	0.0099	0.0163	0.0140	0.0142	0.0380	0.0037	0.0056
F2	0.0139	0.0139	0.1496	0.1496	0.0408	0.0408	0.0503	0.0465	0.0556	0.0517	0.0454	0.0384	0.0362	0.0385
F4	0.0138	0.0138	0.1367	0.1367	0.0411	0.0411	0.0441	0.0404	0.0490	0.0454	0.0394	0.0328	0.0307	0.0330
N2	0.0357	0.0357	0.1174	0.1174	0.0430	0.0430	0.0453	0.0418	0.0504	0.0469	0.0478	0.0037	0.0324	0.0348
CCC3	0.0357	0.0357	0.1174	0.1174	0.0430	0.0430	0.0453	0.0418	0.0504	0.0469	0.0478	0.0037	0.0324	0.0348
N3	0.0357	0.0357	0.1174	0.1174	0.0430	0.0430	0.0453	0.0418	0.0504	0.0469	0.0478	0.0037	0.0324	0.0348
CCC1	0.0387	0.0387	0.1216	0.1216	0.0461	0.0461	0.0491	0.0453	0.0542	0.0504	0.0516	0.0056	0.0354	0.0378
P5	0.0419	0.0419	0.1173	0.1173	0.0097	0.0097	0.0099	0.0077	0.0140	0.0118	0.0119	0.0351	0.0019	0.0037
P1	0.0419	0.0419	0.1173	0.1173	0.0120	0.0120	0.0119	0.0097	0.0163	0.0141	0.0140	0.0351	0.0038	0.0057
P3	0.0516	0.0516	0.1186	0.1186	0.0120	0.0120	0.0167	0.0142	0.0210	0.0142	0.0188	0.0439	0.0076	0.0096
P4	0.0419	0.0419	0.1173	0.1173	0.0120	0.0120	0.0119	0.0097	0.0163	0.0141	0.0140	0.0351	0.0038	0.0057
L4A	0.0390	0.0390	0.1104	0.1104	0.0076	0.0076	0.0080	0.0059	0.0119	0.0097	0.0099	0.0325	0.0000	0.0018
L3	0.0390	0.0390	0.1104	0.1104	0.0076	0.0076	0.0080	0.0059	0.0119	0.0097	0.0099	0.0325	0.0000	0.0018
L5	0.0387	0.0387	0.1077	0.1077	0.0117	0.0117	0.0119	0.0096	0.0160	0.0138	0.0139	0.0324	0.0037	0.0056
L2	0.0419	0.0419	0.1118	0.1118	0.0137	0.0137	0.0142	0.0119	0.0184	0.0160	0.0162	0.0354	0.0056	0.0075
E6	0.0387	0.0387	0.1077	0.1077	0.0117	0.0117	0.0119	0.0096	0.0160	0.0138	0.0139	0.0324	0.0037	0.0056
E1	0.0387	0.0387	0.1077	0.1077	0.0117	0.0117	0.0119	0.0096	0.0160	0.0138	0.0139	0.0324	0.0037	0.0056
N4	0.0357	0.0357	0.1174	0.1174	0.0430	0.0430	0.0453	0.0418	0.0504	0.0469	0.0478	0.0037	0.0324	0.0348
G2	0.0390	0.0390	0.1104	0.1104	0.0076	0.0076	0.0080	0.0059	0.0119	0.0097	0.0099	0.0325	0.0000	0.0018
G1	0.0390	0.0390	0.1104	0.1104	0.0076	0.0076	0.0080	0.0059	0.0119	0.0097	0.0099	0.0325	0.0000	0.0018
H2	0.0343	0.0343	0.1169	0.1169	0.0250	0.0250	0.0263	0.0235	0.0308	0.0280	0.0230	0.0338	0.0159	0.0180
H3	0.0371	0.0371	0.1098	0.1098	0.0226	0.0226	0.0285	0.0257	0.0333	0.0305	0.0253	0.0363	0.0181	0.0202
B5	0.0347	0.0347	0.1107	0.1107	0.0248	0.0248	0.0256	0.0229	0.0302	0.0275	0.0279	0.0229	0.0157	0.0178
B1	0.0315	0.0315	0.1068	0.1068	0.0225	0.0225	0.0229	0.0204	0.0275	0.0250	0.0252	0.0204	0.0135	0.0156
B2	0.0315	0.0315	0.1068	0.1068	0.0225	0.0225	0.0229	0.0204	0.0275	0.0250	0.0252	0.0204	0.0135	0.0156
B4	0.0347	0.0347	0.1107	0.1107	0.0248	0.0248	0.0256	0.0229	0.0302	0.0275	0.0279	0.0180	0.0157	0.0178

Appendix 3.5. Continued.

	I1	F2	F4	N2	CCC3	N3	CCC1	P5	P1	P3	P4	L4A	L3	L5
I1	-													
F2	0.0419	-												
F4	0.0362	0.0037	-											
N2	0.0378	0.0380	0.0325	-										
CCC3	0.0378	0.0380	0.0325	0.0000	-									
N3	0.0378	0.0380	0.0325	0.0000	0.0000	-								
CCC1	0.0410	0.0411	0.0354	0.0018	0.0018	0.0018	-							
P5	0.0057	0.0387	0.0331	0.0349	0.0349	0.0349	0.0378	-						
P1	0.0078	0.0387	0.0331	0.0349	0.0349	0.0349	0.0378	0.0019	-					
P3	0.0118	0.0414	0.0419	0.0436	0.0436	0.0436	0.0469	0.0098	0.0120	-				
P4	0.0078	0.0387	0.0331	0.0349	0.0349	0.0349	0.0378	0.0019	0.0000	0.0120	-			
L4A	0.0037	0.0362	0.0307	0.0324	0.0324	0.0324	0.0354	0.0019	0.0038	0.0076	0.0038	-		
L3	0.0037	0.0362	0.0307	0.0324	0.0324	0.0324	0.0354	0.0019	0.0038	0.0076	0.0038	0.0000	-	
L5	0.0076	0.0411	0.0354	0.0324	0.0324	0.0324	0.0354	0.0057	0.0079	0.0117	0.0079	0.0037	0.0037	-
L2	0.0096	0.0444	0.0385	0.0354	0.0354	0.0354	0.0386	0.0076	0.0097	0.0138	0.0097	0.0056	0.0056	0.0018
E6	0.0076	0.0411	0.0354	0.0324	0.0324	0.0324	0.0354	0.0057	0.0079	0.0117	0.0079	0.0037	0.0037	0.0000
E1	0.0076	0.0411	0.0354	0.0324	0.0324	0.0324	0.0354	0.0057	0.0079	0.0117	0.0079	0.0037	0.0037	0.0000
N4	0.0378	0.0380	0.0325	0.0000	0.0000	0.0000	0.0018	0.0349	0.0349	0.0436	0.0349	0.0324	0.0324	0.0324
G2	0.0037	0.0362	0.0307	0.0324	0.0324	0.0324	0.0354	0.0019	0.0038	0.0076	0.0038	0.0000	0.0000	0.0037
G1	0.0037	0.0362	0.0307	0.0324	0.0324	0.0324	0.0354	0.0019	0.0038	0.0076	0.0038	0.0000	0.0000	0.0037
H2	0.0162	0.0315	0.0263	0.0338	0.0338	0.0338	0.0370	0.0181	0.0181	0.0253	0.0181	0.0159	0.0159	0.0202
H3	0.0183	0.0340	0.0286	0.0362	0.0362	0.0362	0.0394	0.0204	0.0204	0.0278	0.0204	0.0181	0.0181	0.0226
B5	0.0202	0.0370	0.0315	0.0230	0.0230	0.0230	0.0257	0.0179	0.0179	0.0250	0.0179	0.0157	0.0157	0.0157
B1	0.0179	0.0338	0.0285	0.0204	0.0204	0.0204	0.0230	0.0158	0.0158	0.0226	0.0158	0.0135	0.0135	0.0135
B2	0.0179	0.0338	0.0285	0.0204	0.0204	0.0204	0.0230	0.0158	0.0158	0.0226	0.0158	0.0135	0.0135	0.0135
B4	0.0202	0.0370	0.0315	0.0180	0.0180	0.0180	0.0204	0.0179	0.0179	0.0250	0.0179	0.0157	0.0157	0.0157

Appendix 3.5. Continued.

	L2	E6	E1	N4	G2	G1	H2	H3	B5	B1	B2	B4
L2	-											
E6	0.0018	-										
E1	0.0018	0.0000	-									
N4	0.0354	0.0324	0.0324	-								
G2	0.0056	0.0037	0.0037	0.0324	-							
G1	0.0056	0.0037	0.0037	0.0324	0.0000	-						
H2	0.0226	0.0202	0.0202	0.0338	0.0159	0.0159	-					
H3	0.0250	0.0226	0.0226	0.0362	0.0181	0.0181	0.0057	-				
B5	0.0180	0.0157	0.0157	0.0230	0.0157	0.0157	0.0162	0.0184	-			
B1	0.0157	0.0135	0.0135	0.0204	0.0135	0.0135	0.0139	0.0160	0.0018	-		
B2	0.0157	0.0135	0.0135	0.0204	0.0135	0.0135	0.0139	0.0160	0.0018	0.0000	-	
B4	0.0180	0.0157	0.0157	0.0180	0.0157	0.0157	0.0162	0.0184	0.0038	0.0018	0.0018	-

Appendix 3.6. Comparative (relational) species delimitation within the *Aphanicerca capensis* species complex (summarized in Table 3.20). Morphogroups are compared in a pairwise manner, highlighting distributional and morphological differences where appropriate, and summarizing the secondary species criteria that support species designation. Abbreviations: adp = distance between apices of tergite 9 dorsal process lobes; dp = length of tergite 9 dorsal process lobes; epd = distance from epiproct tip to first denticle; epl = epiproct length; epw = epiproct width; hcw = head capsule width; pnw = pronotum width; ppw = paraproct apex width; sp = length of spinous ridge of tergite 9 dorsal process lobes.

B (Figs 3.3A, 3.4A, 3.5A) and ***C*** (Figs 3.3B, 3.4B, 3.5B): Morphogroup ***C*** is the type species, the type locality being Table Mountain, Cape Peninsula (Tillyard 1931). These two morphogroups are allopatric, separated by False Bay (Fig. 3.1), about 45 km across the ocean, or across the inhospitable Cape Flats. The only morphological variable that did not differ between them was pnw ($P > 0.05$). The dorsal process of tergite 9 differs vastly between the two and is alone sufficient to distinguish them. The females differ in minor respects, with the posterior margin of the SGP of ***C*** rounded with a short median protuberance. Morphogroup ***C*** formed its own 0-step clade 30 steps from the main network, and was in fact sister to *Aphanicerca bovina* from the Stellenbosch and Franschhoek areas (northern Hottentots Holland Mountains) in the phylogenetic analyses. This relationship between COI and morphology is incongruent and is a case of the gene tree not being congruent with the species tree. There is no doubt that morphogroup ***C*** is sister to the other members of the species complex rather than to *A. bovina*, judging by the respective morphological similarities and dissimilarities and the mate choice trials. The average number of pairwise differences (pairwise distance method; uncorrected) was 41.00 (7.36%), and their respective clades were separated by a much greater than average number of steps (52) in the nested clade analysis. ***B*** and ***C*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

B (Figs 3.3A, 3.4A, 3.5A) and ***E*** (Figs 3.3C, 3.4C, 3.5C): Morphogroup ***E*** has not been recorded previously, and has only been found in the Swartberg Pass Boegoekloof stream on the southern slopes of the Groot Swartberg Mountains, separated from the range of morphogroup ***B*** by the Little Karoo, the Langeberg and the Riviersonderend Mountains, with intervening inhospitable terrain. The males of the two are very different morphologically and are easily distinguishable on the shape of the dorsal process of tergite 9, and differed significantly in five variables. The females differ in minor respects, with the posterior margin of the SGP of ***E*** rounded with a very short median protuberance. The average number of pairwise differences (pairwise distance method; uncorrected) was 21.00 (3.77%), and their respective clades were separated by a much greater than average number of steps (32) in the nested clade analysis. ***B*** and ***E*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

B (Figs 3.3A, 3.4A, 3.5A) and ***G*** (Figs 3.3D-F, 3.4D, 3.5D-F): Morphogroup ***G*** has not been recorded previously, and has only been found in the Swartberg Pass, Meiringspoort and Seweweekspoort in the Groot Swartberg Mountains, separated from the range of morphogroup ***B*** by the Little Karoo, the Langeberg and the Riviersonderend Mountains, with intervening inhospitable terrain. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in seven out of the nine variables. The females differ in minor respects, with the posterior margin of the SGP of ***G*** more rounded then ***B*** and often with a very short median protuberance. The average number of pairwise differences (pairwise distance method; uncorrected) was 21.20 (3.81%), their respective clades were separated by a much greater than average number of steps (31) in the nested clade analysis, and the population pairwise F_{ST} value was significant. ***B*** and ***G*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

B (Figs 3.3A, 3.4A, 3.5A) and ***L*** (Figs 3.3G-H, 3.4E, 3.5G-I): Morphogroup ***L*** has not been recorded previously, and has only been found in the Langeberg and therefore the two morphogroups have distant allopatric distributions. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in seven out of the nine variables. The females differ in minor respects, with the posterior margin of the SGP of ***L*** bearing a short median protuberance. The average number of pairwise differences (pairwise distance method; uncorrected) was 18.00 (3.23%), and their respective clades were separated by a much greater than average number of steps (28) in the nested clade analysis. ***B*** and ***L*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***B***.

B (Figs 3.3A, 3.4A, 3.5A) and ***N*** (Figs 3.3I, 3.4F, 3.5J): Morphogroup ***B*** is endemic to a small coastal montane area to the east of False Bay in the southern Hottentots Holland Mountains around Betty's Bay (Fig. 3.1). The male is morphologically highly distinct from the other known morphogroups, with the exception of ***N*** where the differences are more subtle, but still easily distinguished at a glance, and up to now have been found to be consistent. Even though they differed in six of the nine morphometric variables (Table 3.3), they are morphologically quite similar. These six include dp, sp, and adp, the most important in discriminatory power, and the most useful in being able to identify morphogroups quickly and without measuring. The females are similar, with the ***N*** subgenital plate (SGP) posterior margin bearing a small median convexity, while the ***B*** SGP posterior margin is more flattened. The two morphogroups ***B*** and ***N*** are sympatric in the southern Hottentots Holland Mountains, but may potentially have parapatric

distributions within that region, with ***N*** occurring in the Fernkloof Nature Reserve in Hermanus. Collecting effort has not been extensive enough in that region to determine that. The fairly subtle morphological differences, the sympatry, the non-significant genetic divergence, their reciprocal monophyly and sharing of a common ancestor on the Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) trees, and their unification in a separate network in the nested clade analysis (NCA) all provided evidence that they are more closely related to each other than to the other morphogroups (i.e. they are sister species), and more recently speciated. The average number of pairwise differences (pairwise distance method; uncorrected) was 7.00 (1.26%) (Table 3.17). See Tables 3.16 and 3.17 for corrected average pairwise distance (Tamura-Nei distance method with $\alpha = 0.145$, and pairwise distance method respectively). Morphogroups ***B*** and ***N*** have not previously been recorded and were not featured as one of Barnard's (1934) varieties of *A. capensis*. ***B*** and ***N*** are regarded as species relative to each other because of intrinsic reproductive isolation (sympatric), morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

B (Figs 3.3A, 3.4A, 3.5A) and ***P*** (Figs 3.3J-N, 3.4G, 3.5K-L): Morphogroup ***P*** has not been recorded previously, and has only been found in the Outeniqua and Langeberg Mountains and therefore the two morphogroups have distant allopatric distributions. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in six out of the nine variables. The females differ in minor respects, with the posterior margin of the SGP of ***P*** being more rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 16.17 (2.90%), their respective clades were separated by a much greater than average number of steps (33) in the nested clade analysis, and the population pairwise F_{ST} value was significant. ***B*** and ***P*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

B (Figs 3.3A, 3.4A, 3.5A) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): Morphogroup ***R*** has not been recorded previously, and has only been found from two streams in the Langeberg Mountains and therefore the two morphogroups have distant allopatric distributions. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in seven out of the nine variables. The females differ very clearly, with the posterior margin of the SGP of ***R*** obviously notched. The average number of pairwise differences (pairwise distance method; uncorrected) was 18.75 (3.37%), and their respective clades were separated by a much greater than average number of steps (27) in the nested clade analysis. ***B*** and ***R*** are regarded as species relative to each other

because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***B***.

B (Figs 3.3A, 3.4A, 3.5A) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): Morphogroup ***S*** has only been found in the Outeniqua and Langeberg Mountains in the Western Cape Province and in the Elandsberge Mountains in the Eastern Cape Province, and therefore the two morphogroups have distant allopatric distributions. Very little collecting has been done in the Eastern Cape. Only males of this morphogroup were collected from Robinson Pass in the Outeniqua Mountains in 1932 and from Montagu Pass also in the Outeniqua range in 1933 (Barnard 1934). Barnard (1934) illustrated the dorsal process in his Fig. 7e and f, including them as a variant of *A. capensis*. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in only three out of the nine variables. The length of the dorsal process lobes (dp) does not differ, but sp, adp and epl do. The females differ greatly in that the posterior margin of the SGP of ***S*** bears a very elongated process. The average number of pairwise differences (pairwise distance method; uncorrected) was 17.17 (3.08%), their respective clades were separated by a much greater than average number of steps (26) in the nested clade analysis, and the population pairwise F_{ST} value was significant. ***B*** and ***S*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***B***.

B (Figs 3.3A, 3.4A, 3.5A) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): Morphogroup ***T*** has not been recorded previously, and has only been found in the Witsenberg Mountains near Wolseley and therefore the two morphogroups have distant allopatric distributions. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in eight out of the nine variables. The females differ in minor respects, with the posterior margin of the SGP of ***T*** being more rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 17.00 (3.05%), and their respective clades were separated by a much greater than average number of steps (26) in the nested clade analysis. ***B*** and ***T*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***B***.

B (Figs 3.3A, 3.4A, 3.5A) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): Morphogroup ***W*** has only been found in the Cederberg and therefore the two morphogroups have distant allopatric distributions. Barnard (1934) recorded a collection from the Cederberg, calling it variety α of *A. capensis*. The males of the two are very different morphologically and are easily distinguishable on the size

and shape of the dorsal process of tergite 9, and differed significantly in six out of the nine variables. The females differ in minor respects, with the posterior margin of the SGP of ***W*** bearing a short median protuberance. The average number of pairwise differences (pairwise distance method; uncorrected) was 15.50 (2.78%), and their respective clades were separated by a much greater than average number of steps (16) in the nested clade analysis. ***B*** and ***W*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

B (Figs 3.3A, 3.4A, 3.5A) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): Barnard (1934) illustrated what appears to be the ***Z*** female SGP in his Fig. 7n on page 524, and called it variety γ of *A. capensis*. He listed three localities: Palmiet River near Kleinmond in the southern Hottentots Holland Mountains, Landdrost in the Hottentots Holland Mountains (presumably the Landdroskop area in the northern Hottentots Holland), and Oudebosch (presumably what is now the farm called Oubos) in the Riviersonderend Mountains. All three localities concur with the distribution of morphogroup ***Z***, and Barnard's two illustrations agree well with the specimens examined in this study. The localities given by Barnard for his other two varieties, α and β, with the exception of the Cederberg as one of the localities given for variety α, also all lie within the known distribution limits of morphogroup ***Z*** where no other morphogroup has yet been discovered. It is presumed then that his female SGP illustrations (his Fig. 7l-n) and male tergite 9 dorsal process illustration (his Fig. 7b and 7d from Wellington, and 7h from the Tulbagh valley) are all morphogroup ***Z***. The female SGP illustrations 7m and 7l most likely represent intraspecific variation, or may be the females of as yet undiscovered morphogroups or species. The SGP of the two morphogroups ***B*** and ***Z*** cannot easily be distinguished; other traits are used instead, such as the degree of protuberance of the cerci (less so in ***B***), and the sclerotization pattern of the abdominal sternites (S1-S4 incomplete anteriorly in ***Z***, and all sternites completely sclerotized in ***B***). ***B*** and ***Z*** are sympatric in the southern Hottentots Holland Mountains, although ***Z*** has a wider distribution into the northern Hottentots Holland and Riviersonderend Mountains. They are also syntopic in at least one locality (Harold Porter Nature reserve in Betty's Bay), which confirms their status as biological species. They are also very distinct morphologically, in seven significant variables in the male (Table 3.3). The females are readily distinguishable as described above. The average number of pairwise differences (pairwise distance method; uncorrected) was 14.50 (2.60%) (Table 3.17), and their respective clades were separated by a much greater than average number of steps (23) in the nested clade analysis. ***B*** and ***Z*** are regarded as species relative to each other because of intrinsic reproductive isolation (syntopic), morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

C (Figs 3.3B, 3.4B, 3.5B) and ***E*** (Figs 3.3C, 3.4C, 3.5C): These have widely separated allopatric distributions across some inhospitable terrain including the Little Karoo (Cape Peninsula and Groot Swartberg respectively). The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in eight variables. Besides body size, there is little to differentiate the females, with both bearing a small median projection on the posterior margin of the SGP. The average number of pairwise differences (pairwise distance method; uncorrected) was 37.00 (6.64%), and their respective clades were separated by a much greater than average number of steps (39) in the nested clade analysis. ***C*** and ***E*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

C (Figs 3.3B, 3.4B, 3.5B) and ***G*** (Figs 3.3D-F, 3.4D, 3.5D-F): These have widely separated allopatric distributions across some inhospitable terrain including the Little Karoo (Cape Peninsula and Groot Swartberg respectively). The males of the two are quite similar morphologically but are distinguishable on subtle features of the dorsal process of tergite 9, and differed significantly in four morphometric variables, none of which is the dorsal process. They are the three epiproct variables and the width of the paraproct tip (Table 3.3). The females differ in minor respects, with the posterior margin median protuberance of the SGP of ***C*** longer than ***G***. The average number of pairwise differences (pairwise distance method; uncorrected) was 37.80 (6.79%), their respective clades were separated by a much greater than average number of steps (38) in the nested clade analysis, and the population pairwise F_{ST} value was significant. ***C*** and ***G*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

C (Figs 3.3B, 3.4B, 3.5B) and ***L*** (Figs 3.3G-H, 3.4E, 3.5G-I): These have widely separated allopatric distributions (Cape Peninsula and Langeberg respectively). The males of the two are quite similar morphologically but are distinguishable on subtle features of the dorsal process of tergite 9, and differed significantly in four morphometric variables, sp, epd, epl and ppw. The females differ in minor respects, with both morphogroups bearing a short median protuberance, but the sternites of ***L*** are completely sclerotized while those of ***C*** are incomplete anteriorly on sternites 3-6. The average number of pairwise differences (pairwise distance method; uncorrected) was 37.00 (6.64%), and their respective clades were separated by a much greater than average number of steps (35) in the nested clade analysis. ***C*** and ***L*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***C***.

C (Figs 3.3B, 3.4B, 3.5B) and ***N*** (Figs 3.3I, 3.4F, 3.5J): These two morphogroups were separated geographically by the Cape Flats and False Bay, about 50 km across the ocean or inhospitable terrain. The males of the two are very divergent morphologically, easily distinguishable on the shape of the dorsal process of tergite 9, and differed significantly in six morphometric variables. The females differ in minor respects, with both morphogroups bearing a short median protuberance, but the sternites of ***L*** are completely sclerotized while those of ***C*** are incomplete anteriorly on sternites 3-6. The average number of pairwise differences (pairwise distance method; uncorrected) was 41.00 (7.36%), and their respective clades were separated by a much greater than average number of steps (46) in the nested clade analysis. ***C*** and ***N*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

C (Figs 3.3B, 3.4B, 3.5B) and ***P*** (Figs 3.3J-N, 3.4G, 3.5K-L): These have widely separated allopatric distributions (Cape Peninsula, and Langeberg and Outeniqua Mountains respectively). The males of the two are quite similar morphologically but are distinguishable on subtle features of the dorsal process of tergite 9, and differed significantly in four morphometric variables, none of which are the dorsal process. They are the three epiproct variables and the width of the paraproct tip (Table 3.3). The females differ very clearly, with ***C*** bearing a posterior margin median protuberance of the SGP (***P*** rounded), and ***P*** sternites completely sclerotized (***C*** S3-6 incomplete anteriorly). The average number of pairwise differences (pairwise distance method; uncorrected) was 37.17 (6.67%), their respective clades were separated by a much greater than average number of steps (42) in the nested clade analysis, and the population pairwise F_{ST} value was significant. ***C*** and ***P*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

C (Figs 3.3B, 3.4B, 3.5B) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): These have widely separated allopatric distributions (Cape Peninsula and Langeberg respectively). The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in all nine morphometric variables. The females differ very clearly, with the posterior margin of the SGP of ***R*** obviously notched. The average number of pairwise differences (pairwise distance method; uncorrected) was 37.25 (6.69%), and their respective clades were separated by a much greater than average number of steps (36) in the nested clade analysis. ***C*** and ***R*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***C***.

C (Figs 3.3B, 3.4B, 3.5B) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): These have widely separated allopatric distributions (Cape Peninsula, and Langeberg and Outeniqua Mountains respectively). The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in all nine morphometric variables. The females differ greatly in that the posterior margin of the SGP of ***S*** bears a very elongated median process, as compared to the short process of ***C***. The average number of pairwise differences (pairwise distance method; uncorrected) was 36.17 (6.49%), their respective clades were separated by a much greater than average number of steps (35) in the nested clade analysis, and the population pairwise F_{ST} value was significant. ***C*** and ***S*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***C***.

C (Figs 3.3B, 3.4B, 3.5B) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These have fairly widely separated allopatric distributions (Cape Peninsula and Witsenberg respectively). The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in six morphometric variables. The females differ very clearly, with the posterior margin of the SGP of ***T*** well rounded and without a median projection. The average number of pairwise differences (pairwise distance method; uncorrected) was 36.00 (6.46%), and their respective clades were separated by a much greater than average number of steps (35) in the nested clade analysis. ***C*** and ***T*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***C***.

C (Figs 3.3B, 3.4B, 3.5B) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These have fairly widely separated allopatric distributions (Cape Peninsula and Cederberg respectively). Mate choice trials showed morphogroup-specific assortative mating when ***C*** males were offered a choice ($P < 0.01$), but not when the same trial was conducted with ***W*** males ($P > 0.05$). This one-way premating reproductive isolation shows sufficient biological differentiation to add support for delimitation as species, and therefore ***C*** and ***W*** are regarded as biological species. The males of the two are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in seven morphometric variables. The females have similar subgenital plates, but differ in sclerotization patterns, with ***W*** sternites completely sclerotized (***C*** S3-6 incomplete anteriorly). The average number of pairwise differences (pairwise distance method; uncorrected) was 35.50 (6.37%), and their respective clades were separated by a much greater than average number of steps (35) in the nested clade analysis. ***C*** and ***W*** are regarded as species relative to each other because of premating isolation (incomplete), morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

C (Figs 3.3B, 3.4B, 3.5B) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These have allopatric distributions (Cape Peninsula, and northern and southern Hottentots Holland and Riviersonderend Mountains respectively), about 45 km across inhospitable terrain. Mate choice trials showed morphogroup-specific assortative mating when ***C*** males were offered a choice ($P < 0.01$), but not when the same trial was conducted with Bain's Kloof and Stellenbosch ***Z*** males ($P > 0.05$). This one-way premating reproductive isolation shows sufficient biological differentiation to add support for delimitation as species, and therefore ***C*** and ***Z*** are regarded as biological species. The males of the two are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in five morphometric variables. The females differ in the posterior margin of the SGP, with ***Z*** being flattened, slightly concave or having a very small median projection. ***C*** has a small but obvious projection. The average number of pairwise differences (pairwise distance method; uncorrected) was 35.50 (6.37%), and their respective clades were separated by a much greater than average number of steps (32) in the nested clade analysis. ***C*** and ***Z*** are regarded as species relative to each other because of premating isolation (incomplete), morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

E (Figs 3.3C, 3.4C, 3.5C) and ***G*** (Figs 3.3D-F, 3.4D, 3.5D-F): These two morphogroups are sympatric in the Groot Swartberg Mountains, but have not been found in the same stream. Being in such close proximity means that gene flow could potentially occur. However, no intermediate forms have been found. They are regarded as biological species relative to each other because of sympatry. The males of the two are morphologically very distinct, and differed significantly in eight morphometric variables (with the exception of ppw). The females are very similar (note that they were not caught *in copulo*, so incorrect association of sexes and species cannot be ruled out). Note that even though support for species status between this pair is strong, the average number of pairwise differences (pairwise distance method; uncorrected) was only 7.40 (1.33%). The population pairwise F_{ST} value was significant. The inference from the NCA was allopatric fragmentation (clade 4-1). ***E*** and ***G*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, intrinsic reproductive isolation (sympatric), morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

E (Figs 3.3C, 3.4C, 3.5C) and ***L*** (Figs 3.3G-H, 3.4E, 3.5G-I): These have allopatric distributions separated by the Little Karoo (Groot Swartberg and Langeberg respectively). The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in eight morphometric variables. The females are very similar, both morphogroups

bearing a short median protuberance on the SGP posterior margin. The average number of pairwise differences (pairwise distance method; uncorrected) was 4.67 (0.84%). ***E*** and ***L*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and monophyly of ***E***.

E (Figs 3.3C, 3.4C, 3.5C) and ***N*** (Figs 3.3I, 3.4F, 3.5J): These have allopatric distributions in the Groot Swartberg and southern Hottentots Holland Mountains respectively, separated by the inhospitable Little Karoo. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in seven morphometric variables. The females are very similar, both morphogroups bearing a short median protuberance on the SGP posterior margin. The average number of pairwise differences (pairwise distance method; uncorrected) was 18.00 (3.23%), and their respective clades were separated by a much greater than average number of steps (25) in the nested clade analysis. ***E*** and ***N*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

E (Figs 3.3C, 3.4C, 3.5C) and ***P*** (Figs 3.3J-N, 3.4G, 3.5K-L): These have allopatric distributions in the Groot Swartberg, and Outeniqua and Langeberg Mountains respectively. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in seven morphometric variables. The females show minor differences, with ***E*** bearing a short median protuberance on the SGP posterior margin and ***P*** rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 19.17 (3.44%), and their respective clades were separated by a much greater than average number of steps (21) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***E*** and ***P*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

E (Figs 3.3C, 3.4C, 3.5C) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): These two morphogroups were separated by the Little Karoo (endemic to the Groot Swartberg and Langeberg respectively). The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in four morphometric variables. The females differ very clearly, with the posterior margin of the SGP of ***R*** obviously notched. The average number of pairwise differences (pairwise distance method; uncorrected) was 5.75 (1.03%). ***E*** and ***R*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***E***.

E (Figs 3.3C, 3.4C, 3.5C) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): These have allopatric distributions in the Groot Swartberg, and Outeniqua and Langeberg Mountains respectively, separated by the Little Karoo. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in six morphometric variables. The females are also very distinctive, with ***E*** bearing a short median protuberance on the SGP posterior margin and ***S*** a very elongated process. The average number of pairwise differences (pairwise distance method; uncorrected) was 5.50 (0.99%). The population pairwise F_{ST} value was significant. ***E*** and ***S*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***E***.

E (Figs 3.3C, 3.4C, 3.5C) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These have distant allopatric distributions across inhospitable terrain with ***E*** endemic to the Groot Swartberg and ***T*** to the Witsenberg Mountains. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in eight morphometric variables. The females differ subtly, with ***E*** bearing a short median protuberance on the SGP posterior margin, and ***T*** smoothly rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 4.00 (0.72%). ***E*** and ***T*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***E***.

E (Figs 3.3C, 3.4C, 3.5C) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): Morphogroup ***E*** is endemic to the Groot Swartberg and ***W*** to the Cederberg and therefore the two morphogroups have allopatric distributions that are widely separated. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in seven out of the nine variables. The female SGP and sternite sclerotization patterns are very similar. The average number of pairwise differences (pairwise distance method; uncorrected) was 11.50 (2.06%), and their respective clades were separated by a greater than average number of steps (14) in the nested clade analysis. ***E*** and ***W*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

E (Figs 3.3C, 3.4C, 3.5C) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): Morphogroup ***E*** is endemic to the Groot Swartberg and ***Z*** to the northern and southern Hottentots Holland and Riviersonderend Mountains, and therefore the two morphogroups have allopatric distributions that are widely separated. The males of the two are very different morphologically and are easily distinguishable on the size and shape of the dorsal process of tergite 9, and differed significantly in eight out of the nine variables. The females differ in sternite sclerotization patterns in that ***E*** is

completely sclerotized and ***Z*** has incomplete sclerotization anteriorly on sternites 2-5, and the SGP posterior margin is flatter than ***E*** but may bear a very small median protuberance. The average number of pairwise differences (pairwise distance method; uncorrected) was 11.50 (2.06%), and their respective clades were separated by a greater than average number of steps (11) in the nested clade analysis. ***E*** and ***Z*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***L*** (Figs 3.3G-H, 3.4E, 3.5G-I): These have allopatric distributions separated by the inhospitable Little Karoo (Groot Swartberg and Langeberg respectively). The males of the two are morphologically fairly similar, but do show discernable differences in the dorsal process of tergite 9 and differed significantly in four morphometric variables. Because of intraspecific variation in the SGP of ***G*** , the females of these two morphogroups can be confused with each other. Note that even though support for species status between this pair is strong, the average number of pairwise differences (pairwise distance method; uncorrected) was only 5.40 (0.97%). The population pairwise F_{ST} value was significant. The inference from the NCA was allopatric fragmentation (clade 4-1). ***G*** and ***L*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, morphological phenetic distinguishability, morphological diagnosability (males), and monophyly of ***G***.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***N*** (Figs 3.3I, 3.4F, 3.5J): These have distant allopatric distributions in the Groot Swartberg and southern Hottentots Holland Mountains respectively, separated by the Little Karoo and other inhospitable terrain. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in five morphometric variables. The females are very similar, both morphogroups bearing a short median protuberance on the SGP posterior margin, although ***G*** may also be smoothly rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 19.20 (3.45%), and their respective clades were separated by a much greater than average number of steps (24) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***G*** and ***N*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***P*** (Figs 3.3J-N, 3.4G, 3.5K-L): These have allopatric distributions in the Groot Swartberg, and Outeniqua and Langeberg Mountains respectively, separated by the Little Karoo. The males of the two are similar morphologically, only differing

significantly in the three epiproct variables. The lobes of the dorsal process of tergite 9 differ slightly in that the outer margin in ***G*** is more convex, and usually in inner margin as well. The lobe shape in ***P*** from the Langeberg (Fig. 3.3N) seems to represent intraspecific variation, and should be subjected to further study. The females are very similar. The average number of pairwise differences (pairwise distance method; uncorrected) was 19.77 (3.55%), and their respective clades were separated by a much greater than average number of steps (30) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***G*** and ***P*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): These have allopatric distributions separated by the inhospitable Little Karoo (Groot Swartberg and Langeberg respectively). The males of the two are morphologically divergent and differed significantly in seven morphometric variables. Although the posterior margin of the SGP of ***G*** may have a slight indentation, the ***R*** females are very distinctive with a prominent notch. Note that even though support for species status between this pair is strong, the average number of pairwise differences (pairwise distance method; uncorrected) was only 6.75 (1.21%). The population pairwise F_{ST} value was significant. The inference from the NCA was allopatric fragmentation (clade 4-1). ***G*** and ***R*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***G***.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): These have allopatric distributions in the Groot Swartberg, and Outeniqua and Langeberg Mountains respectively, separated by the Little Karoo. The males of the two are morphologically divergent and differed significantly in seven morphometric variables. The ***S*** females are very distinctive with a very elongated median process of the SGP. Note that even though support for species status between this pair is strong, the average number of pairwise differences (pairwise distance method; uncorrected) was only 6.10 (1.10%). The population pairwise F_{ST} value was significant. The inference from the NCA was allopatric fragmentation (clade 4-1). ***G*** and ***S*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***G***.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These have distant allopatric distributions with ***G*** endemic to the Groot Swartberg and ***T*** to the Witsenberg Mountains. The males of the two are morphologically similar and differed significantly in only two morphometric variables, namely sp (longer in ***T***) and epw (wider in ***T***). The typical form of ***T***

(Fig. 3.3S) was very easy to differentiate from ***G*** by the concave outer margin of the proximal part of the lobe in the former, and the outward twisting of the spinous part of the lobe. The less common form (Fig. 3.3T) has a straighter outer margin and less of a twist so that it is more difficult to differentiate from ***G*** and ***P***. Nevertheless, the convex outer margin in ***G*** makes differentiation between these two morphogroups straight forward, especially so when taking into account the sp and epw. The females are very similar. The average number of pairwise differences (pairwise distance method; uncorrected) was 4.60 (0.83%). The inference from the NCA was allopatric fragmentation (clade 4-1). ***G*** and ***T*** are regarded as species relative to each other because of allopatric fragmentation, morphological phenetic distinguishability, morphological diagnosability (males), and monophyly of ***G***.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): Morphogroup ***G*** is endemic to the Groot Swartberg and ***W*** to the Cederberg and therefore the two morphogroups have allopatric distributions that are widely separated. The males of the two are fairly similar in the shape of the dorsal process of tergite 9, but nevertheless easily distinguished, and differed significantly in five morphometric variables. The females are very similar, both morphogroups bearing a short median protuberance on the SGP posterior margin, although ***G*** may also be smoothly rounded or even mildly excised. The average number of pairwise differences (pairwise distance method; uncorrected) was 12.70 (2.28%), and their respective clades were separated by a greater than average number of steps (13) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***G*** and ***W*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

G (Figs 3.3D-F, 3.4D, 3.5D-F) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): Morphogroup ***G*** is endemic to the Groot Swartberg and ***Z*** to the northern and southern Hottentots Holland and Riviersonderend Mountains, and therefore the two morphogroups have allopatric distributions that are widely separated by the Little Karoo. The males of the two are very divergent in the shape of the dorsal process of tergite 9, and differed significantly in seven out of the nine morphometric variables. The females are fairly similar, but the ***Z*** SGP posterior margin is flattened and sometimes very slightly concave with or without a very short acute tipped median protuberance, while ***G*** is more smoothly rounded or even mildly excised or may have a very short blunt median projection. The females also differ in sternite pigmentation patterns. The average number of pairwise differences (pairwise distance method; uncorrected) was 12.10 (2.17%), and their respective clades were separated by a greater than average number of steps (10) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***G*** and ***Z*** are

regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***N*** (Figs 3.3I, 3.4F, 3.5J): These have allopatric distributions in the Langeberg and southern Hottentots Holland Mountains respectively. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and yet differed significantly in only three morphometric variables, namely the dorsal process variables dp, sp and adp. The females are not easily distinguished. The average number of pairwise differences (pairwise distance method; uncorrected) was 16.00 (2.87%), and their respective clades were separated by a much greater than average number of steps (21) in the nested clade analysis. ***L*** and ***N*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and monophyly of ***N***.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***P*** (Figs 3.3J-N, 3.4G, 3.5K-L): These two morphogroups have allopatric distributions in the Langeberg and Outeniqua Mountains respectively, except for an area of syntopy at Kristalkloof in the Langeberg (Table 3.9), which probably means that they are parapatric with a small area of sympatry. There is therefore no geographical impediment to gene flow, but no morphological hybrid zone was detectable. The males of the two are subtly distinctive morphologically in the shape of the dorsal process of tergite 9, especially sp which in ***L*** is very straight and slender, and differed significantly in four morphometric variables. As mentioned earlier, the lobe shape in ***P*** from the Langeberg (Fig. 3.3N) seems to represent intraspecific variation, and should be subjected to further study. The females show minor differences, with ***L*** bearing a short median protuberance on the SGP posterior margin and ***P*** rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 16.17 (2.90%), and their respective clades were separated by a greater than average number of steps (17) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***L*** and ***P*** are regarded as species relative to each other because of genetic structure, intrinsic reproductive isolation (syntopic), morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***P***.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): These have sympatric distributions in the Langeberg. The males of the two are highly morphologically divergent and differed significantly in all nine morphometric variables. The ***R*** females are very distinctive with a prominent notch. Note that even though support for species status between this pair is strong, the average number of pairwise differences (pairwise distance method; uncorrected) was only 3.25 (0.58%). The inference from the NCA was allopatric fragmentation (clade 2-3). ***L*** and ***R*** are regarded as species relative to each other because of allopatric fragmentation, intrinsic

reproductive isolation (sympatric), morphological phenetic distinguishability, and morphological diagnosability.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): These two morphogroups have allopatric distributions in the Langeberg and Outeniqua Mountains respectively, sympatric in the Langeberg, and syntopic at Kristalkloof in the Langeberg (Table 3.9). There is therefore no geographical impediment to gene flow, but no morphological hybrid zone was detectable. The males of the two are highly distinctive morphologically in the shape of the dorsal process of tergite 9, and differed significantly in five morphometric variables. The ***S*** females are very distinctive with a very elongated median process of the SGP, while ***L*** bears a short protuberance. The average number of pairwise differences (pairwise distance method; uncorrected) was 2.50 (0.45%), and they share a haplotype at Kristalkloof. ***L*** and ***S*** are regarded as species relative to each other because of intrinsic reproductive isolation (syntopic), morphological phenetic distinguishability, and morphological diagnosability.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These two morphogroups have allopatric distributions in the Langeberg and Witsenberg Mountains respectively. The males of the two are highly distinctive morphologically in the shape of the dorsal process of tergite 9, but differed significantly in just three morphometric variables. The females are easily distinguishable as the posterior margin of the SGP of ***L*** bears a short protuberance, and ***T*** is rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 1.00 (0.18%), and they share a haplotype together with morphogroup ***S***. ***L*** and ***T*** are regarded as species relative to each other because of morphological phenetic distinguishability, and morphological diagnosability.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These two morphogroups have allopatric distributions in the Langeberg and Cederberg Mountains respectively. The males of the two are highly distinctive morphologically in the shape of the dorsal process of tergite 9, and differed significantly in six morphometric variables. The females are very similar. The average number of pairwise differences (pairwise distance method; uncorrected) was 8.83 (1.59%), and their respective clades were separated by a greater than average number of steps (13) in the nested clade analysis. ***L*** and ***W*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and monophyly of ***W***.

L (Figs 3.3G-H, 3.4E, 3.5G-I) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These two morphogroups have allopatric distributions in the Langeberg, and northern and southern Hottentots Holland

and Riviersonderend Mountains respectively. The males of the two are highly distinctive morphologically in the shape of the dorsal process of tergite 9, and differed significantly in five morphometric variables. The females are very similar, but differ in sternite pigmentation patterns. The average number of pairwise differences (pairwise distance method; uncorrected) was 8.50 (1.53%). The population pairwise F_{ST} value was significant. ***L*** and ***Z*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***Z***.

N (Figs 3.3I, 3.4F, 3.5J) and ***P*** (Figs 3.3J-N, 3.4G, 3.5K-L): These have distant allopatric distributions in the southern Hottentots Holland Mountains and Langeberg respectively. The males of the two are highly divergent morphologically in the shape of the dorsal process of tergite 9, and differed significantly in six morphometric variables. The females show minor differences, with ***N*** bearing a short median protuberance on the SGP posterior margin and ***P*** smoothly rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 16.17 (2.90%), and their respective clades were separated by a much greater than average number of steps (28) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***N*** and ***P*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

N (Figs 3.3I, 3.4F, 3.5J) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): These have distant allopatric distributions in the southern Hottentots Holland Mountains and the Langeberg respectively. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in eight of the nine morphometric variables (with the exception of adp). The females are also very distinctive, with ***N*** bearing a short median protuberance on the SGP posterior margin and ***R*** notched. The average number of pairwise differences (pairwise distance method; uncorrected) was 16.50 (2.96%), and their respective clades were separated by a much greater than average number of steps (22) in the nested clade analysis. ***N*** and ***R*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***N***.

N (Figs 3.3I, 3.4F, 3.5J) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): These have distant allopatric distributions in the southern Hottentots Holland Mountains, and the Langeberg and Outeniqua Mountains respectively. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, but differed significantly in just three of the nine morphometric variables (dp, adp and epl). The females are also very distinctive, with ***N*** bearing a short median protuberance on the SGP posterior margin and the median process of ***S*** highly elongate. The

average number of pairwise differences (pairwise distance method; uncorrected) was 16.50 (2.96%), and their respective clades were separated by a much greater than average number of steps (21) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***N*** and ***S*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***N***.

N (Figs 3.3I, 3.4F, 3.5J) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These have distant allopatric distributions in the southern Hottentots Holland Mountains and the Witsenberg respectively. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and differed significantly in six of the nine morphometric variables. The females are also quite distinctive, with ***N*** bearing a short median protuberance on the SGP posterior margin and ***T*** smoothly rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 15.00 (2.69%), and their respective clades were separated by a much greater than average number of steps (21) in the nested clade analysis. ***N*** and ***T*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***N***.

N (Figs 3.3I, 3.4F, 3.5J) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These have distant allopatric distributions in the southern Hottentots Holland Mountains and the Cederberg respectively. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and yet differed significantly in only two of the nine morphometric variables. The females are very similar. The average number of pairwise differences (pairwise distance method; uncorrected) was 13.50 (2.42%), and their respective clades were separated by a greater than average number of steps (11) in the nested clade analysis. ***N*** and ***W*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability (males), and reciprocal monophyly.

N (Figs 3.3I, 3.4F, 3.5J) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These have sympatric distributions in the southern Hottentots Holland Mountains, and ***Z*** also occurs in the northern Hottentots Holland Mountains. There would be no geographical barriers to recurrent gene flow. The males of the two are highly distinctive morphologically especially the dorsal process of tergite 9, and yet differed significantly in only three of the nine morphometric variables. The females are easily distinguished, with the posterior margin of the SGP of ***Z*** more flattened than ***N***, and sternites 2-5 unsclerotized anteriorly. The average number of pairwise differences (pairwise distance method; uncorrected) was 14.50 (2.60%), and their respective clades were separated by a much greater than average number of steps (18) in the nested clade analysis. ***N*** and ***Z*** are regarded as species relative to each other because of intrinsic reproductive isolation

(sympatric), morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

P (Figs 3.3J-N, 3.4G, 3.5K-L) and ***R*** (Figs 3.3O-P, 3.4H, 3.5M): These two morphogroups have sympatric distributions in the Langeberg Mountains (although their ranges may not overlap and may therefore be parapatric; further sampling would be required), and ***P*** additionally occurs in the Outeniqua Mountains. There is therefore no geographical impediment to gene flow. The males of the two are very distinctive morphologically in the shape of the dorsal process of tergite 9, and differed significantly in seven morphometric variables. The females are clearly divergent, with ***R*** SGP posterior margin notched, and ***P*** rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 16.92 (3.04%), and their respective clades were separated by a much greater than average number of steps (18) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***P*** and ***R*** are regarded as species relative to each other because of genetic structure, intrinsic reproductive isolation (sympatric), morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***P***.

P (Figs 3.3J-N, 3.4G, 3.5K-L) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): These two morphogroups have allopatric distributions in the Langeberg and Outeniqua Mountains respectively, except for an area of syntopy at Kristalkloof in the Langeberg (Table 3.9), which probably means that they are parapatric with a small area of sympatry. There is therefore no geographical impediment to gene flow. The males of the two are very distinctive morphologically in the shape of the dorsal process of tergite 9, and differed significantly in five morphometric variables. The females are clearly divergent, with ***S*** SGP posterior margin bearing a highly elongated process, and ***P*** rounded. The average number of pairwise differences (pairwise distance method; uncorrected) was 15.33 (2.75%), and their respective clades were separated by a greater than average number of steps (17) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***P*** and ***S*** are regarded as species relative to each other because of genetic structure, intrinsic reproductive isolation (syntopic), morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***P***.

P (Figs 3.3J-N, 3.4G, 3.5K-L) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These two morphogroups have allopatric distributions in the Langeberg and Outeniqua Mountains, and Witsenberg Mountains respectively. The males of the two are similar morphologically in the shape of the dorsal process of tergite 9, and differed significantly in only three morphometric variables, namely hcw, epd and epw. Additionally, the ratio of the means of sp/dp is 0.55 for ***P*** and 0.61 for ***T***, mainly due to the proximal section of the lobe being longer in ***P***. The ***T*** variant (Fig. 3.3T) also needs

further examination. The females are also very similar. The average number of pairwise differences (pairwise distance method; uncorrected) was 15.17 (2.72%), and their respective clades were separated by a greater than average number of steps (16) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***P*** and ***T*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability (males), and monophyly of ***P***.

P (Figs 3.3J-N, 3.4G, 3.5K-L) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These two morphogroups have distant allopatric distributions in the Langeberg and Outeniqua Mountains, and Cederberg Mountains respectively. The males of the two are easily distinguished by the shape of the dorsal process of tergite 9, and differed significantly in seven morphometric variables. The females are distinguishable on the posterior margin of the SGP which is rounded in ***P*** and bears a small median protuberance in ***W***. The average number of pairwise differences (pairwise distance method; uncorrected) was 15.67 (2.81%), and their respective clades were separated by a greater than average number of steps (17) in the nested clade analysis. The population pairwise F_{ST} value was significant. ***P*** and ***W*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

P (Figs 3.3J-N, 3.4G, 3.5K-L) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These two morphogroups have allopatric distributions in the Langeberg and Outeniqua Mountains, and the southern and northern Hottentots Holland and Riviersonderend Mountains respectively. The males of the two are easily distinguished by the shape of the dorsal process of tergite 9, and differed significantly in seven morphometric variables. The females are subtly distinguishable on the posterior margin of the SGP which is rounded in ***P*** and is flattened, with or without a very small median protuberance in ***Z***. They can also be distinguished on differences in sternite pigmentation. The average number of pairwise differences (pairwise distance method; uncorrected) was 10.17 (1.83%). The population pairwise F_{ST} value was significant. ***P*** and ***Z*** are regarded as species relative to each other because of genetic structure, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

R (Figs 3.3O-P, 3.4H, 3.5M) and ***S*** (Figs 3.3Q-R, 3.4I, 3.5N-O): ***S*** occurs in the Langeberg where it is sympatric (but not syntopic) with ***R***, and in the Outeniqua and Elandsberge Mountains. The males of the two are highly morphologically divergent and differed significantly in six morphometric variables. The ***R*** females are very distinctive with a prominent notch. The ***S*** females are equally distinctive with a highly elongate slender median process of the posterior margin of the SGP. Note that even though support for species status between this

pair is strong, the average number of pairwise differences (pairwise distance method; uncorrected) was only 3.75 (0.67%). The population pairwise F_{ST} value was significant. The inference from the NCA was allopatric fragmentation (clade 4-1). ***R*** and ***S*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, intrinsic reproductive isolation (sympatric), morphological phenetic distinguishability, and morphological diagnosability.

R (Figs 3.3O-P, 3.4H, 3.5M) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These two morphogroups have allopatric distributions in the Langeberg and Witsenberg Mountains respectively. The males of the two are highly morphologically divergent and differed significantly in eight of the nine morphometric variables. The ***R*** females are very distinctive with a prominent notch. The ***T*** females have a rounded posterior margin of the SGP. The average number of pairwise differences (pairwise distance method; uncorrected) was only 2.25 (0.40%). The inference from the NCA was allopatric fragmentation (clade 4-1). ***R*** and ***T*** are regarded as species relative to each other because of allopatric fragmentation, morphological phenetic distinguishability, and morphological diagnosability.

R (Figs 3.3O-P, 3.4H, 3.5M) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These two morphogroups have distant allopatric distributions in the Langeberg and Cederberg Mountains respectively. The males of the two are easily distinguishable and differed significantly in four of the nine morphometric variables. The ***R*** females are very distinctive with a prominent notch. The ***W*** females have a small median projection of the posterior margin of the SGP. The average number of pairwise differences (pairwise distance method; uncorrected) was 10.25 (1.84%), and their respective clades were separated by a greater than average number of steps (11) in the nested clade analysis. ***R*** and ***W*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***W***.

R (Figs 3.3O-P, 3.4H, 3.5M) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These two morphogroups have allopatric distributions in the Langeberg, and the southern and northern Hottentots Holland and Riviersonderend Mountains respectively. The males of the two are easily distinguished by the shape of the dorsal process of tergite 9, and differed significantly in six morphometric variables. The ***R*** female SGP is very distinctive with a prominent median notch, while the SGP is flattened, with or without a very small median protuberance in ***Z***. They can also be distinguished on differences in sternite pigmentation. The average number of pairwise differences (pairwise distance method; uncorrected) was 9.25 (1.66%). The population pairwise F_{ST} value was significant. ***R*** and ***Z*** are regarded as species relative to each other because of

genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***Z***.

S (Figs 3.3Q-R, 3.4I, 3.5N-O) and ***T*** (Figs 3.3S-T, 3.4J, 3.5P): These two morphogroups have allopatric distributions in the Langeberg, Outeniqua and Elandsberge Mountains, and Witsenberg Mountains respectively. The males of the two are highly divergent and differed significantly in eight of the nine morphometric variables. The ***S*** females are very distinctive with a highly elongate slender median process of the posterior margin of the SGP. The ***T*** females have a rounded posterior margin of the SGP. The average number of pairwise differences (pairwise distance method; uncorrected) was only 1.50 (0.27%), and they share a haplotype. ***S*** and ***T*** are regarded as species relative to each other because of morphological phenetic distinguishability, and morphological diagnosability.

S (Figs 3.3Q-R, 3.4I, 3.5N-O) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These two morphogroups have distant allopatric distributions in the Langeberg, Outeniqua and Elandsberge Mountains, and Cederberg Mountains respectively. The males of the two are highly divergent and differed significantly in five of the nine morphometric variables. The ***S*** females are very distinctive with a highly elongate slender median process of the posterior margin of the SGP. The ***W*** females have a small median projection of the posterior margin of the SGP. The average number of pairwise differences (pairwise distance method; uncorrected) was 10.00 (1.80%). The population pairwise F_{ST} value was significant. The inference from the NCA was past gradual range expansion followed by fragmentation (clade 3-3) (Table 3.18). ***S*** and ***W*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***W***.

S (Figs 3.3Q-R, 3.4I, 3.5N-O) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These two morphogroups have allopatric distributions in the Langeberg, Outeniqua and Elandsberge Mountains, and northern and southern Hottentots Holland and Riviersonderend Mountains respectively. The males of the two are highly divergent and differed significantly in seven of the nine morphometric variables. The ***S*** females are very distinctive with a highly elongate slender median process of the posterior margin of the SGP, while the SGP is flattened, with or without a very small median protuberance in ***Z***. The females also differ in sternite pigmentation patterns. The average number of pairwise differences (pairwise distance method; uncorrected) was 7.67 (1.38%). The population pairwise F_{ST} value was significant. The inference from the NCA was past gradual range expansion followed by fragmentation (clade 3-3) (Table 3.18). ***S*** and ***Z*** are regarded as species relative to each other because of allopatric fragmentation, genetic structure, morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***Z***.

T (Figs 3.3S-T, 3.4J, 3.5P) and ***W*** (Figs 3.3U-V, 3.4K, 3.5Q-S): These two morphogroups have allopatric distributions in the Witsenberg and Cederberg Mountains respectively. The males of the two are easily distinguishable, and differed significantly in seven of the nine morphometric variables. The ***W*** females bear a short median process of the posterior margin of the SGP. The ***T*** females have a smoothly rounded posterior margin of the SGP. The average number of pairwise differences (pairwise distance method; uncorrected) was 8.50 (1.53%). ***T*** and ***W*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***W***.

T (Figs 3.3S-T, 3.4J, 3.5P) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These two morphogroups have allopatric distributions in the Witsenberg and northern and southern Hottentots Holland and Riviersonderend Mountains respectively. The males of the two are easily distinguishable, and differed significantly in seven of the nine morphometric variables. The ***T*** females have a smoothly rounded posterior margin of the SGP, while the SGP is flattened, with or without a very small median protuberance in ***Z***. The females also differ in sternite pigmentation patterns. The average number of pairwise differences (pairwise distance method; uncorrected) was 7.50 (1.35%). ***T*** and ***Z*** are regarded as species relative to each other because of morphological phenetic distinguishability, morphological diagnosability, and monophyly of ***Z***.

W (Figs 3.3U-V, 3.4K, 3.5Q-S) and ***Z*** (Figs 3.3W-AA, 3.4L, 3.5U-V): These two morphogroups have distant allopatric distributions in the Cederberg and northern and southern Hottentots Holland and Riviersonderend Mountains respectively. The males of the two are subtly but easily distinguishable, and differed significantly in six of the nine morphometric variables. The ***W*** females bear a short median process of the posterior margin of the SGP, while the SGP is flattened, with or without a very small median protuberance in ***Z***. The females also differ in sternite pigmentation patterns. The inference from the NCA was past gradual range expansion followed by fragmentation (clade 3-3) (Table 3.18). The average number of pairwise differences (pairwise distance method; uncorrected) was 8.00 (1.44%). ***W*** and ***Z*** are regarded as species relative to each other because of allopatric fragmentation, morphological phenetic distinguishability, morphological diagnosability, and reciprocal monophyly.

Chapter 4
A morphological and molecular phylogeny of southern African notonemourid stoneflies (Plecoptera)

Phylogenetic data on the Notonemouridae stoneflies (Plecoptera) of southern Africa can provide a key to understanding the evolution and biogeography of the palaeogenic fauna of the region. Their cryptic speciation, low vagility and restriction to temperate montane refugia make the Notonemouridae an ideal model for examining possible drivers of speciation. Forty of the forty four species (including 13 undescribed) across the six genera of southern African stoneflies were included in a morphological and mitochondrial DNA molecular (39 species) analysis to test the monophyly of the genera and to estimate phylogenetic relationships between genera and species. All morphological characters were newly conceived for separate maximum parsimony and Bayesian inference analyses. Under the parsimony criterion, five weighting schemes (equal, *a priori*, successive approximations, implied and self) were employed. Partial sequences of cytochrome oxidase subunit I were used in parsimony, maximum likelihood and Bayesian analyses, and in combined analyses with the morphology data in parsimony and Bayesian analyses. Branch confidence was measured using Bremer and relative Bremer supports, as well as the bootstrap and jackknife resampling procedures. All five morphology parsimony weighting scheme and BI morphology cladograms were in agreement on the monophyly of the genera, the clade (*Aphanicercella*, *Balinskycercella*), and the clade (*Afronemoura*, *Aphanicerca*). The model based analyses (Bayesian inference and maximum likelihood) of both the mtDNA partition and combined analyses were regarded as less reliable than the parsimony (morphological and molecular) analyses due to recovery of nonmonophyly of two of the genera. Morphological and molecular parsimony cladograms were largely in agreement, and were congruent in generic relationships. The generic relationships under the parsimony criterion could be divided into those that were stable and those that were unstable. Stable clades were common to all trees of all parsimony weighting methods used. These were: (*Aphanicercella*, *Balinskycercella*), and (*Afronemoura*, *Aphanicerca*). The unstable clades were those that were present in some strict or majority rule consensus cladograms but not in others. To summarize generic relationships, the most conservative consensus was a polytomy of four clades, namely *Aphanicercopsis*, *Desmonemoura*, (*Aphanicercella*, *Balinskycercella*), and (*Afronemoura*, *Aphanicerca*); when better resolved under weighted optimizations, consensus cladograms recovered *Aphanicercopsis* as the sister group to the remaining genera. Then *Desmonemoura* either formed part of the remaining tritomy, or became sister to (*Afronemoura*, *Aphanicerca*). The combined *a priori* morphology and molecular consensus cladogram (*Aphanicercopsis* ((*Aphanicercella*, *Balinskycercella*), (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*)))) was favoured because: 1) at the generic level it was fully resolved; 2) it was congruent with the Bayesian inference morphology tree in the generic relationship (*Desmonemoura* (*Afronemoura*, *Aphanicerca*)), and with the implied weighting, self weighting, successive approximations weighting and majority rule equal weighting cladograms in recovering *Aphanicercopsis* as the sister group to the other genera; and 3) because of the perceived importance of the characters that were weighted in the *a priori* analysis. Unambiguous character states that defined the stable and unstable clades were given for cladograms with equal and *a priori* weights. Paraproct glands were described for the first time in Plecoptera, and possibly in Insecta. Unusual paired structures (probably spermathecae), not previously

described in Plecoptera but with possible homology in *Capnioneura* (Capniidae), were described in female *Aphanicercopsis* except *A. outeniquae* Barnard. Some important and phylogenetically useful characters were degree of fusion of ventral nerve cord abdominal ganglia, male paraproct glands (occurrence and form), and accessory glands of the male seminal vesicle. Two main biogeographic areas were defined on species composition, namely the Eastern Highlands and the Cape Folded Mountains, with some overlap, and one additional minor zone, the Namaqua Highlands. Cluster analysis showed that mountains ranges had a more similar species composition to geographically proximate mountains than they had to more distant mountains. The intersection zone of the Southern Folded Mountains and Western Folded Mountains was particularly species-rich (20 of the 44 species). Dissimilarity in species composition between mountains was the norm, indicating that local endemism at the mountain range scale was common. Endemism was found to be widespread, with almost 41% of the species endemic to a single mountain range group. Eighty percent of the ecoregion endemics were found in the Southern and Western Folded Mountains. A hypothesis forwarded for the evolution of the southern African Notonemouridae proposes that the common ancestor of the six genera dispersed from a Cape Folded Mountains origin, to become widespread across the montane areas of the southern tip of the African continent after the separation from Gondwanaland, including the Cape Folded Mountains, Amatola and Drakensberg regions. Because allopatric speciation is believed to be far more prevalent than sympatric speciation, and because there are four genera present in the Cape Folded Mountains and usually multiple genera within one stream, it is likely that populations of this most recent common ancestor of these genera became separated by vicariant events (or surrogates such as topographical complexity) within the Cape Folded Mountains, allowing the genera to evolve. Species within these genera subsequently underwent cycles of range expansion and speciation in allopatry. Secondary contact would ultimately have occurred resulting in generic sympatry.

Key words: Notonemouridae, phylogeny, biogeography, systematics, morphology, cytochrome oxidase, mitochondrial DNA, Miocene, Pliocene, Pleistocene

INTRODUCTION

The Plecoptera is a minor, basal, aquatic order of the lower Neoptera, with about 3500 species worldwide (Fochetti & Tierno de Figueroa 2008), occurring on all continents except for Antarctica (Theischinger 1991). Of the 16 extant families of Plecoptera, only two occur in southern Africa, namely the Perlidae and the Notonemouridae (Balinsky 1962; Zwick 1973). The Gondwanan relictual Notonemouridae are represented by 31 described species in six genera (Picker & Stevens 1999) in southern Africa. The remaining 90 species are distributed among Australia, New Zealand, Madagascar and South America (Fochetti & Tierno de Figueroa 2008). It is apparent that the centre of adaptive radiation for African Notonemouridae is the south-western region of the Western Cape Province, with most of the southern African species being narrow endemics (Stevens & Picker 1999).

The larvae of the Notonemouridae occur in cold, low order, fast-flowing streams with stony substrates and even in small moss-covered seepages on mountainsides, providing these are

perennial or flow underground during the dry season. Adults are typically found on rocks within and adjacent to streams and on riparian vegetation, but also on vegetation some distance from streams. The larvae generally are intolerant of thermal or organic pollution and so are useful indicators of water quality (Dallas & Day 1993).

Southern African notonemourid stoneflies have been the subject of only ten taxonomic published papers between 1931 and 1999. Tillyard (1931) first assigned these notonemourids to Nemourinae (Nemouridae). He recognized two new genera, *Aphanicerca* Tillyard (type species *A. capensis* from Table Mountain on the Cape Peninsula) and *Desmonemoura* Tillyard (type species *D. pulchellum* Tillyard). *Aphanicerca* was assigned three species based on wing and genitalic characters, namely *A. capensis* Tillyard, placed in the subgenus *Aphanicerca*, and *A. denticulata* Tillyard and *A. barnardi* Tillyard that were assigned to the subgenus *Aphanicercella*. Using wing venation characters (which are only useful for distinguishing higher taxonomic groupings above generic level) as the primary distinguishing features of the genera, Tillyard assigned two of the three species erroneously to *Aphanicerca*. He did however, evidently appreciate the wide variation in genitalic structure when erecting the two subgenera within *Aphanicerca*. Barnard (1934) transferred *Aphanicerca denticulata* to a new genus *Aphanicercopsis* Barnard, and elevated the subgenus *Aphanicercella* to genus rank, with *Aphanicercella barnardi* as the type species.

Barnard (1934) correctly placed emphasis on male, and to a lesser extent, female genitalia for generic and specific characterization. He did not elaborate on larval identification or phylogenetically relevant features, but stated that larvae of the genera are "practically indistinguishable" (Barnard 1934). He dissolved the subgenus category and redescribed *Aphanicerca capensis* Tillyard, and additionally described five new *Aphanicerca* species, namely *A. uncinata* Barnard, *A. lyrata* Barnard, *A. bicornis* Barnard, *A. bovina* Barnard, and *A. tereta* Barnard (Table 4.1). Of importance was the recognition by Barnard of different allopatric "varieties" of *A. capensis* males from Wellington, Montagu Pass and Tulbagh (based on the shape of the dorsal process of tergite 9), as well as females (with variably-shaped subgenital plates from various localities); however, he stated that the slight variations in male and female genitalia did not justify assigning varietal names to them. This variation first detected by Barnard in *A. capensis* was shown to underlie a species complex of 12 species (Chapter 3). Chapter 3 provided evidence for splitting *A. capensis* into 12 morphologically defined groups which were then subjected to morphometric, biological and mitochondrial DNA techniques in order to test these 12 species hypotheses. That study showed sufficient lines of evidence for this according to the unified (general lineage) species concept (de Queiroz 1998, 1999, 2007) to justify according species status to those 12 morphogroups.

Table 4.1. Taxonomic history of the southern African Notonemouridae. All 31 described species are listed in the column on the right together with the author and year of publication of both original descriptions and name changes to reflect new genera. Where names have changed, the original designations of those species are provided in the left hand column together with the author and date of publication.

Aphanicercopsis amatolae Balinsky (1956)	*Afronemoura amatolae* (Balinsky); Illies (1980)
Aphanicercopsis spinulata Balinsky (1967)	*Afronemoura spinulata* (Balinsky); Illies (1980)
	Afronemoura stuckenbergi Picker & Stevens (1999)
	Aphanicerca bicornis Barnard (1934)
	Aphanicerca bovina Barnard (1934)
Aphanicerca subg. *Aphanicerca capensis* Tillyard (1931)	*Aphanicerca capensis* Tillyard; Barnard (1934)
	Aphanicerca chanae Picker & Stevens (1999)
	Aphanicerca gnua Picker & Stevens (1999)
	Aphanicerca lyrata Barnard (1934)
	Aphanicerca tereta Barnard (1934)
	Aphanicerca uncinata Barnard (1934)
Aphanicerca subg. *Aphanicercella barnardi* Tillyard (1931)	*Aphanicercella barnardi* Tillyard; Barnard (1934)
	Aphanicercella bifurcata Barnard (1934)
	Aphanicercella bullata Stevens & Picker (1999)
	Aphanicercella cassida Barnard (1934)
	Aphanicercella clavata Stevens & Picker (1999)
	Aphanicercella flabellata Stevens & Picker (1999)
	Aphanicercella nigra Barnard (1934)
	Aphanicercella quadrata Barnard (1934)
	Aphanicercella scutata Barnard (1934)
	Aphanicercella securata Stevens & Picker (1999)
	Aphanicercella spatulata Stevens & Picker (1999)
Aphanicerca subg. *Aphanicercella denticulata* Tillyard (1931)	*Aphanicercopsis denticulata* (Tillyard); Barnard (1934)
	Aphanicercopsis hawaquae Barnard (1934)
	Aphanicercopsis outeniquae Barnard (1934)
	Aphanicercopsis tabularis Barnard (1934)
Aphanicercella fontium Balinsky (1956)	*Balinskycercella fontium* (Balinsky); Stevens & Picker (1995)
Aphanicercella gudu Balinsky (1956)	*Balinskycercella gudu* (Balinsky); Stevens & Picker (1995)
Aphanicercella tugelae Balinsky (1956)	*Balinskycercella tugelae* (Balinsky); Stevens & Picker (1995)
	Desmonemoura brevis Picker & Stevens (1999)
	Desmonemoura pulchellum Tillyard (1931)

Barnard (1934) described three new species of *Aphanicercopsis*; *A. tabularis* Barnard, *A. outeniquae* Barnard, and *A. hawaquae* Barnard (Table 4.1), as well as five new species of *Aphanicercella*; *A. scutata* Barnard, *A. cassida* Barnard, *A. bifurcata* Barnard, *A. quadrata* Barnard, and *A. nigra* Barnard (Table 4.1). As with *Aphanicerca capensis*, *Aphanicercella barnardi* was recognized by Barnard as a variable species with "transitional forms" that did not justify unique names. This morphological variation within *A. barnardi* was used in conjunction with mate choice trials, to justify division into six species (Table 4.1; Stevens & Picker 1999; as outlined in Chapter 2 of this thesis). Finally, Barnard (1934) redescribed *Desmonemoura* Tillyard, and corrected Tillyard's error by describing the female of *D. pulchellum* which Tillyard had previously described as the *A. barnardi* female. Barnard (1936) provided additional distributional records for seven of the above species.

Balinsky (1956) described five new species of stonefly from the eastern, summer rainfall region of southern Africa, which he reclassified together with the rest of the southern African taxa as belonging to the family Leuctridae. He was of the opinion that similarity in wing

venation placed the notonemourids in the Leuctridae, in spite of the fact, as he pointed out, that the paraproct is more similar to that of the Nemouridae (Balinsky 1956). He described two new species from Grahamstown and the Hogsback (Amatolae Mountains) in the Eastern Cape Province, which he assigned to *Aphanicercopsis*, namely *A. amatolae* Balinsky (Balinsky 1956) and *A. spinulata* Balinsky (Balinsky 1967). As pointed out by Illies (1980), Balinsky (1956) acknowledged that *A. amatolae* "differs very considerably from the four species of the genus listed by Barnard". With regard to the female, Balinsky stated that the subgenital plate was quite typical for *Aphanicercopsis* and thus based his generic allocation on this character. Balinsky (1956) stated that "The classification of my species as an *Aphanicercopsis* would appear to be based mainly on negative characters if only the ♂♂ are taken into consideration". Yet, he described the species accurately, providing unique characters such as the pair of sharp spines on the posterior margin of the ninth tergite, broadly and uniformly convex posterior margin of ninth tergite, and 10th tergite comprising two broad heavily chitinised plates. Balinsky seems to have viewed the absence of appendages on tergite 9 (e.g. in *Aphanicerca* and *Desmonemoura*), and the absence of a clasper-like structure on the tenth pleurite (as in *Aphanicercella*), as characters, instead of focusing on what actually was present. Fitzhugh (2006) points out that coding a character as "absent" can only be interpreted as "a shorthand term for what actually is observed". So, the absence of processes on tergite nine is better described (coded for in a cladistic sense) as "posterior edge broadly and uniformly convex, bearing a pair of sharp spines near the midline…", which is a direct quotation from Balinsky's (1956) description. Had Balinsky focused on his own description and not on the characters that his new species lacked in relation to existing genera, he may have erected a new genus himself. Instead, this was done later by Illies (1980) who established the genus *Afronemoura* Illies to accommodate Balinsky's *Aphanicercopsis amatolae* and *A. spinulata* (Balinsky 1967) (Table 4.1). Illies also described the larva of *Afronemoura*, which has a unique feature not found in the other genera, namely a tuft of bristles about one third of the way up the antennae. He also pointed out the non-overlapping distributions of *Aphanicercopsis* and *Afronemoura* (Illies 1980). Balinsky (1956) also described three new species which he assigned to *Aphanicercella*, namely *A. tugelae* Balinsky, *A. gudu* Balinsky and *A. fontium* Balinsky. As he noted for the *Afronemoura* species, these new species formed a morphologically distinct unit (Balinsky 1956). A new genus, *Balinskycercella* Stevens & Picker (Stevens & Picker 1995; Chapter 2 of this thesis), was erected to accommodate this distinctive clade which was regarded as the sister group of *Aphanicercella* (Table 4.1). Subsequently, the genera *Desmonemoura*, *Aphanicerca*, *Afronemoura*, *Aphanicercopsis* and *Balinskycercella* were revised (Picker & Stevens 1999; Chapter 2 of this thesis), and included descriptions of four new species (Table 4.1).

Knowledge of species distributions and phylogenetic relationships within the notonemourid stoneflies of southern Africa are needed to provide a hypothesis for the evolution of other members of the well-represented palaeogenic fauna (basal taxa currently occupying relictual habitats) of the region (Stuckenberg 1962). Ecologically, the Notonemouridae share a number of features characteristic of other members of the relictual invertebrate fauna of southern Africa; cryptic speciation, low vagility and restriction to temperate montane refugia. This makes them an ideal model for examining possible drivers of speciation. Up to the present though, scant attention has been paid to the biogeography of southern African stoneflies. Balinsky (1962) and Stuckenberg (1962) emphasized the family's Gondwanan origins and distributional similarities with other local relictual montane faunal invertebrates, particularly within the Cape Folded Mountains (CFM) and the Eastern Highlands (EH). It is thought that the relictual fauna of southern Africa are currently restricted to small temperate refugia as a result of their once wider distributions being contracted following gradual climate warming and aridification that occurred as Africa moved northwards following the fragmentation of Gondwanaland (Day 2005). These organisms have survived in temperate refugia (mountain streams, caves, forest) present in the complex geological formations of the CFM, a region rich in both fauna and flora (Taylor 1978). Stuckenberg (1962) partly attributed the general species richness of the CFM to the antiquity of the landscape (post-Ecca and pre-Cretaceous with further folding in the mid-Cretaceous), the varied topography, and the climate (particularly the predictable winter rainfall pattern). Price *et al*. (2007) discussed the controversy regarding climatic conditions present in the Cape Floristic Region (CFR) during the Pliocene and Pleistocene, namely stability versus rapid, dramatic change. One view is that the CFR may have been spared the climatic cycles that caused extinctions of flora in northern temperate areas during this time period (Barraclough 2006). Pleistocene glaciation was largely a northern phenomenon, from which southern Africa was largely spared (Barraclough 2006). However, the glaciation that did occur in southern Africa is thought to have been more extreme in the south-eastern Cape region than in the south-western Western Cape Province, leading to more extinctions in the former (Cowling *et al*. 1996); indeed, the Cape Folded Mountains were evidently not high enough to have been glaciated (Deacon 1983). It is likely that a combination of rapid speciation and low extinction rates led to the overall species richness of the flora in this region (Cowling *et al*. 1996). However, this high floral species richness is not necessarily matched by the species richness of the herbivorous insects of the CFR (Giliomee 2003), and therefore extrapolation from the flora to the invertebrate fauna may not always be appropriate. Nevertheless, climatic factors may have similar effects on both groups. Overall, there is no consensus on a causal relationship between any one main environmental variable and the high levels of speciation of the palaeogenic (relictual) invertebrates in the CFM, and the answer probably lies in a multiplicity of factors (Day 2005), including those which resulted in the remarkable diversification of the flora.

The concept of using a short segment of mitochondrial DNA (mtDNA), the cytochrome oxidase subunit I (COI) gene, as a taxon barcode to identify any taxa to species level was developed by Hebert *et al.* (2003) as a response to the decline in taxonomic expertise availability. While the idea itself is highly appealing, the practicality and feasibility are controversial. In particular, it is well known that mtDNA may not be definitive at species level in cases of closely related species (Sperling 2003). This incongruence between gene trees and species trees may be due to factors including incomplete lineage sorting and hybridization (Hendrixson & Bond 2005). In spite of the views of numerous proponents (e.g. Stoeckle 2003, Whitfield 2003, Kress *et al*. 2005) it has been shown that this approach would fail in achieving its aims (e.g. Sperling 2003, Will & Rubinoff 2004), and in the process be catastrophic for the field of taxonomy and the study of biodiversity. The near demise of the original idea of a simple barcode has birthed a more complex approach using multiple genes and likelihood methods (Pons *et al*. 2006), but still with the aim of automated identification using DNA. The study of the *Aphanicerca capensis* species complex (Chapter 3) concluded that COI was not suitable as a barcode marker for species delimitation in that group. The present study affords an opportunity to evaluate the utility of the COI DNA barcode in the remainder of the southern African notonemourids. Another reason for choosing this gene was that it is widely used in phylogenetics (Beheregaray 2008) and is therefore useful for comparative purposes (Caterino *et al*. 2000).

The broad aim of this chapter is to produce a phylogeny using morphological and molecular data for the southern African Notonemouridae, the first molecular and combined analyses to include all six genera and almost all species. Morphological characters are useful in that they broadly sample the nuclear genome and are sometimes more useful at deeper nodes due to morphological similarity at species level. Mitochondrial DNA data complement the analysis as they are often better able to resolve species relationships (Wiens & Penkrot 2002). The resulting cladograms of hypotheses of relationships will act as a framework for discussion of morphology and current and historical distribution patterns of the African Notonemouridae.

The specific aims of this chapter are: 1) To establish a consensus of generic and species relationships of the southern African Notonemouridae; 2) To assess the monophyly of the southern African notonemourid genera; 3) To identify morphological synapomorphies that define the genera. The inclusion of morphological data in the present study provides cladistic characters that may contribute towards developing a uniform set of characters that would be useful in resolving relationships within the Notonemouridae as a whole, and adds to the limited body of knowledge of notonemourid anatomy and morphology; and 4) To describe the current distribution patterns and attempt to hypothesize a time frame for cladogenesis of the local

notonemourid taxa, and thereby to increase knowledge of the current distribution and historical biogeography of Gondwanan taxa in southern Africa. The data obtained will be useful for biogeographic inferences in future studies, as it is through comparison of the phylogenies of numerous different groups and the discovery of congruencies that will further our understanding of the biogeography of the invertebrate fauna (Stuckenberg 1995).

MATERIALS AND METHODS

Taxon sampling

Fresh material was collected live and immediately immersed in 70% ethanol and then frozen at -10°C for both morphological and mtDNA data collection. Because males are more easily distinguishable to species than females, all specimens used for the molecular analysis were males, except for the following which could be unambiguously determined; one *Aphanicercella nigra* (specimen EE7) female, one *Aphanicercella clavata* Stevens & Picker (specimen CC7) female, two *Aphanicerca mclellani* sp. n. females (specimens E1 and L4a, with the latter caught *in copulo* in the field with specimen L3 which was also included in the analysis), and one *Balinskycercella tugelae* female (OO3). *Afronemoura* Illies comprises three species of which two were included in the study. The third, *A. stuckenbergi* Picker & Stevens, was described in Picker & Stevens (1999) (Chapter 2), but sufficient fresh material for dissection and sequencing was not available. *Aphanicerca tereta* Barnard was the only species in the genus to be excluded, as efforts to find it were in vain. *Aphanicerca gnua* Picker & Stevens (1999) (Chapter 2) was excluded from the mtDNA and the combined partition analyses due to lack of fresh material. The 11 new *Aphanicerca* species recognized and named in Chapter 3, but as yet not described, were all included. All 11 described *Aphanicercella* Tillyard species, including the five new species described in Stevens & Picker (1999) (Chapter 2), were included. Additionally, a new as yet undescribed species, *Aphanicercella pauletteae* sp. n. was included. A second new species, *Aphanicercella namaquaensis* sp. n. came to light too late for inclusion in the analysis, but was included in the distribution data and maps. These two new species are easily diagnosed on morphological criteria and raise the number of species in the genus to 13. All four described *Aphanicercopsis* Barnard species were included. *Balinskycercella* Stevens & Picker (1995) (Chapter 2), comprises three species, of which *B. fontium* (Balinsky) was excluded from all analyses due to lack of material. Both *Desmonemoura* species, namely *D. pulchellum* and *D. brevis* Picker & Stevens (1999) were included. For the molecular partition, *Aphanicercella quadrata* was the only exemplar for which multiple samples could not be obtained. In summary, the morphological analysis included 40, and the molecular and combined analyses 39 out of the 44 possible species. *Aphanicerca bainii* sp. n. is an undescribed species that was used as an outgroup in Chapter 3 (Figs 3.10-3.13; Appendix 3.4). This species has not yet been analysed morphologically and as such was not incorporated as part of the 44 possible species.

Table 4.2. Distributional data for the 102 individuals of the southern African notonemourids sampled for the mtDNA analysis. H-H = Hottentots Holland, KZN = KwaZulu-Natal.

Genus	Species	Field code	Locality, collector	Mountain range	Latitude	Longitude
Afronemoura	*amatolae*	Q2, Q3	Madonna and Child Falls, Hogsback. DM Stevens.	Amatola	-32.605300	26.962600
Afronemoura	*spinulata*	R2, R3	Katberg Hotel Red Trail. DM Stevens.	Amatola	-32.488500	26.681200
Aphanicerca	*austrocapensis* sp. n.	M2	Kristalkloof, Garcia's Pass, near Riversdale. DM Stevens.	Langeberg	-33.958600	21.230400
Aphanicerca	*austrocapensis* sp. n.	N2	Bergplaas-Kleinplaat road, NE of George. DM Stevens.	Outeniqua	-33.872275	22.687287
Aphanicerca	*austrocapensis* sp. n.	CCC3	Keur River bridge, Montagu Pass, George. DM Stevens.	Outeniqua	-33.907175	22.418134
Aphanicerca	*austrocapensis* sp. n.	CCC1	Keur River bridge, Montagu Pass, George. DM Stevens.	Outeniqua	-33.907175	22.418134
Aphanicerca	*austrocapensis* sp. n.	N3, N4	Prince Alfred's Pass, N of Knysna. DM Stevens.	Outeniqua	-33.860994	23.171860
Aphanicerca	*bicornis*	S3, S4	Karmel Camp, Franschhoek Pass. DM Stevens.	H-H (northern)	-33.917900	19.161900
Aphanicerca	*bicornis*	S5	Leopard's Kloof, Harold Porter Botanic Reserve, Betty's Bay. DM Stevens.	H-H (southern)	-34.346690	18.930410
Aphanicerca	*bovina*	T1	Swartboskloof, Stellenbosch. DM Stevens.	H-H (northern)	-33.991700	18.954200
Aphanicerca	*bovina*	T2	Jonkershoek Nature Reserve, Stellenbosch. DM Stevens.	H-H (northern)	-33.989800	18.956900
Aphanicerca	*breviloba* sp. n.	C1, C2	Boegoekloof, Swartberg Pass. DM Stevens.	Groot Swartberg	-33.357400	22.058500
Aphanicerca	*brevispina* sp. n.	D3, D4	Harold Porter Botanic Reserve, Betty's Bay. DM Stevens.	H-H (southern)	-34.352300	18.927000
Aphanicerca	*capensis*	A2	Boschenheuwel Arboretum, Kirstenbosch. DM Stevens.	Cape Peninsula	-33.987460	18.437190
Aphanicerca	*capensis*	A1	Slangolie Ravine, Twelve Apostles. DM Stevens.	Cape Peninsula	-33.977700	18.385100
Aphanicerca	*cederbergensis* sp. n.	H2	11.2 km S of Algeria forest station. DM Stevens.	Cederberg	-32.425600	19.131800
Aphanicerca	*cederbergensis* sp. n.	H3	Eikeboom, 16.4 km S of Algeria, Cederberg. DM Stevens.	Cederberg	-32.454900	19.169600
Aphanicerca	*chanae*	U3, U4	Marloth Nature Reserve, Swellendam. DM Stevens.	Langeberg	-33.999200	20.456200
Aphanicerca	*incisura* sp. n.	P3, P5	Bergheim, between Montagu and Barrydale. DM Stevens.	Langeberg	-33.932800	20.380900
Aphanicerca	*incisura* sp. n.	P1, P4	Ravenna, between Montagu and Barrydale. DM Stevens.	Langeberg	-33.918500	20.378800
Aphanicerca	*longiloba* sp. n.	I1, I3	Kristalkloof, Garcia's Pass, near Riversdale. DM Stevens.	Langeberg	-33.958600	21.230400
Aphanicerca	*longiloba* sp. n.	I2	Tradouw Pass. DM Stevens.	Langeberg	-33.982738	20.708599
Aphanicerca	*lyrata*	W2, W3	Jonkershoek Nature Reserve, Stellenbosch. DM Stevens.	H-H (northern)	-33.989800	18.956900
Aphanicerca	*mclellani* sp. n.	L2, L5	Cloete's Pass, NW of Mossel Bay. DM Stevens.	Langeberg	-33.919800	21.742100
Aphanicerca	*mclellani* sp. n.	L3, L4a	Kristalkloof, Garcia's Pass, near Riversdale. DM Stevens.	Langeberg	-33.958600	21.230400
Aphanicerca	*mclellani* sp. n.	E1	Keur River bridge, Montagu Pass, George. DM Stevens.	Outeniqua	-33.907175	22.418134
Aphanicerca	*mclellani* sp. n.	E6	Kom se Pad, Gouna Forest, Knysna. DM Stevens.	Outeniqua	-33.947500	23.141100
Aphanicerca	*pickeri* sp. n.	F2, F4	Fernkloof Nature Reserve, Hermanus. DM Stevens.	H-H (southern)	-34.390000	19.269100

Table 4.2. Continued.

Genus	Species	Field code	Locality, collector	Mountain range	Latitude	Longitude
Aphanicerca	*swartbergensis* sp. n.	O1, O2	Malvadraai, Swartberg Pass. DM Stevens.	Groot Swartberg	-33.299600	22.050100
Aphanicerca	*swartbergensis* sp. n.	DDD2	Oudemuragie road, near Meiringspoort. DM Stevens.	Groot Swartberg	-33.391800	22.355900
Aphanicerca	*swartbergensis* sp. n.	J2	Seweweekspoort. DM Stevens.	Groot Swartberg	-33.412100	21.408700
Aphanicerca	*swartbergensis* sp. n.	J1	Seweweekspoort. DM Stevens.	Groot Swartberg	-33.394300	21.399200
Aphanicerca	*uncinata*	X3, X4	Leopard's Kloof, Harold Porter Botanic Reserve, Betty's Bay. DM Stevens.	H-H (southern)	-34.346690	18.930410
Aphanicerca	*witsenbergensis* sp. n.	G1, G2	Witsenberg Game Park, near Wolseley. DM Stevens.	Witsenberg	-33.382737	19.213298
Aphanicerca	*zwicki* sp. n.	B2	Bain's Kloof Pass, 1st stream N Wellington. DM Stevens.	H-H (northern)	-33.645158	19.070927
Aphanicerca	*zwicki* sp. n.	B1	Jonkershoek Nature Reserve, Stellenbosch. DM Stevens.	H-H (northern)	-33.989100	18.968400
Aphanicerca	*zwicki* sp. n.	B4, B5	Oubos farm, Riviersonderend. DM Stevens.	Riviersonderend	-34.082000	19.829100
Aphanicercella	*barnardi*	Y4	Algeria Forest Station. DM Stevens.	Cederberg	-32.374100	19.062000
Aphanicercella	*barnardi*	Y1	Hex River Mountain & Ski Club Hut, below Milner Ridge Peak. J. Wakeling.	Hex River Mts	-33.487600	19.465000
Aphanicercella	*bifurcata*	Z3	Marloth Nature Reserve, Swellendam. DM Stevens.	Langeberg	-33.999200	20.456200
Aphanicercella	*bifurcata*	Z1	Keur River bridge, Montagu Pass, George. DM Stevens.	Outeniqua	-33.907175	22.418134
Aphanicercella	*bullata*	AA7	Stream 19.5 km after Algeria on road to Sanddrift. DM Stevens.	Cederberg	-32.463600	19.195900
Aphanicercella	*bullata*	AA2	Oudemuragie road, near Meiringspoort. DM Stevens.	Groot Swartberg	-33.413400	22.383000
Aphanicercella	*bullata*	AA5	Gouna pump station, Gouna Forest. DM Stevens.	Outeniqua	-33.990700	23.040700
Aphanicercella	*bullata*	AA6	Oubos farm, Riviersonderend. DM Stevens.	Riviersonderend	-34.082000	19.829100
Aphanicercella	*cassida*	BB1	Oudemuragie road, 11.6 km E off R328 from “Rust en Vrede” signboard. DM Stevens.	Groot Swartberg	-33.411200	22.354100
Aphanicercella	*cassida*	JA5	Lisbon River, Sabie area. J. van Alphen-Stahl.	Mpumalanga Drakensberg	-24.862001	30.835997
Aphanicercella	*cassida*	JC1	Seweweekspoort. DM Stevens.	Groot Swartberg	-33.394300	21.399200
Aphanicercella	*cassida*	JD2	Prince Alfred's Pass, N of Knysna. DM Stevens.	Outeniqua	-33.860994	23.171860
Aphanicercella	*clavata*	CC8, CC9	Boschenheuwel Arboretum, Kirstenbosch. DM Stevens.	Cape Peninsula	-33.987460	18.437190
Aphanicercella	*clavata*	CC2, CC7	Bain's Kloof Pass, 1st stream N of Wellington. DM Stevens.	H-H (northern)	-33.645158	19.070927
Aphanicercella	*flabellata*	DD1	Bain's Kloof Pass, 1st stream N of Wellington. DM Stevens.	H-H (northern)	-33.645158	19.070927
Aphanicercella	*flabellata*	DD2	Swartboskloof, Stellenbosch. DM Stevens.	H-H (northern)	-33.991700	18.954200
Aphanicercella	*nigra*	EE6	Tsitsikamma National Park, Red Trail. DM Stevens.	Tsitsikamma	-34.018040	23.889230
Aphanicercella	*nigra*	EE7	Tsitsikamma National Park. DM Stevens.	Tsitsikamma	-34.032580	23.973730
Aphanicercella	*pauletteae* sp. n.	AB1, AB2	Gouna pump station, Gouna Forest. DM Stevens.	Outeniqua	-33.990700	23.040700

Table 4.2. Continued.

Genus	Species	Field code	Locality, collector	Mountain range	Latitude	Longitude
Aphanicercella	*quadrata*	FF2	Hex River Mountain & Ski Club Hut, below Milner Ridge Peak. J. Wakeling.	Hex River Mts	-33.487600	19.465000
Aphanicercella	*scutata*	GG2	Concrete bridge 11.2 km after Algeria on road to Sanddrift. DM Stevens.	Cederberg	-32.425600	19.131800
Aphanicercella	*scutata*	GG1	Bain's Kloof Pass, Steenbok Park. DM Stevens.	H-H (northern)	-33.555860	19.149920
Aphanicercella	*securata*	HH1	Franschhoek Pass, Villiersdorp side. DM Stevens.	H-H (northern)	-33.973000	19.175700
Aphanicercella	*securata*	HH4	Harold Porter Botanic Reserve, Betty's Bay. DM Stevens.	H-H (southern)	-34.352300	18.927000
Aphanicercella	*spatulata*	II3, II5	Tweede Tol, Bain's Kloof Pass. DM Stevens.	H-H (northern)	-33.569600	19.138500
Aphanicercopsis	*denticulata*	JJ2, JJ3	Boschenheuwel Arboretum, Kirstenbosch. DM Stevens.	Cape Peninsula	-33.987460	18.437190
Aphanicercopsis	*denticulata*	JJ4, JJ5	Bain's Kloof, sharp bend with white brick wall. DM Stevens.	H-H (northern)	-33.594720	19.121140
Aphanicercopsis	*hawaquae*	KK1	Cloete's Pass, NW of Mossel Bay. DM Stevens.	Langeberg	-33.919800	21.742100
Aphanicercopsis	*hawaquae*	KK2	Oudemuragie road, 11.6 km E off R328 from “Rust en Vrede” signboard. DM Stevens.	Groot Swartberg	-33.411200	22.354100
Aphanicercopsis	*outeniquae*	LL1	Keur River bridge, Montagu Pass, George. DM Stevens.	Outeniqua	-33.907175	22.418134
Aphanicercopsis	*outeniquae*	LL2	Gouna pump station, Gouna Forest. DM Stevens.	Outeniqua	-33.990700	23.040700
Aphanicercopsis	*tabularis*	MM1	Slangolie Ravine, Twelve Apostles. DM Stevens.	Cape Peninsula	-33.977700	18.385100
Aphanicercopsis	*tabularis*	MM4	Theresa Avenue stream, Camps Bay. DM Stevens.	Cape Peninsula	-33.967920	18.382010
Balinskycercella	*gudu*	NN1, NN2	Tugela Gorge. DM Stevens.	KZN Drakensberg	-28.745700	28.913500
Balinskycercella	*tugelae*	OO2, OO3	Tugela Gorge. DM Stevens.	KZN Drakensberg	-28.745700	28.913500
Desmonemoura	*brevis*	PP1, PP3	Rust en Vrede Waterfall, Oudemuragie Road. DM Stevens.	Groot Swartberg	-33.391800	22.355900
Desmonemoura	*pulchellum*	QQ1	Du Toit's Kloof Pass. DM Stevens.	H-H (northern)	-33.722100	19.182100
Desmonemoura	*pulchellum*	QQ3	Keur River bridge, Montagu Pass, George. DM Stevens.	Outeniqua	-33.907175	22.418134

Table 4.3. The distribution of the 44 notonemourid species by mountain range group and associated Level 1 River Ecoregion (*sensu* Kleynhans *et al*. 2005). Grey blocks show species endemic to a mountain range group. Also given are species number per mountain range group, species number per ecoregion, number of ecoregion endemics, percentage ecoregion endemics, and percentage of African Notonemouridae species that are ecoregion endemics.

Level 1 River Ecoregion	Eastern Escarpment Mtns				Namaqua Highlands	North Eastern Uplands	Northern Escarpment Mtns		South Eastern Uplands			Southern Folded Mountains + Southern and South Eastern Coastal Belts												Western Folded Mountains + South Western Coastal Belt								TOTAL
Mountain range group	Eastern Cape Province Southern Drakensberg	KwaZulu-Natal Drakensberg	Lesotho Drakensberg	Maluti	Kamiesberg, Namaqualand	Northern KwaZulu-Natal Midlands	Mpumalanga Drakensberg (northern)	Mpumalanga Drakensberg (southern)	Amatolae	KwaZulu-Natal Midlands	Southern KwaZulu-Natal	Baviaanskloof (E. Cape)	Cape Peninsula	Elandsberge (E. Cape)	Groot Swartberg	Groot-Winterhoekberge (E. Cape)	Hottentots Holland (southern)	Kougaberge (E. Cape)	Langeberg	Outeniqua	Riviersonderend	Southern Eastern Cape Province Highlands	Swartberg	Tsitsikamma	Cedeberg	Groot-winterhoek, Tulbagh	Hex River	Hottentots Holland (northern)	Kouebokkeveld	Piketberg	Skurweberge	Witsenberg	
Afronemoura amatolae								•	•													•									3
Afronemoura spinulata		•				•			•	•	•											•									6
Afronemoura stuckenbergi								•																							1
Aphanicerca austrocapensis sp. n.																			•	•											2
Aphanicerca bicornis																	•										•	•			3
Aphanicerca bovina																												•			1
Aphanicerca breviloba sp. n.															•																1
Aphanicerca brevispina sp. n.																	•														1
Aphanicerca capensis													•																		1
Aphanicerca cederbergensis sp. n.																									•						1
Aphanicerca chanae																			•												1
Aphanicerca gnua																														•	1
Aphanicerca incisura sp. n.																			•												1
Aphanicerca longiloba sp. n.																			•												1
Aphanicerca lyrata																	•											•			2
Aphanicerca mclellani sp. n.														•					•	•											3
Aphanicerca pickeri sp. n.																	•														1
Aphanicerca swartbergensis sp. n.															•																1
Aphanicerca tereta																					•										1
Aphanicerca uncinata																	•											•			2
Aphanicerca witsenbergensis sp. n.																														•	1
Aphanicerca zwicki sp. n.																	•				•							•			3
Aphanicercella barnardi																									•		•		•		3
Aphanicercella bifurcata																		•	•	•	•			•		•	•				7
Aphanicercella bullata														•	•			•	•	•	•				•						7
Aphanicercella cassida	•	•					•	•	•	•		•		•	•	•			•	•		•									13
Aphanicercella clavata													•															•			2
Aphanicercella flabellata													•															•		•	3
Aphanicercella namaquaensis sp. n.					•																										1
Aphanicercella nigra														•		•		•		•				•	•			•			7
Aphanicercella pauletteae sp. n.																				•											1
Aphanicercella quadrata																									•		•				2
Aphanicercella scutata																	•						•		•			•		•	5
Aphanicercella securata																	•											•			2
Aphanicercella spatulata																	•											•			2
Aphanicercopsis denticulata													•		•		•		•				•				•	•			7
Aphanicercopsis hawaquae															•		•		•				•		•			•			7
Aphanicercopsis outeniquae																			•	•				•							3
Aphanicercopsis tabularis													•																		1
Balinskycercella fontium		•																													1
Balinskycercella gudu		•	•	•																											3
Balinskycercella tugelae		•	•																												2
Desmonemoura brevis															•					•											2
Desmonemoura pulchellum																			•	•	•			•	•	•	•	•				8
TOTAL spp per mountain group	1	5	2	1	1	1	1	3	3	2	1	1	5	4	7	2	11	3	12	10	5	3	3	4	8	3	5	14	1	1	1	3	
Total spp per ecoregion	5				1	1	3		3			33													21							
Number of endemics in ecoregion	3				1	0	1		0			14													6							
% endemics within ecoregion	60				100	0	33.3		0			42.4													28.6							
% endemics of African Notonemouridae	6.8				2.3	0	2.3		0			31.8													13.6							

Locality coordinates were collected using a Garmin E-Trex GPS handset in most cases, and by calculating from a 250 000 scale map in a few instances when the GPS was not available, or when museum specimen labels did not provide that information. Subsequently, data points were adjusted to Google Earth (beta version 4.3.7204.0836) coordinates, in cases where I was familiar with the locality, to allow visualization of the actual locality when known, to facilitate future collections. Localities for the specimens used in the mtDNA analyses are given in Table 4.2. Some mountain ranges were grouped together and called a mountain range group to reduce the number of biogeographic areas (Appendix 4.1; Fig. 4.17; Table 4.3). This was done for small adjacent ranges with similar species composition.

Morphological characters and character states

No previous morphological analyses of the southern African Notonemouridae have been done, therefore all cladistic characters and their states were newly conceived (Appendix 4.2). The morphological data matrix is given in Appendix 4.3. Because of difficulty in obtaining foreign material, only a single outgroup was used in the analyses. However, this outgroup (*Notonemoura latipennis* Tillyard), a New Zealand notonemourid, provided larval, adult male and adult female morphological characters, as well as mtDNA data. Additionally, the aim of this analysis was not to assess the monophyly of the southern African fauna, which had been confirmed in a global analysis by Terry & Whiting (2003), but instead to investigate evolutionary relationships among southern African notonemourids. Reductive character coding was the preferred method, following Strong & Lipscomb (1994). Because the larvae of only relatively few species are known with 100% certainty (those with visible adult genitalia in the final instar larva), larval characters chosen were limited to those that are known to be consistent without exception at genus level. All examined larvae were, without exception, easily diagnosed to genus using the selected characters. Character state numbers were arbitrarily assigned and 0 does not imply a plesiomorphic state. The plesiomorphic state for each character, which was obtained as a result of the parsimony analysis, is given in Appendix 4.4. Forty-eight characters were coded; 4 larval, 33 adult male, 6 adult female and 5 general adult. All characters were unordered, 11 were multistate and 37 binary. Inapplicable characters were coded '-'.

In only a few cases were useful species level characters discovered, because of the close morphological similarities, especially within the *Aphanicerca capensis* and *Aphanicercella barnardi* species complexes and between *Aphanicercopsis* species. Where it was apparent that characters were autapomorphies and not phylogenetically useful, they were mostly excluded for brevity. Identically coded species were included as separate terminals because it is useful to know that they are in fact identical in those characters to their congeners, although they would have no effect on cladogram topology, and because they usually differed in the molecular data

set. Potentially, morphometric characters can be included, for example using actual values and ranges in TNT or step-matrix gap-weighting (Wiens 2001).

Phylogenetic analyses of morphological characters

The morphological data comprised 40 ingroup taxa, one outgroup notonemourid from New Zealand, and 48 characters, of which one was uninformative. NONA version 2.0 (Goloboff 1999) was used through WinClada version 1.00.08 (Nixon 2002) for a heuristic equal weighting (EW) maximum parsimony (MP) analysis, using 20000 replications, 1 starting tree per replication (hold/), and tree bisection reconnection (TBR) branch swapping with a second round of TBR (mult*/max*). The same settings were used for an additional analysis using *a priori* (*AP*) weights. Characters 31, 32, 36, 38, 42, and 44 were assigned a weight of two, and the rest of the characters remained at a weight of one. *A priori* weighting is not often done because the ideal analysis technique will minimize subjective input. However, it is more easily justified to apply some basic common sense to morphological weighting than to arbitrarily decide between four or five weighting methods, and then arbitrarily decide on the many options within each, or to simply accept the default settings because they have been found to work well in most situations, but which have nothing to do with the anatomical and taxonomic considerations of the group under examination. In this case, the characters were selected for weighting because they were subjectively regarded as representing the products of deeper morphological evolution compared to other (more 'superficial') characters. These can be regarded as more 'groundplan' than the others and are more likely to affect the deeper nodes. These decisions were based on the assumption that such characters e.g. presence of a reproductive structure and ventral abdominal nerve chain shortening, would be more taxon-inclusive and therefore more informative of generic sister group relationships, and thus more likely to be constant within genera. Uninformative sites were excluded from ensemble consistency (CI) and retention (RI) indices calculations. Branch support was assessed in WinClada/NONA by bootstrap (Felsenstein 1985) and jackknife resampling methods, using settings of 1000 replications, 10 search replications (mult*N), and one starting tree per replication (hold/). As there is no consensus on what constitutes a significant bootstrap or jackknife value, all values were reported for all analyses. Additionally, absolute (Bremer 1988, 1994) and relative Bremer supports (Goloboff & Farris 2001) were calculated using TNT version 1.1 (Goloboff *et al*. 2003). Available memory was increased five times in steps of 10000 starting at 10000 and ending at 60000. Wagner trees obtained from the initial traditional TBR search (1000 replications, holding 10 trees per replication) were saved to RAM and then submitted to another round of TBR. The first Bremer analysis was on most parsimonious trees only, and the subsequent five analyses retaining suboptimal trees from one step to five steps longer in memory. The consensus was examined at each step to determine the end point. Bremer support is the number of extra steps required for a

consensus of suboptimal trees to eliminate a branch from the consensus of all most parsimonious trees. The maximum value possible for Bremer support of a branch is the branch length (number of characters on the branch), and therefore these values are not comparable across data sets, and can be problematical to interpret when weighting schemes are used (Goloboff & Farris 2001). Also, absolute Bremer support simply provides the "net" support of the branch, and does not take into account the amount of contradictory evidence. These problems are circumvented by relative Bremer support which compares supportive and contradictory evidence in computing the relative fit difference which varies between zero (unsupported) and one (uncontradicted) (Goloboff & Farris 2001). This can be scaled from zero to 100 as in TNT. Strict and 50% majority rule consensus trees were produced.

Trees using implied weights (Goloboff 1993) (IW) were produced using TNT with 10000 replications and saving 10 trees per replication in a tree bisection reconnection (TBR) branch swapping traditional heuristic search. All trees (trees to be kept in memory was set at 80000) were saved to RAM and then subjected to a second round of TBR. The default concavity value of k = 3 was used. Implied weighting does not rely on an initial set of weights, and is a non-iterative procedure that weights characters *a posteriori* according to their homoplasy by means of a concave homoplasy function, with characters that are more homoplasious being less influential (Goloboff 1993). Branch support was assessed in TNT using the bootstrap (TBR, 100 replications), the jackknife (TBR, 100 replications, holding 10 trees per replication, 36 character removal probability), and absolute and relative Bremer supports as described above except that trees held in memory were started at 1000 and increased in steps of 1000.

Trees using self weighting or auto weighting (Goloboff 1997) (SW) were produced using TNT with 1000 traditional search replications and saving 10 trees per replication in a TBR branch swapping heuristic search. All trees (trees to be kept in memory was set at 80000) were saved to RAM and then subjected to a second round of TBR. The default concavity value of k = 3 was used. Branch support was assessed using bootstrap (100 replications), jackknife (100 replications, holding 2 trees per replication), and absolute and relative Bremer supports. Bremer supports were calculated as described above under implied weighting, but with 500 replications and holding one tree per replication.

Successive approximations character weighting (Farris 1969) (SAW) was used for a MP heuristic search in PAUP* version 4.0b10 for Windows® (Swofford 2002). Characters were reweighted by the maximum value of the rescaled consistency indices. A TBR search of 20000 replications, holding one tree per stepwise addition was performed. The weights of six reweighted characters stabilized on the third iteration. These weights were input into TNT for

analysis (20000 replications) to produce the consensus tree and the resampling support values. Character weights were rescaled to 100, or a fraction thereof for the six reweighted characters. The bootstrap and jackknife analyses were run with 2000 replications each, keeping one tree per replication.

The commonly employed pluralistic approach to phylogenetic analysis of using multiple analytical methods with differing underlying assumptions and philosophical bases has been criticized (Giribet *et al*. 2002). If there is one true topology, however, then all methods should be able to find it regardless of their philosophical approach. Concordance then between multiple methods does provide a qualitative idea of robustness of the data and resulting cladogram. In the event of discordance, making a subjective choice between competing cladograms aided by other evidence is preferable to deliberately refusing to fully investigate the data. Even within an *a priori* selected single optimality criterion analysis, competing cladograms are obtainable, requiring the investigator to select a preferred topology, for example, choosing between different equally parsimonious cladograms under various weighting schemes. In order to test the morphology and molecular data as thoroughly as possible, both cladistic parsimony and model-based methods were used. For the morphology model-based approach, a Bayesian inference (BI) analysis was performed using MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001), implementing the Markov k + gamma model (Lewis 2001). Four Metropolis-coupled MCMC chains (one cold and three heated) were employed for each of the two simultaneous runs, with the temperature of the heated chains set to 0.075. The remaining settings were left at default values. One million generations of two runs were performed, with trees sampled every 100 generations. The first 2500 samples (25%) were discarded as burnin.

Mitochondrial DNA (COI)*: *DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from a small piece of thoracic muscle tissue from each stonefly. The tissue was homogenized using a plastic pestle in a 1.5ml microcentrifuge tube containing 350 µl 2x CTAB buffer (cetyltrimethyl ammonium bromide) (modified CTAB extraction, Doyle & Doyle 1987), to which 1 µl of proteinase K (10 mg/ml) was added. The sample was then incubated at 60°C for 2 hours, 350 µl 24:1 chloroform:isoamylalcohol added, vortexed, then centrifuged for four minutes at 13000 rpm. The supernatant (300 µl) was transferred to a new microcentrifuge tube, precipitated with 300 µl ice-cold isopropanol, and then frozen overnight. The pelleted mix was then centrifuged for 25 minutes at 13000 rpm, supernatant discarded, washed with 100 µl ice-cold 96% ethanol, and centrifuged again for 5 minutes at 13000 rpm. The supernatant was discarded, the pellet dried in a desiccation jar for 2 hours and then dissolved in 50 µl sterile distilled water for 30 minutes.

A 557 base pair (bp) fragment of the mtDNA cytochrome oxidase subunit I (COI) gene was amplified from each individual DNA extract using forward (LCO1490: 5′-GGTCAA CAAATCATAAAGATATTGG-3′) and reverse (HCO2198: 5′-TAAACTTCAGGGTGACC AAAAAATCA-3′) primers designed to amplify a 710-bp fragment (Folmer *et al.* 1994), with poor quality sequence ends resulting in the shorter useful fragments. The 30 µl polymerase chain reaction (PCR) volume contained 3 µl 10 x NH_4 buffer, 3 µl 25 mM $MgCl_2$, 1.2 µl each of dATP, dCTP, dGTP and dTTp (each 25 mM), 17.65 µl sterile distilled water, 1 µl of each 10 µM primer, 0.15 µl of 5 units / ml Taq (*Thermus aquaticus*) DNA polymerase, and 3 µl of stonefly DNA. The amplification parameters used for 35 cycles on a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems) were as follows: an initial denaturing step of 95°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 1.5 min. This was followed by a final extension step of 72°C for 7 min. The resultant amplified DNA concentrations were estimated by running 3 µl of PCR product on a 1% agarose gel (with ethidium bromide) next to a marker and then visualized under ultraviolet light. The PCR double stranded products were purified using the QIAquick PCR purification kit (Qiagen). Direct sequencing of both strands of the purified PCR product was achieved by first cycle-sequencing (GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems)) using the following in 10 µl reactions (BigDye® Terminator v3.1, Applied Biosystems): 2 µl Terminator Ready Reaction premix, 1 µl BigDye® 5x sequencing buffer, 0.16 µl primer (10 µM), 2.84 ml double distilled water and 1-4 µl DNA. The cycle-sequencing routine was 30 cycles of 96°C for 15s, 50°C for 15s and 60°C for 4 min. Sequencing was run on an ABI 3100 genetic analyzer at the Core DNA Sequencing Facility at the University of Stellenbosch, South Africa.

Sequence alignment and phylogenetic analyses: Mitochondrial DNA

COI sequences were assembled from forward and reverse trace files and aligned using CLC Gene Workbench 2 (CLC bio, Aarhus, Denmark). To test the monophyly of each species, and because evidence of incomplete lineage sorting or mitochondrial introgression was discovered in the *Aphanicerca capensis* species complex (Chapter 3 of this thesis), multiple haplotypes for each taxon were included in the analyses (except for *Aphanicercella quadrata*), so that the 39 species were represented by 102 unique haplotypes.

NONA and WinClada were again used for the molecular COI equal weighting parsimony analysis, with a maximum of 20000 trees to be kept in memory, 10000 replications, one starting tree per replication (hold/), TBR branch swapping followed by another round of TBR (mult*/max*). Uninformative sites were excluded from ensemble consistency (CI) and retention (RI) indices calculations. Bootstrap and jackknife support were assessed in NONA/WinClada using 500 replications, 10 searches within each replication, one starting tree per search, "don't

do max* TBR", and a maximum of 10000 trees kept in memory. Bremer and relative Bremer branch supports were calculated in TNT by increasing trees in memory in steps of 1000 for each suboptimal level, and 1000 replications, keeping one tree per replication.

Maximum likelihood (ML) analysis was performed in PhyML version 2.4.4 (Guindon & Gascuel 2003). The General Time Reversible (GTR) model with a proportion of invariable sites (+ I) and a gamma distribution (+ gamma) was selected as the most appropriate model of DNA substitution using the Akaike Information Criterion (AIC) (Akaike 1973) as implemented in MODELTEST version 3.06 (Posada & Crandall 1998) in tandem with PAUP* version 4.0b10 for Windows® (Swofford 2002); parameters were estimated in PHYML. Branch support was estimated by 1000 non-parametric bootstrap pseudoreplicates (Felsenstein 1985). A BI analysis was conducted using MrBayes under the GTR + I + gamma model. The proportion of invariable sites and the gamma distribution shape parameter priors were not fixed, but were instead left at the default uniform distribution settings of MrBayes. The default uninformative (flat Dirichlet) priors were used for estimation of base substitution rates and nucleotide frequencies. Four Metropolis-coupled MCMC chains (one cold and three heated) were employed for each of the two simultaneous runs. Initial runs failed to achieve satisfactory stationarity, so the number of chains was increased to six (one cold and five heated), and the temperature of the heated chains reduced to 0.05. Three million generations of two runs were performed, with trees sampled every 100 generations. The first 7500 samples (25%) were discarded as burnin, resulting in 22501 trees per run. Stationarity was assumed to have been achieved by examining the generation – log likelihood plot, chain mixing, the average standard deviation of split frequencies between the two runs and the potential scale reduction factor.

Combined analysis of morphological and DNA data partitions

Homogeneity between the morphological and molecular data sets was assessed using 100 replications of the Incongruence Length Difference Test (Farris *et al*. 1994) as implemented in WinClada (Nixon 2002), although the test has been shown to be unreliable in some circumstances (summarized and expanded upon in Ramírez (2006)). Uninformative characters were included in the ILD Test run. As there was no significant incongruence between the morphological and COI data partitions (ILD Test, $P = 0.1782$), they were merged for a combined analysis; this comprised 103 taxa, 605 characters (48 morphological and 557 mtDNA bases) of which 253 were informative (48 morphological and 205 molecular). Equal weighting parsimony analysis was carried out using WinClada and NONA, with 20000 replications using TBR and a second round of TBR. Bootstrap, jackknife, Bremer and relative Bremer supports were calculated in TNT as for the mtDNA analysis. The analysis was repeated with the 2x *a priori* weights for the same six morphology characters as outlined above. A combined partition

BI analysis was performed using MrBayes. As before, the GTR + I + gamma model was used for the COI partition with the same settings as for the DNA-only analysis. For the morphology partition, a Markov k + gamma distribution model was used (Lewis 2001). Again, two runs of six chains each were used, the five heated chains temperature set to 0.05, and 7200000 generations performed with chain sampling every 100 generations. The burnin fraction to be discarded was 18000 samples. Trees from MrBayes and PhyML throughout were produced in TreeView version 1.6.6 (Page 1996) and modified in Corel®Draw™ version 11 (Corel, Ottowa, Canada).

Biogeographical patterns

Locality data were obtained largely from material collected for this study, supplemented by localities obtained from museum collections and the literature. Species distributions were plotted using ArcView version 3.1 (ESRI, California, U.S.A.) on overlays of the Level 1 River Ecoregions of South Africa (Kleynhans *et al*. 2005) and provincial boundaries. These first level ecoregions provide the ecological framework for level 2 river classification which follows the same criteria but at a more detailed level. The 31 ecoregions are intended to reflect river and not terrestrial ecoregions, and therefore may not exactly coincide with purely terrestrial ecosystem boundaries. They were delineated based on physiography (including relief, slope shape, drainage density, stream frequency, and altitude), climate (rainfall variables), geology, soils and vegetation. In order to look for patterns of species distribution between mountain ranges, cluster analysis and non-metric multidimensional scaling (MDS) were performed in Primer 5 for Windows version 5.1.2 (Clarke 1993). Similarity matrices for these two analyses were produced by analysis of samples (mountains) using the Bray-Curtis dissimilarity measure on presence/absence data. The group average algorithm was used for cluster analysis of mountain ranges. *Aphanicercella cassida* was included and excluded in two separate runs of both cluster and MDS analyses, as it is a widespread species and as such may obscure patterns. The MDS was run using 100 iterations, and *Aphanicercella namaquaensis* sp. n. was excluded as the distribution of this species is unknown, and it is currently known only from one unique locality. Stress values were 0.01 and 0.03 in the *A. cassida*-excluded and the *A. cassida*-included three dimensional MDS analyses respectively.

RESULTS AND DISCUSSION

Results are discussed as follows:

A. Character distribution and evolution
B. Intergeneric relationships and monophyly of the genera based on morphology
C. Intergeneric relationships and monophyly of the genera based on mtDNA

D. Intergeneric relationships and monophyly of the genera based on combined morphology and mtDNA partitions
E. Interspecies relationships based on morphology
F. Interspecies relationships based on mtDNA
G. Interspecies relationships based on combined morphology and mtDNA
H. Clade relationships
I. Biogeography.

A. *Character distribution and evolution*

The character consistency (Ci) and retention (Ri) indices (Farris 1989) from the EW and *AP* analyses are given in Appendix 4.4, together with the plesiomorphic states for each character. The double weighted characters that contributed to the topology differences between the EW and *AP* cladograms were 31 (Figs. 4.1-4.2), 36 (Figs. 4.1-4.2) and 44. Weighted characters 32, 38 and 42 (Fig. 4.3) did not result in any differences. The *AP* and EW strict consensus cladograms under unambiguous and accelerated transformation (ACCTRAN) were used to determine the polarities given. ACCTRAN optimization favours reversals rather than parallelisms, and was preferred over delayed transformation (DELTRAN) as it preserves the primary homology presupposition for longer (de Pinna 1991).

Character 5 (the lobate processes of male tergite 9) (Figs 2.11, 2.14, 2.21, 2.26A, F, 3.3A-AA): Under both EW and *AP* weighting, state 0 (absence) was plesiomorphic and state 1 (presence) was a homoplasious apomorphy. Under EW (Figs 4.4-4.5), state 1 was a parallelism in *Desmonemoura* and *Aphanicerca*. The lobate processes on tergite 9 are therefore seemingly not homologous in the two genera, but may have arisen independently. The character failed the test of congruence. Whether or not it failed the test of similarity was not immediately obvious. The lobes in the two genera were topologically similar enough to warrant testing for homology by inclusion in the analysis, although there were differences that represented variation within the homology. The similarity lay in the presence of the lobes and the tergite involved. The dissimilarity lay in the structure of the lobe (see character 6 description). Because there was identifiable dissimilarity, it would be preferable to regard the character as having failed the similarity test, and to diagnose the character as convergent. The character was included in order to test its potential homology. Excluding the character did not alter the topology of the consensus cladogram. In the *AP* unambiguous optimization (Fig. 4.7), polarity could not be determined. In the *AP* ACCTRAN cladogram (Fig. 4.8), the state underwent secondary loss in *Afronemoura*. Although state 1 was a non-homoplasious apomorphy in the *AP* ACCTRAN cladogram, it was not a synapomorphy as it did not define a monophyletic group, but rather a

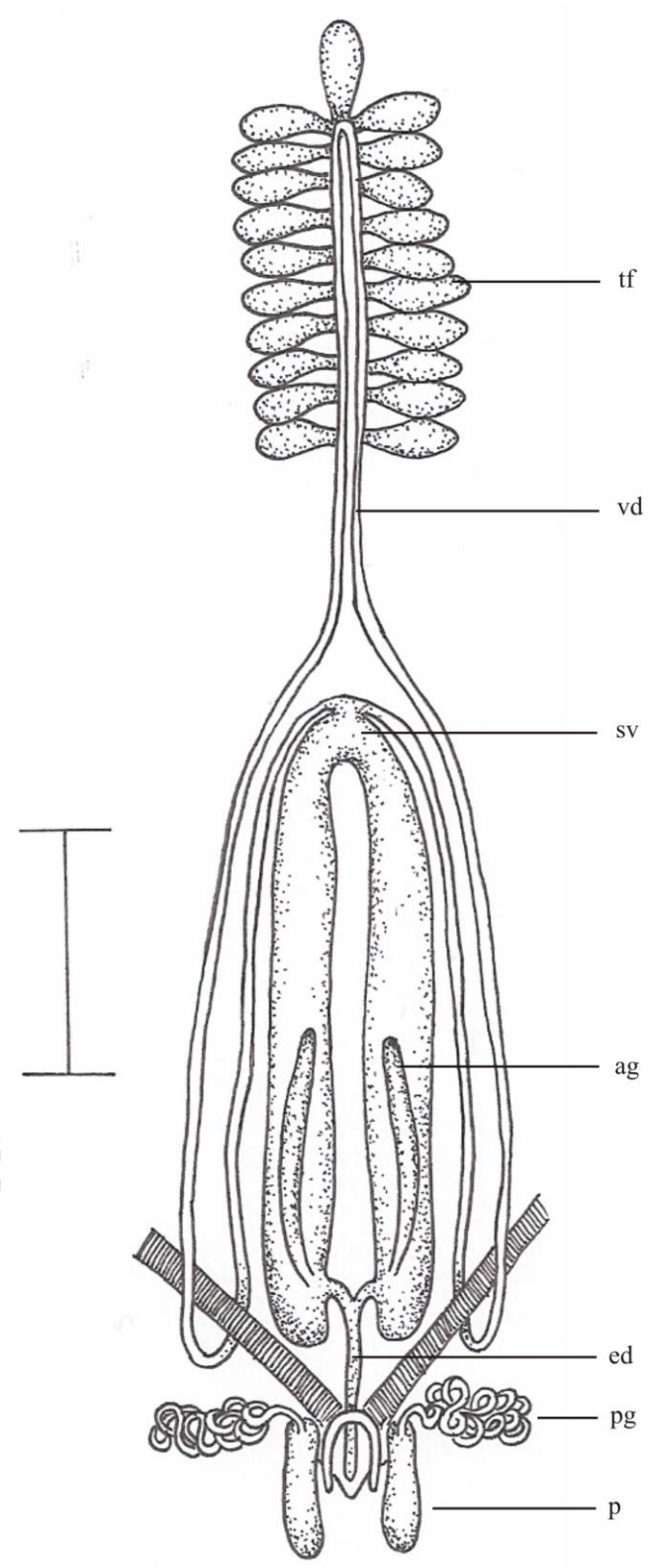


Fig. 4.1. Male internal reproductive system; semi-schematic. *Afronemoura amatolae*. Abbreviations: ag = accessory gland of the seminal vesicle; ed = ejaculatory duct; p = paraproct; pg = paraproct gland; sv = seminal vesicle; tf = testicular follicle; vd = vas deferens. Scale bar = 0.5 mm.

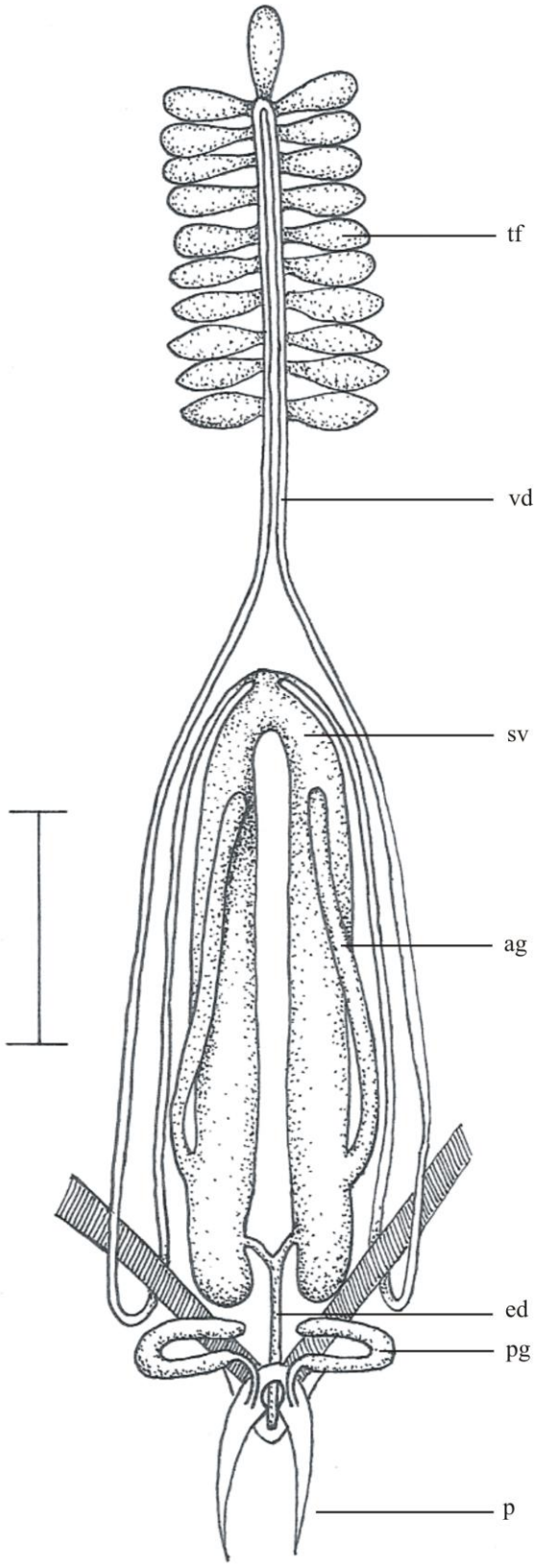


Fig. 4.2. Male internal reproductive system; semi-schematic. *Balinskycercella gudu*. Abbreviations: ag = accessory gland of the seminal vesicle; ed = ejaculatory duct; p = paraproct; pg = paraproct gland; sv = seminal vesicle; tf = testicular follicle; vd = vas deferens. Scale bar = 0.5 mm.

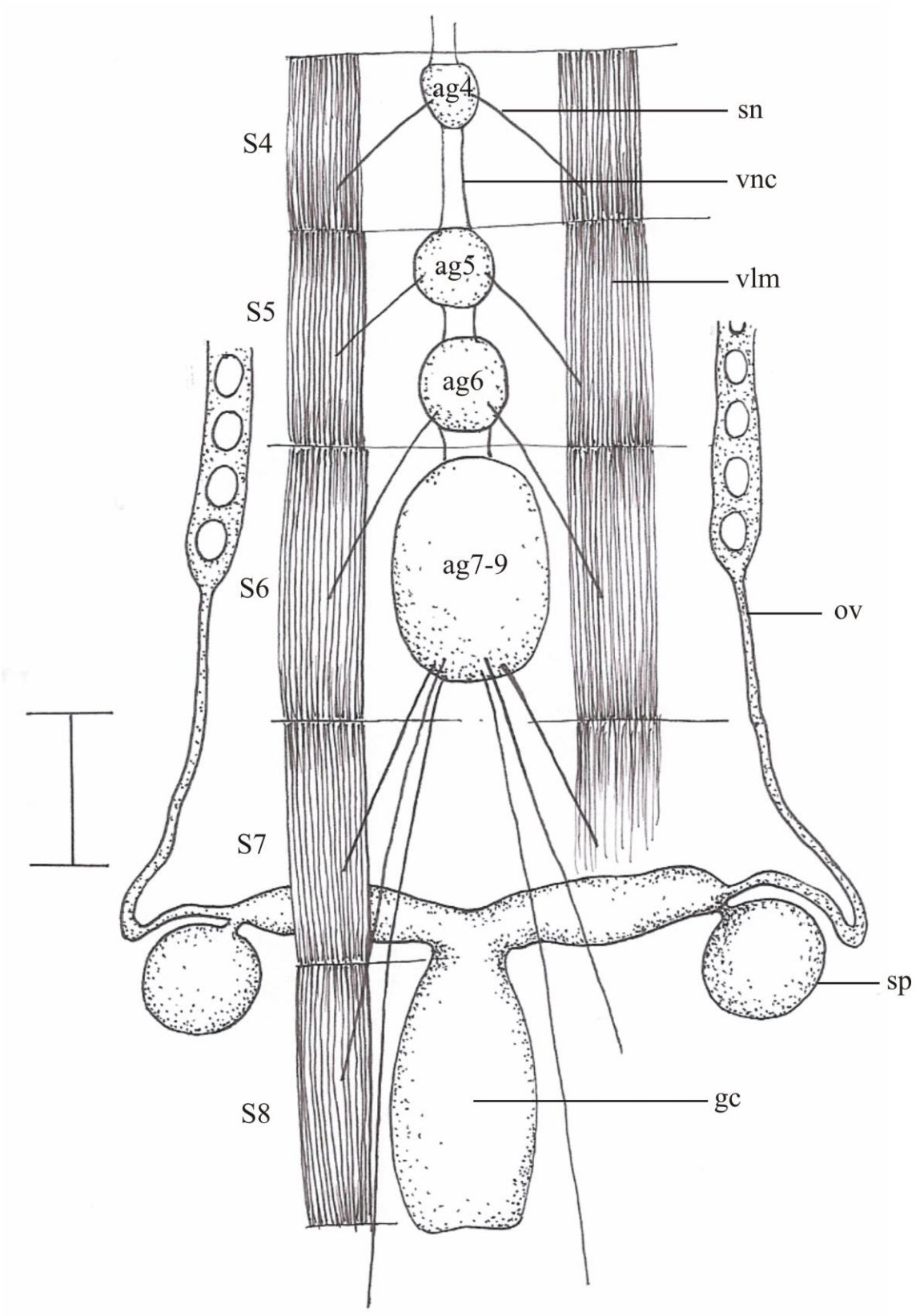


Fig. 4.3. Caudal section of female *Aphanicercopsis tabularis* reproductive system, and of ventral nerve chord; semi-schematic. Abbreviations: ag = abdominal ganglion; gc = genital chamber; ov = oviduct; S = abdominal segment; sn = segmental nerve; sp = spermatheca; vlm = abdominal internal ventral longitudinal muscle. Scale bar = 0.25 mm.

paraphyletic one (Farris 1974). Further analysis would be required to determine the relationship of the tergite 9 dorsal processes between the two genera.

Character state 15:1 (Fig. 2.18I1-K1), the incised apex of the epiproct, was hypothesized to have been a synapomorphy, but was shown to be a homoplasious apomorphy in the three taxa *Aphanicercella bifurcata*, *Aphanicercella nigra*, and *Aphanicercella quadrata*, and therefore may have arisen independently. The mtDNA partition analysis also did not place them as sister taxa.

Character state 16:1, the minute ventral ventrally-directed projection of the male epiproct apex, was a homoplasious apomorphy representing a parallelism in the *Aphanicercella barnardi* species complex and *A. cassida*. This character was a feature of the *A. barnardi* species complex, but also of *A. cassida*.

Character state 18:1 (Figs 2.18A1-K1, 2.21), the elongated arm-like extensions of the male tenth pleurite, was a synapomorphy of *Balinskycercella* and *Aphanicercella*, but did not define a monophyletic group as it was a homoplasy at a deeper level. In the EW and *AP* unambiguous optimized cladograms, polarity could not be determined, but it was clear that the character was a homoplasious apomorphy. In EW ACCTRAN it was a parallelism of the (*Aphanicercella*, *Balinskycercella*) clade and the *Desmonemoura* clade, arising twice by convergence. In *AP* ACCTRAN, the character underwent reversal in *Afronemoura* and *Aphanicerca*. This character may therefore be homologous in *Aphanicercella* and *Balinskycercella*, but not necessarily with the pleurite arm of *Desmonemoura*.

Character 28 (medial supporting sclerite of male paraproct): (Figs 2.12, 2.15, 2.18A3-K3 2.22). A large amount of variation in this character occurs in *Aphanicercella*, with five character states. This variation was significant in species delimitation (Stevens & Picker 1999) (Chapter 2 of this thesis). All other genera were characterized by one autapomorphic state each, with the exception of state 1 which was a synapomorphy for (*Afronemoura*, *Aphanicerca*).

Character 31, the presence or absence of male paraproct glands (Figs 4.1-4.2), was weighted double in the *AP* analysis. These are tubular structures called "glands" here but their histological structure and function are unknown. Under EW unambiguous optimization, polarity could not be determined, but under ACCTRAN, presence (state 1) was apomorphic, with *Aphanicercopsis* undergoing reversal to the plesiomorphic condition. State 1 was also apomorphic in *AP* unambiguous and ACCTRAN optimizations, and was a synapomorphy for the "non-*Aphanicercopsis*" clade ((*Balinskycercella*, *Aphanicercella*), *Desmonemoura*, (*Afronemoura*,

Aphanicerca)). *AP* double weighting improved the EW Ci and Ri scores from 50 and 75 respectively to 100. These structures are interesting and important as they have not been described previously in Plecoptera (P. Zwick, personal communication), and possibly not in Insecta. What makes it likely that these paraproct glands are previously undescribed structures, is that the accessory glands of the seminal vesicle found in this study are assumed to be homologues of the accessory glands found in other plecopteran taxa, leaving no other structures that may be homologous with the paraproct glands. Methods and organs of sperm transfer are highly varied in the Plecoptera (Brinck 1956, Zwick 2000), so a variety of structures and glands would not be unexpected. The glands opened into the membranous base of the paraprocts and took one of two forms, either short and thick with a single loop (Fig. 4.2) (Character 32:0), or long, thin and convoluted (Fig. 4.1) (Character 32:1). The polarity of the shape of the gland was unresolved. Under EW, presence was the plesiomorphic condition, with absence a synapomorphy for (*Balinskycercella*, *Aphanicercella*), while under *AP* ACCTRAN absence was plesiomorphic and presence a synapomorphy of (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*)).

The accessory gland of the seminal vesicle (Figs 4.1-4.2) (character 36) was assumed to be homologous with the accessory glands of other Plecoptera (Brinck 1956; Zwick 1973), but may prove to be a separate structure with a different function, such as a simple diverticulum. As was the case with character 31, under EW unambiguous optimization, polarity could not be determined, but under ACCTRAN, presence (state 1) was apomorphic, with *Aphanicercopsis* undergoing reversal to the plesiomorphic condition. State 1 was also apomorphic in *AP* unambiguous and ACCTRAN optimizations, and was a synapomorphy for the "non-*Aphanicercopsis*" clade ((*Balinskycercella*, *Aphanicercella*), *Desmonemoura*, (*Afronemoura*, *Aphanicerca*)). *AP* double weighting improved the EW Ci and Ri scores from 50 and 75 respectively to 100.

Another double weighted character in the *AP* analysis was the position of the subgenital plate bearing the female genital pore. Sternite 8 (character 38:1) was the plesiomorphic condition. State 0 (sternite 7) was a synapomorphy for (*Balinskycercella*, *Aphanicercella*). The double weight in the *AP* analysis did not result in any changes from the EW cladogram.

Also double weighted was character 42 (Fig. 4.3), paired spermathecae with ducts opening into the oviducts and not into the common oviduct or genital chamber (absent = 0; present = 1). State 1 was a synapomorphy of *Aphanicercopsis denticulata*, *A. hawaquae* and *A. tabularis* (to the exclusion of *A. outeniquae*). Although it is not yet conclusive that these structures are not found in *A. outeniquae*, numerous dissections failed to find them. Although they are called "spermathecae" here, their structure and function are not yet known. They may be "accessory

glands", paired spermathecae or have another function. While reproductive tract accessory glands are common in Plecoptera (e.g. Brinck 1956; Zwick 1973), these particular structures have not been noted in other Plecoptera, but sausage-shaped sperm storage structures in the same position in *Capnioneura* have been described (Zwick 1973) (P. Zwick, personal communication) and therefore may be homologous. Spermathecae in the Plecoptera are single (or bifid and close together) and open into the genital chamber (Brinck 1956). The EW and *AP* cladograms did not differ in regard to this character.

Ventral abdominal nerve cord anatomy in the Plecoptera is complex due to variation within the order (K. Klass, pers. comm.), and Klug & Klass (2007) advised revision of designations in earlier work of ganglia to segments. Within the order, the Notonemouridae is the only family with a variable degree of ganglionic chain shortening (Zwick 1973), which may suggest that the family should be re-examined with a view to subdivision. Zwick (1973) regarded this shortening of the abdominal ganglion chain, which may be due to both anterior and posterior ganglionic fusion, as an important character that is useful phylogenetically, in particular showing a close relationship between Notonemouridae and Nemouridae, the latter having only five free abdominal ganglia. Zwick (2000) stated that in the Nemouridae (*sensu lato*) (i.e. Notonemouridae + Nemouridae), no more than six free ganglia are found. However, it was found in this study, as in Illies (1961) that two conditions exist within the African Notonemouridae, namely either six or seven free ganglia. In three ingroup genera and the notonemourid outgroup, there were seven free ganglia. Illies (1961) also illustrated seven free ganglia in the South American notonemourid *Neonemura*.

Illies (1961) depicted the notonemourid ventral abdominal nerve cord as being shortened anteriorly with fusion of the first abdominal ganglion with the metathoracic ganglion, and posteriorly with fusion from ganglion 8 in *Aphanicercopsis* and from ganglion 7 in *Aphanicerca*. His diagrams show the transverse nerves from a given ganglion (n) supplying the previous segment (n-1); so for example ganglion 3 supplies transverse nerves to the muscles of segment 2. The dissections in the present study showed that the first free ganglion posterior to the metathoracic ganglion supplied the ventral internal longitudinal muscles of segment 1, which is the same as depicted in Illies (1961). Nerve branches were traced from the ganglia to the ventral internal longitudinal muscles of the respective abdominal segments (Fig. 4.3). My interpretation, based on Klug & Klass (2007) and contrary to Illies, is that the first abdominal ganglion was not fused with the metathoracic ganglion, but was actually ganglion 1 and innervates the first abdominal segment. My interpretation of Illies' figures and my dissections therefore, was that fusion occurs from ganglion 7 in *Aphanicercopsis* (and *Aphanicercella* and *Balinskycercella*) and from ganglion 6 in *Aphanicerca* (and *Afronemoura* and *Desmonemoura*).

A cautionary note though, is that nerves may not originate at the point from where they appear to, and this has led to confusion as to whether the origin is the ganglion in front of or behind the point of apparent origin (Klug & Klass 2007). Because the present study did not focus on the central nervous system and was limited in its approach to the problem, more detailed studies should be undertaken before reaching any firm conclusions.

Because of the potential phylogenetic usefulness of nervous system characters (Zwick 1973; Klug & Klass 2007), this variable shortening of the nerve cord (character 44) was double weighted in the *AP* analysis. The condition of six free ganglia (state 0) represents increased ganglionic fusion and was a synapomorphy of (*Desmonemoura* (*Afronemoura*, *Aphanicerca*) in the *AP* analysis. In the EW cladogram, apomorphic state 0 was a parallelism in *Desmonemoura* and (*Afronemoura*, *Aphanicerca*). Zwick's (2000) assertion therefore, that the longer chain is plesiomorphic was corroborated in this study.

B. ***Intergeneric relationships and monophyly of the genera based on morphology***

The strict consensus equal weighting maximum parsimony analysis revealed a polytomy of four clades, namely (*Desmonemoura*, *Aphanicercopsis*, (*Balinskycercella*, *Aphanicercella*), (*Afronemoura*, *Aphanicerca*)) (Figs 4.4-4.5). Cladogram branch lengths and statistics are given in the figure legends. Support values are given in Fig. 4.6. The *a priori* weighting (Figs 4.7-4.9), implied weighting (Fig. 4.10), self weighting (Appendix 4.8) and successive weighting (Appendix 4.9) strict consensus cladograms were all better resolved, with all four forming a sister group relationship between *Aphanicercopsis* and the other five genera. The only other difference between the five cladograms was that in the *AP* analysis, which was the best resolved strict consensus cladogram with no generic polytomies, *Desmonemoura* became a sister group to the (*Afronemoura*, *Aphanicerca*) clade. All the genera within the morphological cladograms were monophyletic. The IW, SW and SAW cladograms were congruent. The splitting of *Aphanicercopsis* as sister group to the other genera was fairly well supported in *AP* (Fig. 4.9) with a Bremer support of 3, and in SAW (Appendix 4.9) with a bootstrap value of 80% and jackknife 86%. The EW majority rule consensus cladogram (Appendix 4.10), like the other four weighted analyses, removed *Aphanicercopsis* from the basal polytomy of the strict consensus, and placed it as sister group to the other genera. The generic relationships of the EW majority rule cladogram and the strict consensus of the IW, SW and SAW cladograms were congruent.

Although the Bayesian morphology cladogram (Appendix 4.11) provided a slightly different topology, the BI and *AP* generic relationships were very similar in the following respects: an *Aphanicercopsis* clade, an (*Aphanicercella*, *Balinskycercella*) clade, and a (*Desmonemoura (Afronemoura*, *Aphanicerca*)) clade.

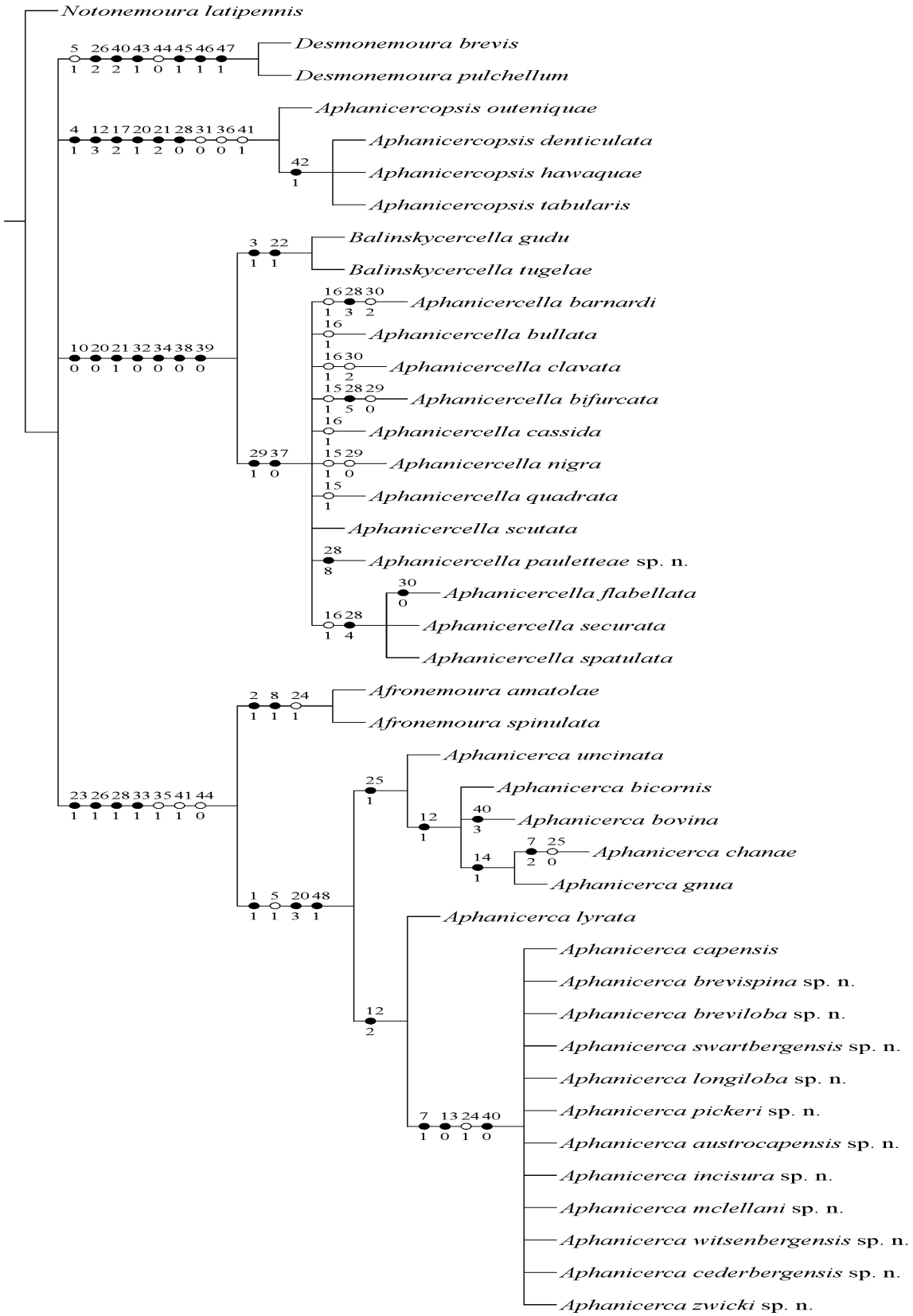


Fig. 4.4. Strict consensus tree (L = 96, CI = 77, RI = 94) of 372 most parsimonious cladograms (L = 84, CI = 88, RI = 97) using morphological characters under equal weighting unambiguous optimization. Filled circles are non-homoplasious apomorphies and open circles are homoplasious apomorphies, with the character number above and state below.

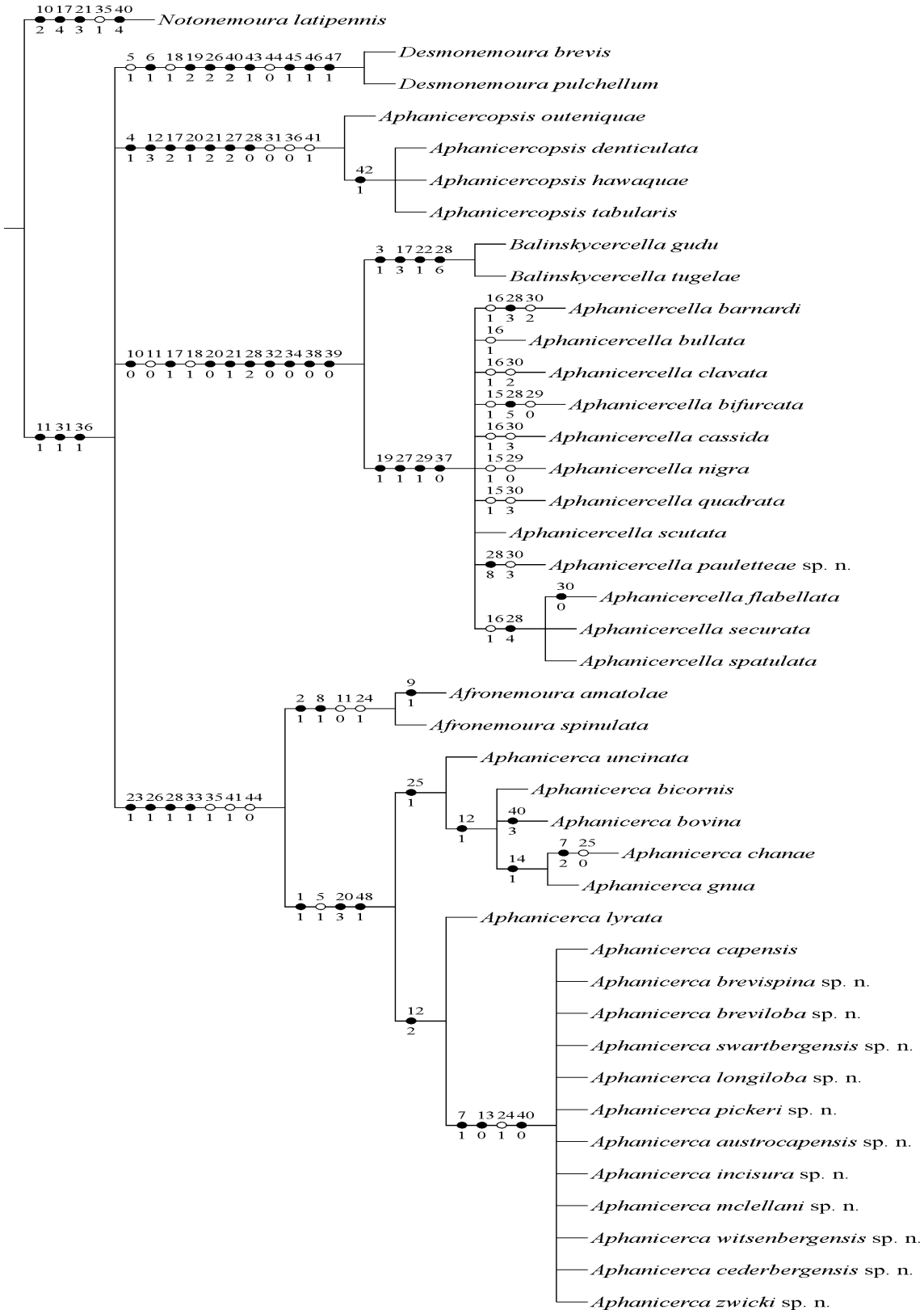


Fig. 4.5. Strict consensus tree (L = 96, CI = 77, RI = 94) of 372 most parsimonious cladograms (L = 84, CI = 88, RI = 97) using morphological characters under equal weighting ACCTRAN optimization. Filled circles are non-homoplasious apomorphies and open circles are homoplasious apomorphies, with the character number above and state below.

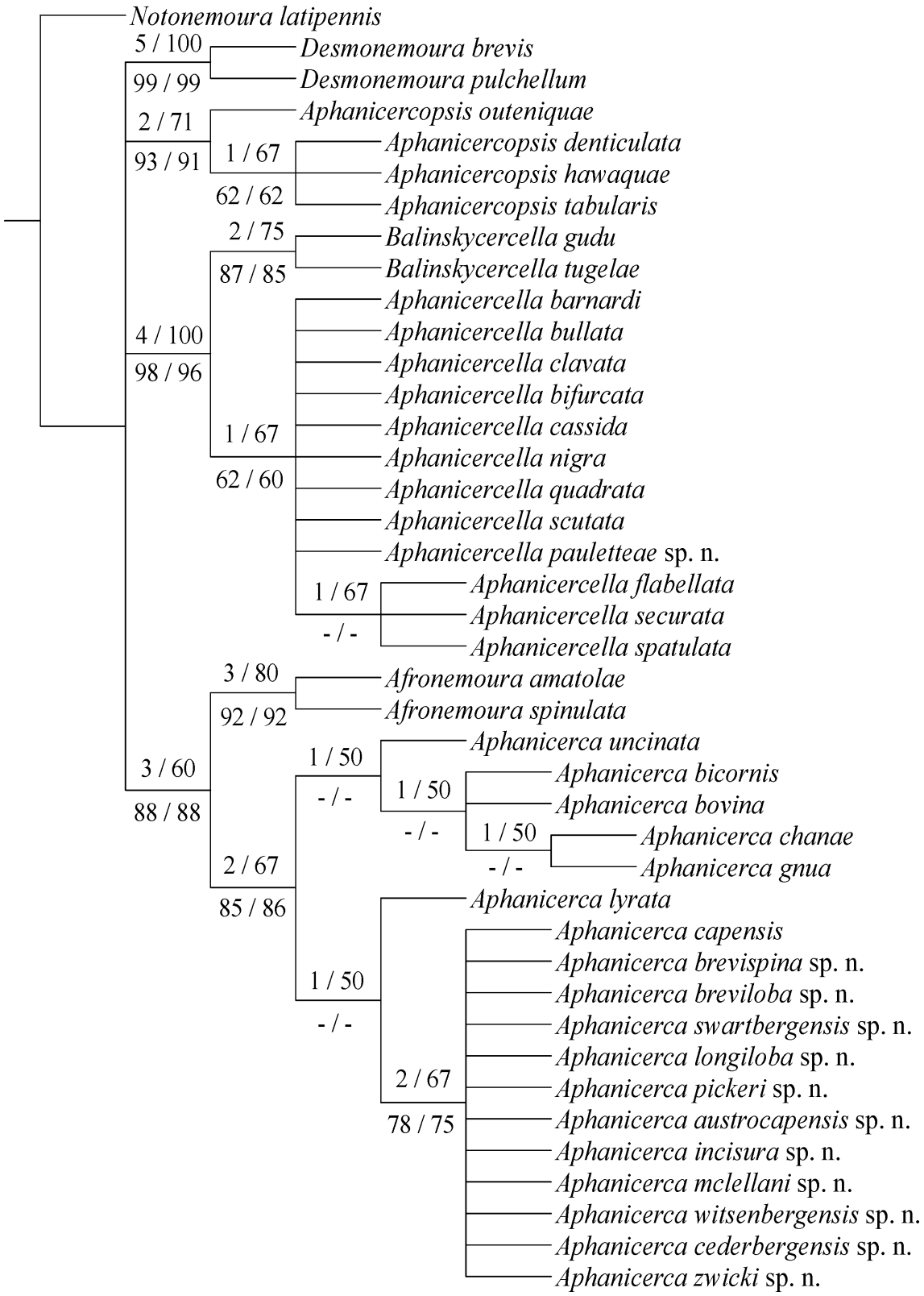


Fig. 4.6. Branch support for the morphology strict consensus cladogram under equal weighting. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A dash indicates failure to recover the node.

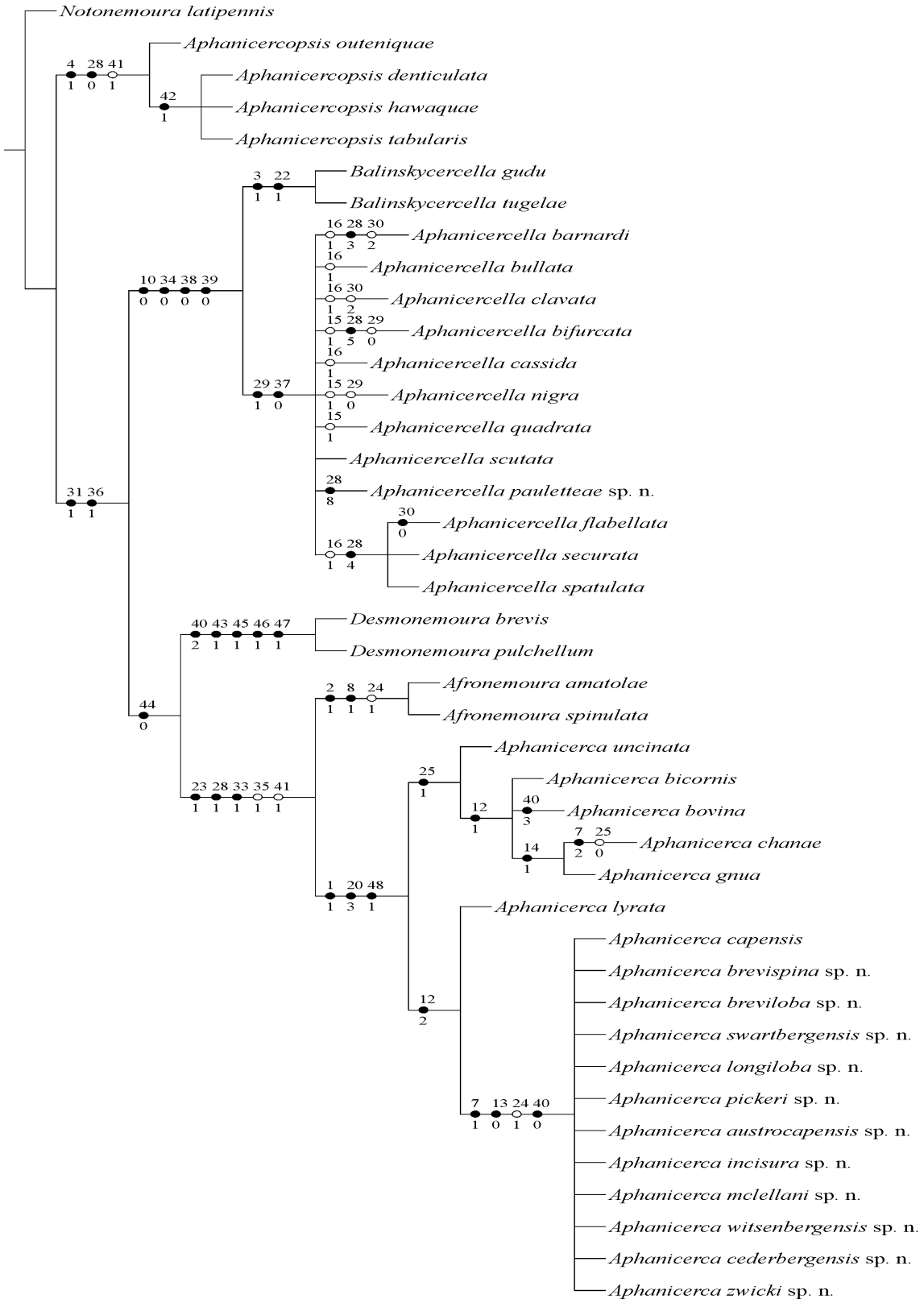


Fig. 4.7. Strict consensus tree (L = 99, CI = 80, RI = 96) of 124 most parsimonious cladograms (L = 90, CI = 88, RI = 97) using morphological characters under *a priori* weighting unambiguous optimization, where all characters were weighted 1 except for characters 31, 32, 36, 38, 42 and 44 which were weighted 2. Filled circles are non-homoplasious apomorphies and open circles are homoplasious apomorphies, with the character number above and state below.

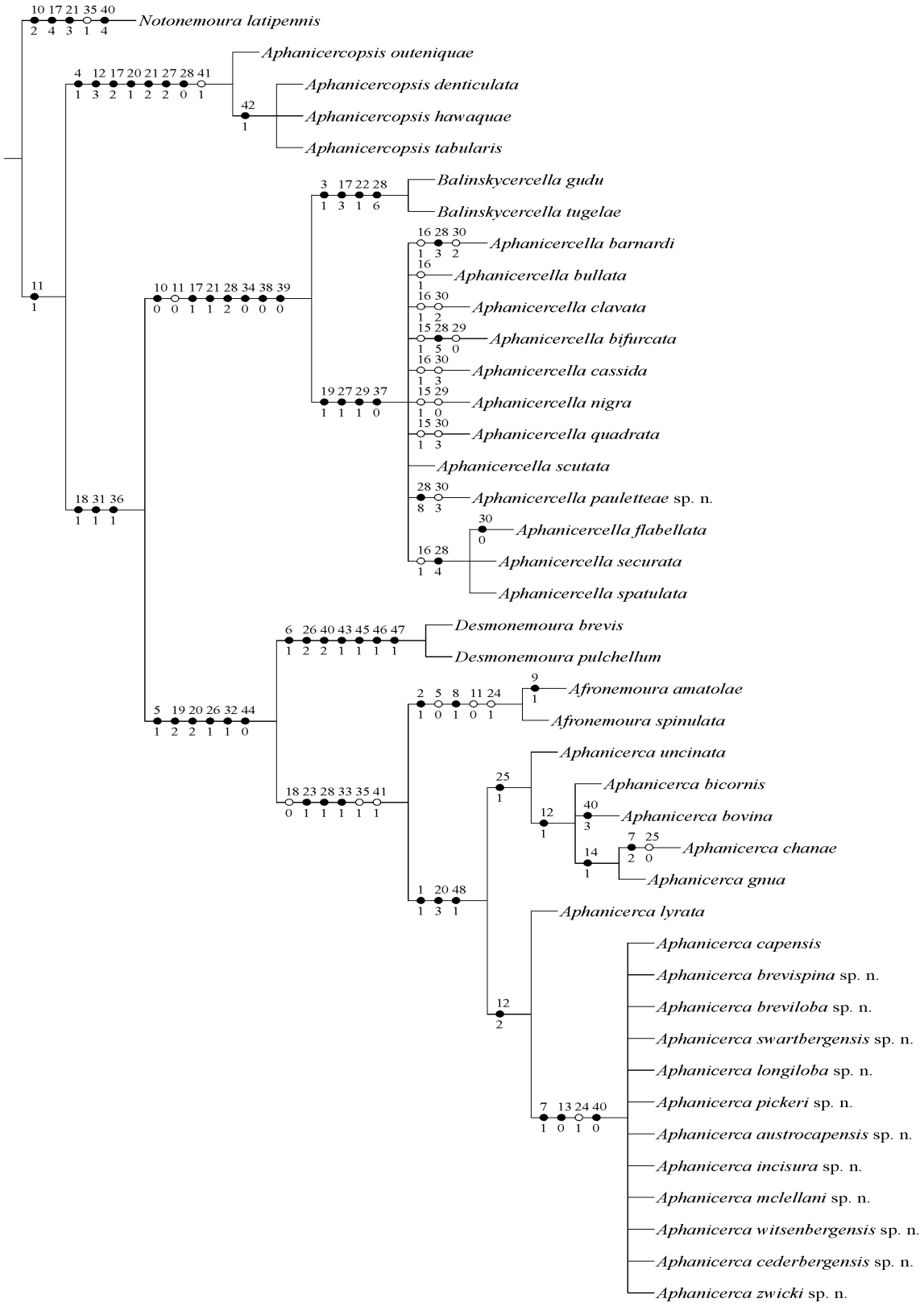


Fig. 4.8. Strict consensus tree (L = 99, CI = 80, RI = 96) of 124 most parsimonious cladograms (L = 90, CI = 88, RI = 97) using morphological characters under *a priori* weighting ACCTRAN optimization, where all characters were weighted 1 except for characters 31, 32, 36, 38, 42 and 44 which were weighted 2. Filled circles are non-homoplasious apomorphies and open circles are homoplasious apomorphies, with the character number above and state below.

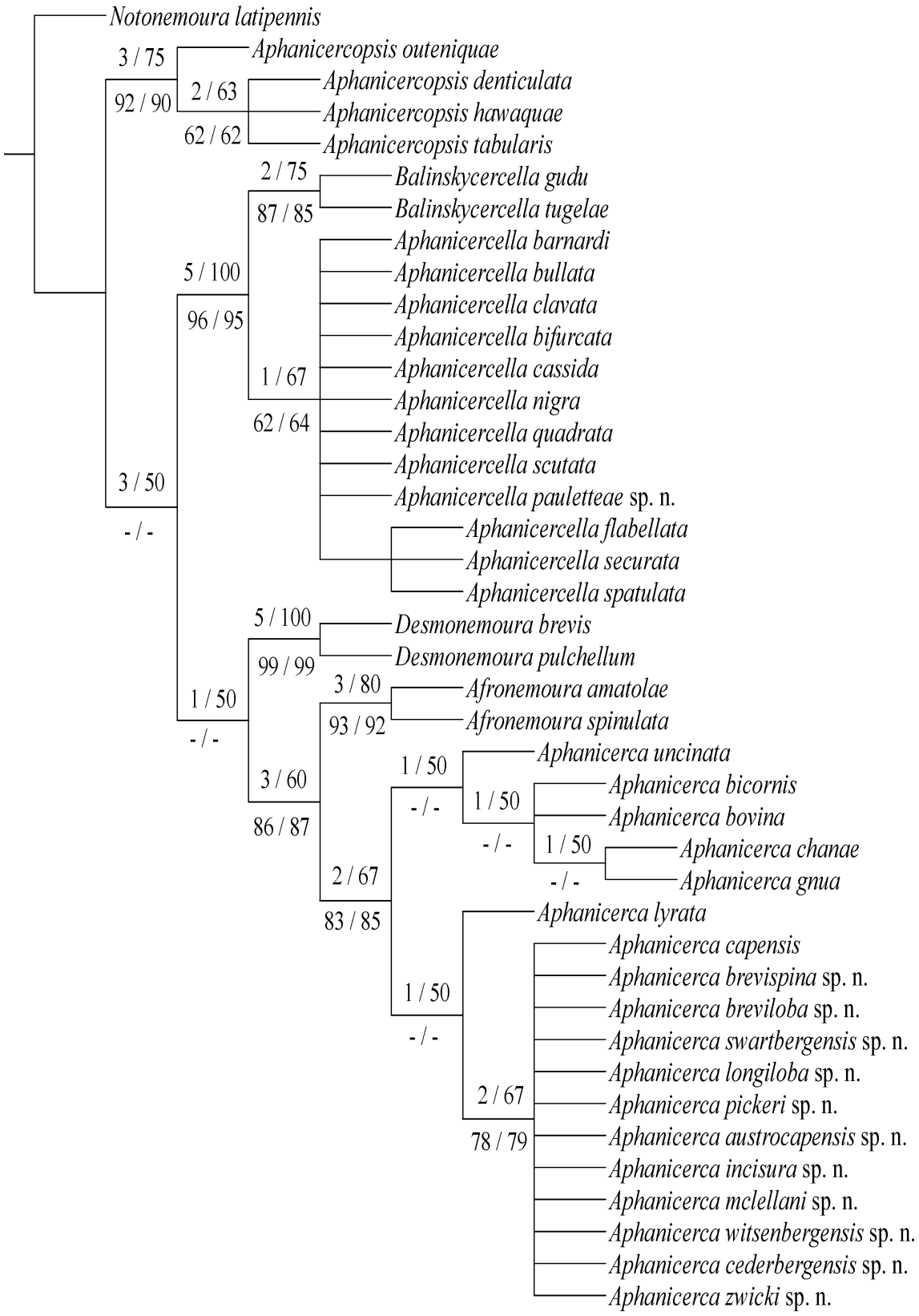


Fig. 4.9. Strict consensus tree (L = 99, CI = 80, RI = 96) of 124 most parsimonious cladograms (L = 90, CI = 88, RI = 97) using morphological characters under *a priori* weighting, where all characters were weighted 1 except for characters 31, 32, 36, 38, 42 and 44 which were weighted 2. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A dash indicates failure to recover the node.

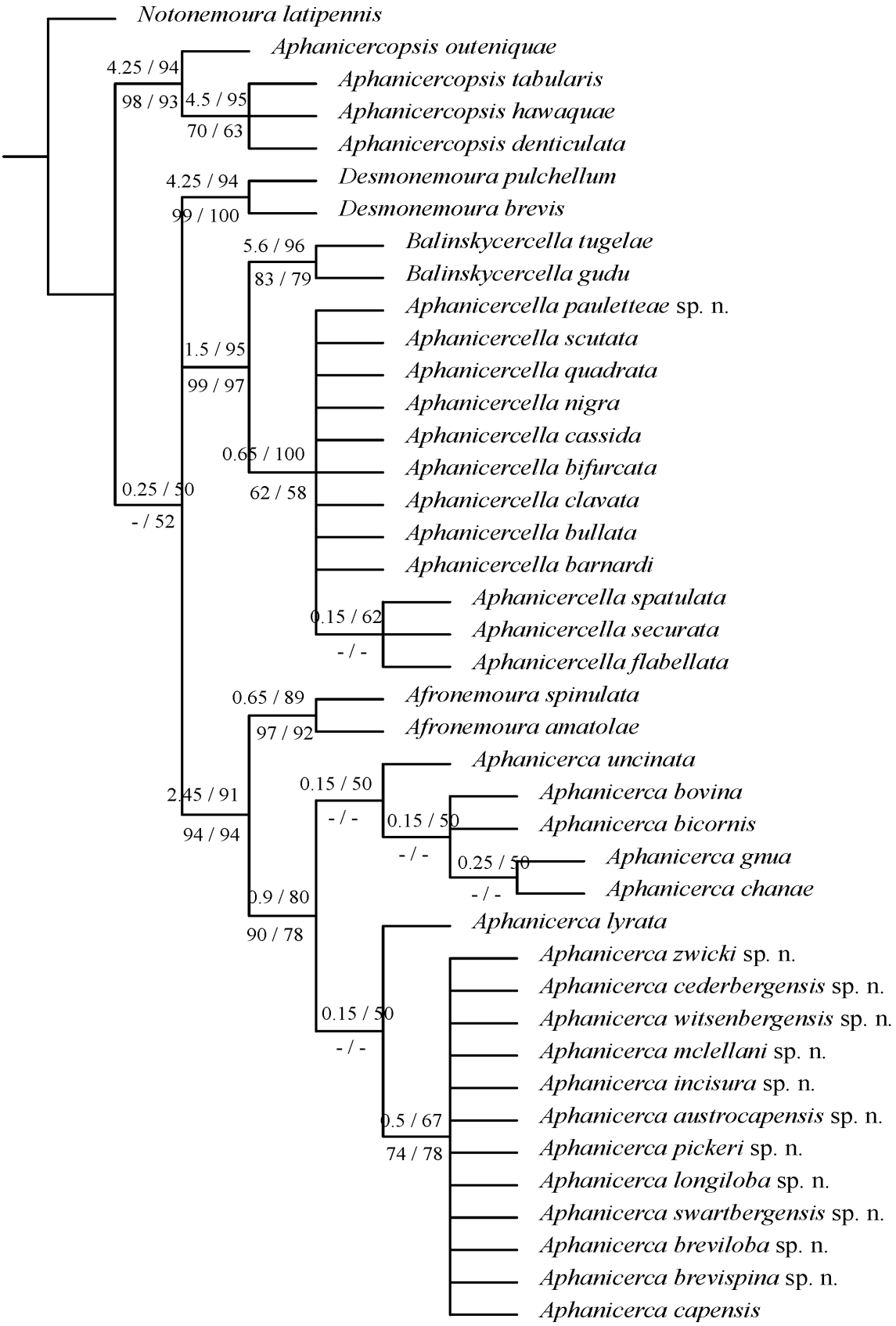


Fig. 4.10. Strict consensus tree of 248 most parsimonious cladograms using morphological characters under implied weighting with k = 3. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A dash indicates failure to recover the node.

In summary, all five morphology MP weighting schemes and the BI cladogram were in agreement on the monophyly of the genera, the clade (*Aphanicercella*, *Balinskycercella*), and the clade (*Afronemoura*, *Aphanicerca*) with high support values. The MP *AP* (Figs 4.7-4.8) and BI (Appendix 4.11) analyses additionally recognized *Desmonemoura* as the sister group to *(Afronemoura*, *Aphanicerca*). The EW majority rule cladogram (Figs 4.4-4.5) and the strict consensus of the IW (Fig. 4.10), SW (Appendix 4.8) and SAW (Appendix 4.9) cladograms were congruent for generic relationships, namely (*Aphanicercopsis* (*Desmonemoura*, (*Aphanicercella*, *Balinskycercella*), (*Afronemoura*, *Aphanicerca*))).

The (*Balinskycercella*, *Aphanicercella*) clade was supported in EW by the unambiguous synapomorphies: male sternite 9 short (10:0), male pleurites 10 large and mobile relative to lateral dorsal plates (20:0), median dorsal plate of male tergite 10 subtriangular (crescentic) (21:1), male paraproct glands short and thick with a single loop (32:0), male paraproct membranous apex folded over (34:0), seventh sternite (subgenital plate) bears female genital pore (38:0), and female subgenital plate not produced caudad to the attachment to the membranous part of the sternite (39:0). These, with the exception of characters 20, 21, and 32 were the same as for the *AP* unambiguous optimization (Figs 4.7-4.8). Additional synapomorphies under ACCTRAN optimization can be read off the respective EW ACCTRAN and *AP* ACCTRAN cladograms (Figs 4.5, 4.7).

The clade (*Afronemoura*, *Aphanicerca*) was supported under both unambiguous and ACCTRAN optimizations of EW by the synapomorphies: male tergite 10 lateral dorsal plates arise from the posterior margin of the tergite (23:1), lateral supporting sclerite of male paraproct is a robust, short, broad plate (26:1), medial supporting sclerite of male paraproct (= arch process) is a flat subrectangular plate, parallel to and shorter than lateral sclerite (28:1), and male paraproct membranous tip is not apically acute (33:1). These, with the exception of character 26, were the same as those recovered in the *AP* unambiguous and ACCTRAN optimization analyses.

The clade (*Desmonemoura* (*Afronemoura*, *Aphanicerca*)) was not recovered by the EW analyses. The only *AP* unambiguously optimized synapomorphy that supported this clade was increased fusion of ventral nerve cord ganglia (44:0), a double weighted character (Figs 4.3, 4.7). There were another five apomorphies that supported this clade in the ACCTRAN optimized *AP* cladogram ((5:1), (19:2), (20:2), (26:1), (32:1)), but the only one that was a synapomorphy for the three genera was character state 32:1, male paraproct glands long, thin and convoluted. The other characters either underwent a reversal, a transformation or were not applicable to some terminals.

The clade ((*Aphanicercella*, *Balinskycercella*), (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*))), the "non-*Aphanicercopsis*" clade, was not recovered by the EW analysis. In the *AP* cladogram, the unambiguous synapomorphies were: the presence of male paraproct glands (31:1), and the presence of bilateral accessory glands of the male seminal vesicle (36:1). Both of these characters were double weighted.

The monophyly of *Afronemoura* was supported unambiguously in EW and *AP* by two synapomorphies: a group of setal tufts one-third from the larval antennal base (2:1) and the presence of spines on the posterior margin of tergite 9 (8:1). Although only two non-homoplasious characters long, the branch was fairly well supported (Bremer = 3; Relative Bremer = 80). More importantly than statistics though, the characters were strong (large, obviously unique, consistent) and important in defining the genus. The monophyly was also supported by one homoplasious apomorphy (24:1), which also occurred in the *Aphanicerca capensis* species complex. ACCTRAN optimization revealed one additional supporting homoplasy in both EW and *AP* (11:0), and another homoplasy (5:0) in *AP*.

The monophyly of *Aphanicerca* was supported unambiguously in EW and *AP* by three synapomorphies: hairs on the proxomedial aspect of the larval antennae are longer than other antennal hairs (1:1), male pleurites 10 are fused to each other anterior to the lateral dorsal plates (20:3) and the presence of the large and very obvious clear patch on the forewings (48:1). There was an additional supporting homoplasious apomorphy in the EW cladogram, the presence of the dorsal processes of tergite 9, also arising independently in *Desmonemoura*. Reformulating this character is required, as this character was a defining character of the genus. (Bremer = 2; Relative Bremer = 67) (Fig. 3.3A-AA).

The monophyly of *Aphanicercella* was supported unambiguously in EW and *AP* by two apomorphies: the presence of a basal supporting process of the male paraproct (29:1), although it reverted to absence (state 0) in two species, and the accessory gland of the seminal vesicle extends the entire length of the seminal vesicle (37:0). Under ACCTRAN EW and *AP*, two additional supporting synapomorphies were recovered: male pleurite 10 has an arm-like extension of the posterodorsal margin with articulation (19:1) and male paraprocts largely membranous with one or more heavily sclerotized long thin sclerites (27:1). Support for this clade was not strong (Bremer = 1; Relative Bremer = 67).

The monophyly of *Aphanicercopsis* was supported unambiguously in EW by six synapomorphies (4:1, 12:3, 17:2, 20:1, 21:2, 28:0) and in *AP* (4:1, 28:0) by two. Under ACCTRAN, the EW and *AP* recovered the identical seven synapomorphies: pronotum appears

rounded to oval in dorsal view, with rounded corners and equal or subequal width and length (4:1), male epiproct denticulation protruding laterally on lateral margins (12:3), base of male epiprocts curved sclerites as continuations of epiproct lateral sclerites (17:2), male pleurites 10 reduced and fused over whole width to lateral dorsal plates (20:1), median dorsal plate of male tergite 10 broad anteriorly and deeply excised posteriorly (21:2), male paraproct contains long, very thin lateral supporting sclerite which lies on lateral margin (27:2), and medial supporting sclerite of male paraproct has broad base, is long and tapers to a thin apex which is fused with or terminates near the lateral sclerite (28:0). Additional support for monophyly of the genus was provided in both EW and *AP* by one homoplasious apomorphy: the female paraprocts (subanal plates) are elongated, extending beyond the cerci (41:1), also occurring independently in *Afronemoura* and *Aphanicerca*. The EW unambiguous and ACCTRAN optimizations provide another two homoplasies: a reversal to the plesiomorphic condition of absent male paraproct glands (31:0), and a reversal to the plesiomorphic condition of absent accessory glands of the seminal vesicle (36:0). The fact that *Aphanicercopsis* differed from the other local genera in the absence of two important internal genitalic characters (31 and 36), lends support to preferring the *AP* over the EW cladogram, in regarding the genus as sister group to the other five genera. In addition, the presence of spermathecae (42:1) was a major departure from the other local genera.

The monophyly of *Balinskycercella* was supported unambiguously in EW and *AP* by two synapomorphies: complete whorls of long setae on all abdominal segments of the larva, save for mid-ventrally (3:1), and median dorsal plate of male tergite 10 elongated anterad with a prominent hook (22:1). These two characters are important diagnostic features of the genus. Both EW and *AP* ACCTRAN recovered an additional two synapomorphies: base of male epiproct comprises curved sclerites as continuations of lateral sclerites - one anterad and one mediad (17:3), and medial supporting sclerite of male paraproct horseshoe shaped (28:6).

The monophyly of *Desmonemoura* was supported unambiguously in EW by six synapomorphies: lateral supporting sclerite of male paraproct is an elongated broad plate abruptly narrowed at apical quarter (26:2), elongated female subgenital plate produced caudad to the attachment to the sternite and has a broad median incision (40:2), sternite 7 of female with swelling at posterior margin (43:1), brown pronotum (45:1), adult setation of abdominal tergites comprises numerous thicker longer hairs in addition to fine clothing hairs (46:1), and a banded wing pattern (47:1). Monophyly of the clade was also supported by two homoplasious apomorphies in the EW unambiguous optimization: dorsal lobate processes of tergite 9 (5:1), and the sixth abdominal ganglion comprises fusion of posterior ganglia of the ventral abdominal nerve cord (44:0). EW ACCTRAN added two additional synapomorphies (male tergite 9

posteriorly directed dorsal lobate process comprises two separate processes with widely separated bases (6:1), and elongated male pleurites 10 comprise long appendage with circular base (19:2)) and one additional homoplasious apomorphy (male pleurites 10 elongated posterad (18:1)) which also arose independently in *Balinskycercella* and *Aphanicercella*. The *AP* unambiguous optimization supported monophyly with the same characters as EW unambiguous except 26:2. *AP* ACCTRAN optimization recovered the same synapomorphies as EW ACCTRAN, except for character state 19:2, and did not recover any homoplasies.

C. *Intergeneric relationships and monophyly of the genera based on mtDNA*

The MP analysis produced a polytomy of five branches, with the only multi-genus clade being (*Afronemoura*, *Aphanicerca*) (Appendix 4.12; statistics provided in the figure legend). This differed from morphology in not recognizing an (*Aphanicercella*, *Balinskycercella*) clade. Also differing from morphology was that *Afronemoura* became paraphyletic in the MP, ML and BI analyses. The remaining genera were monophyletic in the MP analysis. In the ML (Appendices 4.13 - phylogram, 4.14 - cladogram to more clearly show relationships and support values)) and BI (Appendix 4.15) analyses, *Afronemoura spinulata* was the sister group to the rest of the taxa. In both ML and BI, *Aphanicercella* became paraphyletic. In ML, *Balinskycercella* was the sister group to *Desmonemoura*. In BI, *Balinskycercella* was sister to *Aphanicercella nigra*, and *Desmonemoura* had a sister group relationship to an (*Aphanicercopsis* (*Aphanicercella* spp, (*Aphanicercella* sp., *Balinskycercella*), *Aphanicercella* spp) clade. In all three analyses, *Aphanicerca*, *Aphanicercopsis*, *Balinskycercella* and *Desmonemoura* were monophyletic, but sister relationships differed between all methods. There were no other common elements at generic level between the three methods. The ML phylogram had a likelihood value of -5572.67692, a gamma shape parameter of 1.640, and the proportion of invariant sites 0.609. The nucleotide frequencies were A = 0.27155, C = 0.20041, G = 0.17322, and T = 0.35481.

D. *Intergeneric relationships and monophyly of the genera based on combined morphology and mtDNA partitions*

The MP EW combined analysis strict consensus cladogram recovered a polytomy of four clades, namely (*Desmonemoura*, *Aphanicercopsis*, (*Aphanicercella*, *Balinskycercella*), (*Afronemoura*, *Aphanicerca*)) (Fig. 4.11; see legend for cladogram statistics). This topology of generic relationships was congruent with the morphology EW strict consensus MP cladogram, with all genera being monophyletic. Similarly, the *AP* combined analysis strict consensus cladogram (Fig. 4.12) was congruent in generic relationships with morphology (Figs 4.7-4.8). The BI combined analysis recovered an (*Aphanicercella* sp, ((*Aphanicercella* sp., *Balinskycercella*), *Aphanicercella* spp)) clade, and a (*Desmonemoura* (*Afronemoura*,

Aphanicerca)) clade (Appendix 4.16). *Balinskycercella* nested within *Aphanicercella* (paraphyletic) was the same pattern obtained in the BI COI partition analysis (Appendix 4.15). *Aphanicercopsis* became the sister group to the above mentioned clades. As in the COI MP analysis, branch support for the *Aphanicercella* clade was low, as was support for the (*Aphanicercella, Balinskycercella*) clade.

To summarize, monophyly of the genera was confirmed in all morphology and combined analyses. There are useful morphological synapomorphies of all genera. The mtDNA partition MP analysis yielded monophyly of all genera except *Afronemoura* which was paraphyletic. *Afronemoura* and *Aphanicercella* were paraphyletic in the ML and BI analyses, and the other four genera were monophyletic. Generic relationships differed between all three molecular analyses. It is not clear why *Aphanicercella* showed paraphyly in likelihood and Bayesian analyses but not in parsimony, and why generic relationships differed between methods, but it is possible that likelihood parameters were difficult to estimate. A data set with more markers would probably lead to monophyly in these instances of paraphyly.

The intergeneric relationships recovered from the EW morphology and combined analyses were (*Desmonemoura*, *Aphanicercopsis*, (*Aphanicercella*, *Balinskycercella*), (*Afronemoura*, *Aphanicerca*)), and from the *AP* morphology and combined analyses were (*Aphanicercopsis*, ((*Aphanicercella*, *Balinskycercella*), (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*)))), with the latter cladogram preferred as it is better resolved. These intergeneric relationships do not concur with those of Terry & Whiting (2003) in a recent global phylogeny of Plecoptera. Their large six-gene and morphological analysis included five exemplars of southern African notonemourids from five of the six genera. The morphological component of that study comprised characters taken from Zwick (2000) and was therefore only applicable to the level of family and did not inform relationships at generic or species level (at least in the case of the southern African exemplars). Their generic relationships were based on DNA alone as the scope of their study was too large for detailed morphological study at the generic and species level. The intergeneric relationships (*Aphanicerca*, (*Aphanicercopsis*, (*Desmonemoura*, (*Afronemoura*, *Aphanicercella*) obtained from those six genes conflict with the present COI, morphological and combined cladograms. None of the trees produced here was congruent with their phylogeny. In spite of the thoroughness of using numerous genes, it may be that such large studies investigating deeper phylogenetic relationships may recover spurious relationships at genus level. Potentially, COI may have fortuitously simply been better suited to the particular taxa and level of evolutionary divergence of this study. Probably most likely, though is the effect of taxon sampling, considering that Terry & Whiting (2003), because of the size and aims of their study,

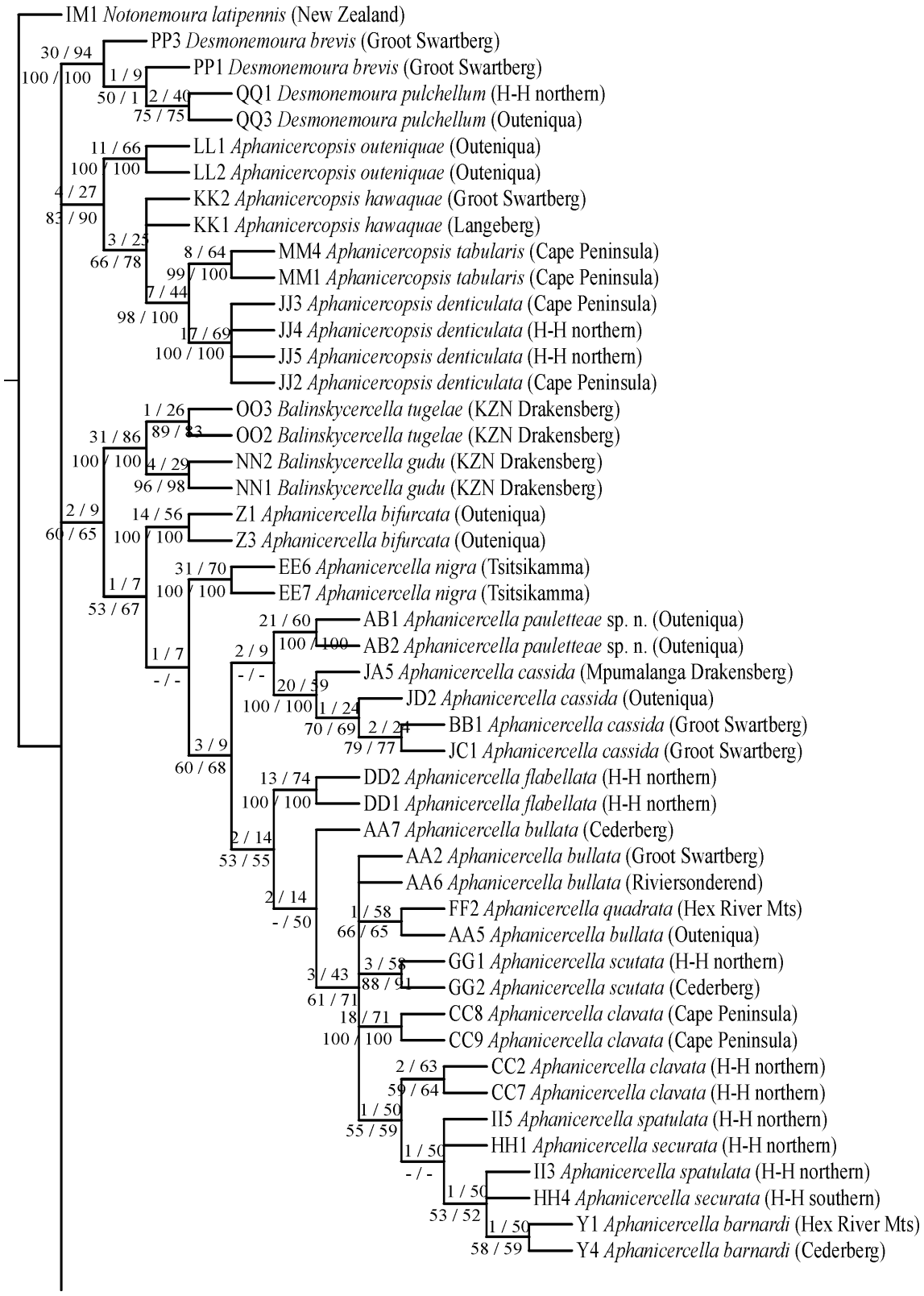


Fig. 4.11. Strict consensus tree (L = 1245, CI = 35, RI = 86) of 78 most parsimonious cladograms (L = 1209, CI = 36, RI = 87) using 48 morphological and 557 mtDNA COI characters under equal weighting. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A support value represented by a dash indicates that the node was not recovered. Taxon names are preceded by the sample field code. The mountain range indicates only the sample locality and not the entire range of the species. H-H = Hottentots Holland Mountains; KZN = KwaZulu-Natal. Fig. 4.11 continued overleaf.

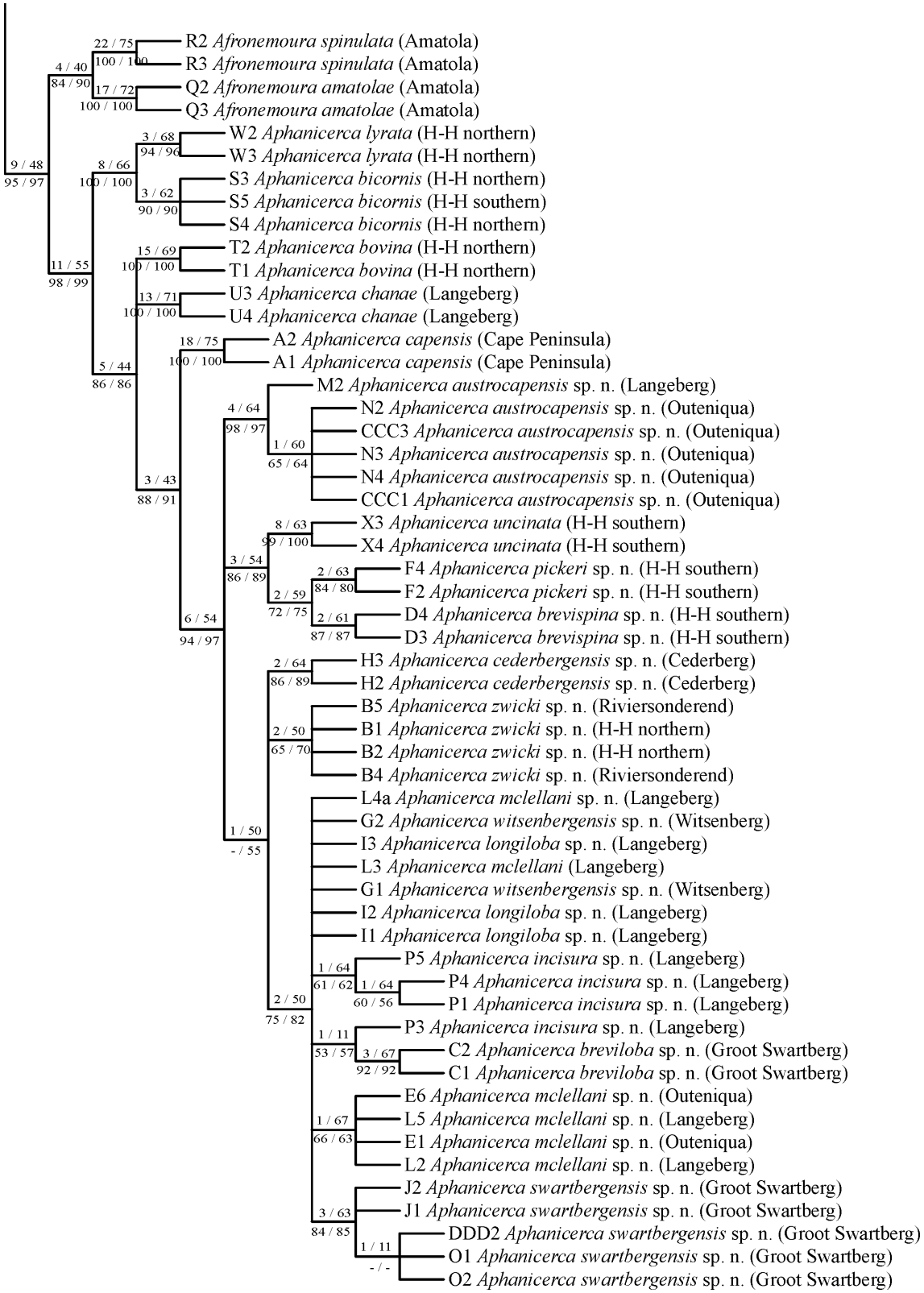


Fig. 4.11. Continued.

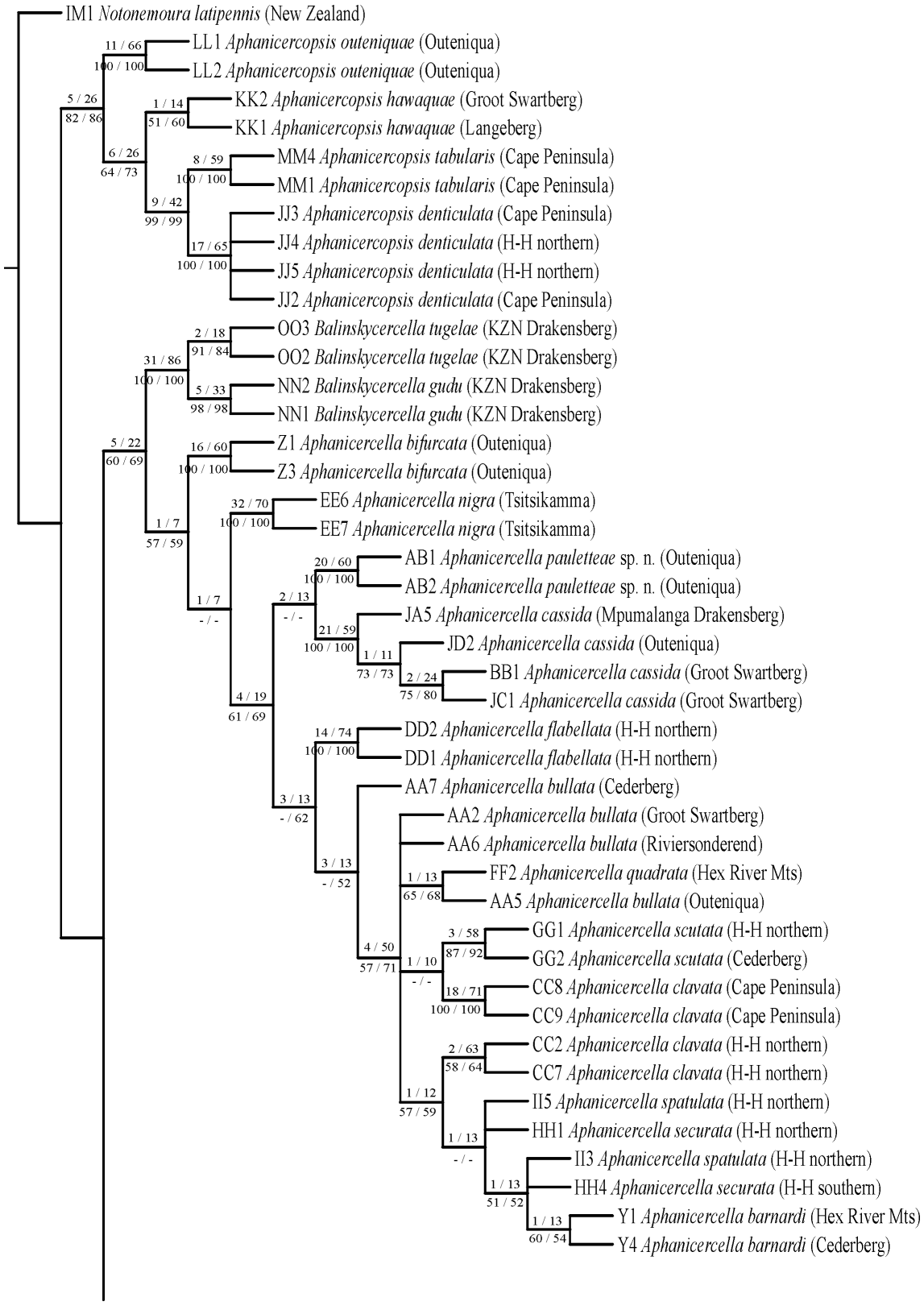


Fig. 4.12. Strict consensus tree (L = 1221, CI = 36, RI = 87) of 24 most parsimonious cladograms (L = 1215, CI = 36, RI = 87) using 48 morphological and 557 mtDNA COI characters under *a priori* weighting. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A support value represented by a dash indicates that the node was not recovered. Taxon names are preceded by the sample field code. The mountain range indicates only the sample locality and not the entire range of the species. H-H = Hottentots Holland Mountains; KZN = KwaZulu-Natal. Fig. 4.12 continued overleaf.

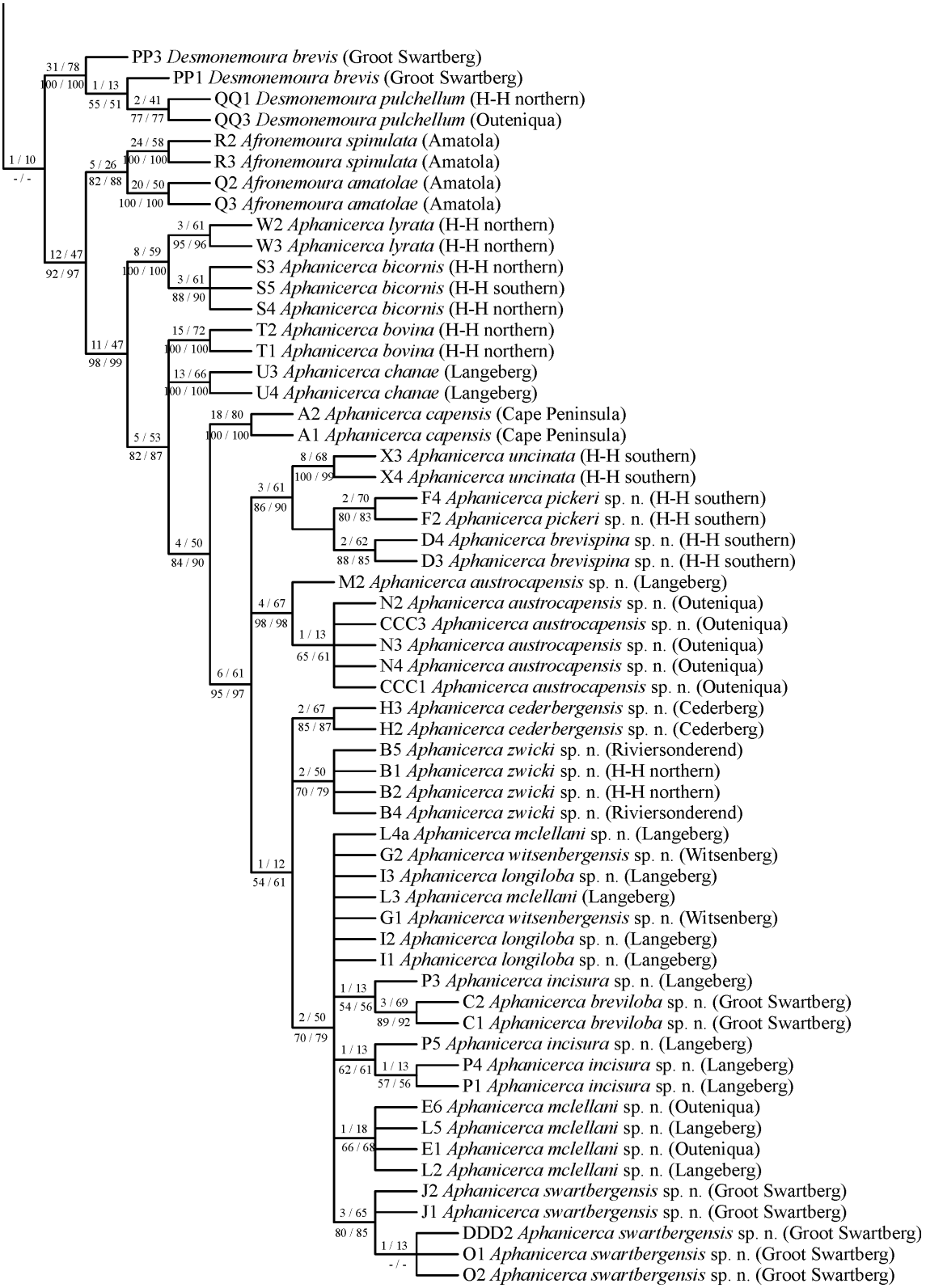


Fig. 4.12. Continued.

used five exemplars to represent the African notonemourid group versus the 102 exemplars of the present molecular and combined analyses. It has been convincingly shown that phylogenetic accuracy increases as taxon sample size increases (Graybeal, 1998; Pollock *et al*. 2002; Zwickl & Hillis 2002). Their study had a different focus and was designed to accurately reflect interfamilial and not intergeneric relationships.

E. *Interspecies relationships based on morphology*

Aphanicercopsis outeniquae was consistently the sister group to the other three *Aphanicercopsis* species due to the latter's synapomorphy of paired spermathecae (character 42: state 1) (Figs 4.3-4.5, 4.7-4.8). Consensus MP trees produced just one subclade within *Aphanicercella*, namely the tritomy (*A. securata* Stevens & Picker, *A. spatulata* Stevens & Picker, *A. flabellata* Stevens & Picker), due to the synapomorphic character 28 (state 4), the shape of the medial supporting sclerite (arch process) of the male paraproct. The same tritomy was also recovered by the BI analysis (Appendix 4.11). The majority rule (Appendix 4.10) and BI trees also recognized an (*A. bifurcata*, *A. nigra*) clade, and *A. cassida* nested within the *A. barnardi* species complex clade, although the latter clade was not well supported. All parsimony cladograms had *Aphanicerca lyrata* as sister taxon to the *A. capensis* species complex, while in the BI phylogram *A. lyrata* was joined by the rest of the *Aphanicerca* species. The species relationships among the latter group differed between the parsimony and Bayesian approaches. The parsimony criterion recognized that *A. uncinata* falls outside of the *A. bicornis*, *A. bovina*, *A. chanae* Picker & Stevens, *A. gnua* group based on the position of male epiproct denticulation (character 12).

F. *Interspecies relationships based on mtDNA*

In the consensus parsimony cladogram (Appendix 4.12), the two *Balinskycercella* species were reciprocally monophyletic. *Desmonemoura pulchellum* was monophyletic and *D. brevis* paraphyletic. *Aphanicercopsis outeniquae*, *A. tabularis* and *A. denticulata* were all monophyletic and *A. hawaquae* paraphyletic. *A. outeniquae* was sister taxon to the rest of the genus. The following *Aphanicercella* species were monophyletic: *A. bifurcata*, *A. nigra*, *A. pauletteae* sp. n., *A. cassida*, *A. flabellata*, and *A. scutata*. *A. clavata* on the Cape Peninsula was morphologically very similar to *A. clavata* in the northern Hottentots Holland Mountains, and so they remain synonymous, although further investigation may lead to division of the two as separate species. This species was polyphyletic and the two populations reciprocally monophyletic. *A. quadrata* and *A. scutata* were nested within the *A. barnardi* species complex, of which they are not members, i.e. morphologically distinct. An odd result was the sister group relationship between *A. bullata* Stevens & Picker from the Outeniqua Mountains and *A. quadrata* from the Hex River Mountains, two species which are very different from each other

morphologically, and are also geographically disjunct. Neither incomplete lineage sorting nor hybridization is likely to be the cause. Three members of the *A. barnardi* species complex, *A. spatulata*, *A. securata* and *A. barnardi* were not monophyletic. The two *Afronemoura* species were reciprocally monophyletic within the paraphyletic genus. *Aphanicerca* was monophyletic, as were the following species: *A. lyrata*, *A. bicornis*, *A. chanae*, *A. capensis*, *A. bovina*, *A. uncinata*, *A. pickeri* sp. n., *A. brevispina* sp. n., *A. austrocapensis* sp. n., *A. cederbergensis* sp. n., *A. zwicki* sp. n., *A. breviloba* sp. n., and *A. swartbergensis* sp.n. The following *Aphanicerca* species were paraphyletic or polyphyletic: *A. mclellani* sp. n., *A. witsenbergensis* sp. n., *A. longiloba* sp. n., and *A. incisura* sp. n. The *A. capensis* species complex was paraphyletic, and was investigated in Chapter 3 of this thesis. It is clear from the relationships within *Aphanicercella* and *Aphanicerca* that this fragment of the COI gene was inadequate on its own to adequately distinguish between species. At genus level, parsimony analysis of COI proved suitable, but the model approaches fared less successfully.

In the ML (Appendices 4.13-4.14) and BI (Appendix 4.15), *Aphanicercopsis hawaquae* was sister to the three other species, and not *A. outeniquae* as in the MP analysis. Species relationships within *Aphanicercella* differed between MP, ML and BI, although the monophyletic species in MP remained so in ML and BI.

G. *Interspecies relationships based on combined morphology and mtDNA*

Species relationships within *Desmonemoura*, *Balinskycercella* and *Afronemoura* in the strict consensus EW combined MP cladogram (Fig. 4.11) were congruent with the combined *a priori* MP (Fig. 4.12) and combined BI cladograms (Appendix 4.16). *Aphanicercopsis* species relationships were congruent in all three cladograms. The first two cladograms were also congruent in species relationships within *Aphanicercella*, except that *A. scutata* became sister group to the Cape Peninsula form of *A. clavata* in the *a priori* weighted analysis. *Aphanicercella* species relationships in the BI cladogram conflicted with the parsimony results. Within *Aphanicerca*, the equal weights and *a priori* weights cladograms were congruent. The three combined analyses were congruent in the sister group relationship between *A. lyrata* and *A. bicornis*, and (*A. uncinata* (*A. pickeri*, *A. brevispina*)), and for much of the remainder of the *A. capensis* species complex.

H. *Clade relationships*

Incongruent cladograms were obtained using the various phylogenetic methods. Use of multiple analytical techniques has been criticized (e.g. Grant & Kluge 2003). However, it is clear from the present analyses, that broader exploration of the data was possible by using different techniques. In the present study, a prior decision to employ solely a model based

approach would have resulted in unchallenged acceptance of numerous anomalous relationships including the nonmonophyly of *Aphanicercella* (BI and ML) which was refuted by the parsimony approach and by morphology. Separate analyses, not favoured by some (e.g. Nixon & Carpenter 1996), in combination with the various approaches, showed that the main source of the discrepancies was the mtDNA data set and not morphology. Further studies using numerous genes would no doubt improve the performance of the molecular component.

The model based analyses (BI and ML) of both the mtDNA partition and combined analyses did not perform as well as parsimony as evidenced by the recovery of generic nonmonophyly in the former. For this reason, a molecular clock was not applied to the data, and should be done when a more comprehensive data set is available. Parsimony analyses of the mtDNA and combined data also produced increased homoplasy of some morphological characters when compared to the morphology data alone (Characters 7, 12, 13, 24, 25, 28, and 40). Under the parsimony criterion therefore, the morphology partition cladogram was preferred over the combined analysis, although this was not a serious consideration as the generic relationships were congruent between the two analyses.

The generic relationships under parsimony can be divided into those that were stable and those that were unstable. Stable clades were common to all trees of all parsimony methods used. These were: (*Aphanicercella*, *Balinskycercella*), and (*Afronemoura*, *Aphanicerca*). Note also that these two stable clades were also recovered in the BI morphology analysis. The unstable clades were those that were present in some strict or majority rule consensus cladograms but not in others. One of the unstable clades was a common feature of the majority rule equal weighting and the strict consensus cladograms under *a priori*, implied weighting, self weighting and successive weighting parsimony analyses of the morphological data, and of the *AP* combined analysis, where *Aphanicercopsis* was the sister group to the other five genera. Within this first unstable clade scenario, there were a further two unstable clades, namely ((*Desmonemoura*) *Afronemoura*, *Aphanicerca*)) which occurred in the *AP* morphological and *AP* combined analyses (and BI morphology and combined data), and (*Desmonemoura*, (*Aphanicercella*, *Balinskycercella*), (*Afronemoura*, *Aphanicerca*)) which occurred in the IW, SW, SAW and majority rule EW MP analyses, where the unstable tritomy was sister group to *Aphanicercopsis*. To summarize generic relationships under the parsimony criterion (and partially under BI) further, the most conservative consensus was a polytomy of four clades, namely *Aphanicercopsis*, *Desmonemoura*, (*Aphanicercella*, *Balinskycercella*), and (*Afronemoura*, *Aphanicerca*); when better resolved consensus cladograms recovered *Aphanicercopsis* as the sister group to the remaining genera, then *Desmonemoura* formed part of the remaining tritomy, or became sister to (*Afronemoura*, *Aphanicerca*). It is very likely that the stable clades will

remain when other outgroups are included in the analyses, and also that some unstable clades will fall away and others will be recovered. While the morphology EW MP cladogram would be regarded as the most compromising estimate of phylogenetic relationships until further outgroups and data sets (more genes and more morphological characters) become available, further resolution using the unstable clades can be validly applied to biogeographic hypotheses as they are well supported. Where a single tree is not obtained, the consensus cladogram is not the most parsimonious hypothesis of relationships and is therefore not as valuable as a more fully resolved cladogram. The *AP* morphology cladogram was favoured in this regard because 1) at generic level it was fully resolved even though it was a strict consensus tree; 2) it was also congruent with the BI morphology tree in the generic relationship (*Desmonemoura* (*Afronemoura*, *Aphanicerca*)), and with the IW, SW, SAW and majority rule EW cladograms in recovering *Aphanicercopsis* as the sister group to the other genera; and 3) because of the perceived importance of the characters that were weighted in the *AP* analysis. In addition, the ensemble consistency and retention indices were higher in the *AP* than the EW strict consensus cladograms. No characters showed a decrease in Ci and / or Ri in the *AP* cladogram when compared to EW. The combined and morphological *AP* cladograms had congruent generic relationships. Using combined morphology and genetic data is the ideal way to approximate true evolutionary relationships, as congruent topologies between the complex evolution of morphological traits and the more simplistic evolution of a neutral marker are not guaranteed.

I. *Biogeography*

This section describes patterns of species richness and endemism and the relationship between some species pairs and their genetic divergence; in addition a historical biogeographical scenario is proposed for the southern African notonemourid fauna.

Notonemourid distribution follows the mountains and high altitude land in a narrow band tracking the coastline from the dry Namaqualand in the north-west, down to the south and up to the Great Escarpment of the north-east (Fig. 4.16A-F). This distribution covers winter, summer and all year rainfall patterns. It is interesting to note from the species distribution maps (Appendix 4.7) that localities are often at the perimeter of the Folded Mountains and the South Western, Southern and South Eastern Coastal Belts. This is because the roads where collecting was most easily done are situated at the foothills of the mountains. The streams obviously originate high up in the mountains themselves, and as long as the stream order is sufficiently high due to land gradient, stoneflies may also be found in ecoregions adjacent to that of the stream origin. Where the mountains abut the coastline, such as in the Tsitsikamma (South Eastern Coastal Belt) and Betty's Bay (South Western Coastal Belt) areas, stoneflies are found just tens of metres from the ocean (pers. obs.).

Distribution of the six genera (Figs 4.13-4.16; Table 4.3; Appendix 4.7)

Afronemoura occurs from the Northern Escarpment Mountains through to the eastern limits of the Southern Folded Mountains (SFM) (Fig. 4.16A). *A. amatolae* and *A. spinulata* both occur in the SFM near Grahamstown and in the Amatola Mountains of the South Eastern Uplands. *A. amatolae* and *A. stuckenbergi* are both found in the Northern Escarpment Mountains (Mpumalanga Drakensberg), the latter more northerly. *A. spinulata* is additionally found in the Eastern Escarpment Mountains (KwaZulu-Natal Drakensberg foothills), South Eastern Uplands (KwaZulu-Natal Midlands and Southern KwaZulu-Natal), and North Eastern Uplands (northern KwaZulu-Natal Midlands).

Balinskycercella is endemic to the KwaZulu-Natal and Lesotho Drakensberg and the Maluti Mountains of Lesotho (all part of the Eastern Escarpment Mountains) (Fig. 4.16E).

Aphanicerca, *Aphanicercella* (with the two exceptions *A. namaquaensis* sp. n. and *A. cassida*), *Aphanicercopsis*, and *Desmonemoura* are restricted to the CFM (and adjoining ecoregions) (Fig. 4.16). The widespread species *Aphanicercella cassida* does not fit this arrangement, and is found throughout the full extent of the EH and into the central parts of the CFM (Appendix 4.7Z). The other *Aphanicercella* species with an unusual distribution, *A. namaquaensis* sp. n. is found north of the Western Folded Mountains (WFM) on Rooiberg in the Kamiesberg Uplands in the Namaqua Highlands (*sensu* Kleynhans *et al*. 2005) (Appendix 4.7X). Several other palaeoendemic insect groups occur in the Kamiesberg Uplands, for example, the blepharicerid midge *Elporia anisonyx* (Barnard 1947; Stuckenberg 1962) and *Anisonyx* spp monkey beetles (Scarabaeidae: Hopliini) (Colville 2006). This was the most northerly locality on the west coast, and remarkable given the effective isolation of this temperate upland, a few hundred kilometres from the nearest suitable temperate montane habitat. The Kamiesberg Upland is the northernmost refugial montane habitat for relictual invertebrate fauna of Africa (Colville 2006).

Although the two *Desmonemoura* species (Appendix 4.7QQ-RR) have not yet been found syntopically, they are sympatric in the Outeniqua Mountains. This locality is an extremity of the distribution ranges of both species, with that of *D. brevis* centred in the Groot Swartberg Mountains, and of *D. pulchellum* in the western SFM and the WFM. The Outeniqua area may be a more recent range expansion following vicariant allopatric speciation.

Allopatric species pairs and genetic divergence

Harrison & Barnard (1971) pointed out that the Cape Peninsula (Fig. 4.17I) is species-poor across many invertebrate stream faunas compared to the inland mountains. Certainly the genera

on the Peninsula are species-poor, with five notonemourid species representing three genera (see Appendix 4.7A-RR for all species distribution maps). Of these, *Aphanicerca capensis* (*sensu strictu*) and *Aphanicercopsis tabularis* are the two species endemic to the Cape Peninsula. The occurrence of the other three of these Peninsula species, namely *Aphanicercella clavata*, *Aphanicercella flabellata* and *Aphanicercopsis denticulata*, on both the Cape Peninsula and the inland Hottentots Holland Mountains of the south-western Western Cape supports the hypothesis of an early mountainous sandstone connection between the two ranges. This conjecture is based on the very narrow eco-physiological requirements of stoneflies (high oxygen saturation of the water, fast flowing, pristine, cold water, uplands and foothill mountain streams). This hypothesized mountain bridge is thought to have eroded away between the late Cretaceous Period and the end of the "Tertiary" (Walker 1952) (i.e. the end of the Pliocene Epoch (2.6 million years ago (mya)). The inference is that the fauna was spread across the entire mountain chain until this vicariant event, with subsequent genetic divergence and allopatric speciation (Wishart & Day 2002), and also extinctions. Presently the two mountain ranges are separated by very sandy, arid lowlands (the Cape Flats) (the terrain between Fig. 4.17I and D), forming an effective migration barrier for most relictual invertebrates, including stoneflies.

Because the Cape Flats, which separates the Peninsula from the inland mountains, is inhospitable to stream fauna (Harrison & Barnard 1971), and therefore assuming no recurrent gene flow across this divide, the degree of genetic divergence between each conspecific pair within and out of the Peninsula, should be similar. *Aphanicerca capensis* (*sensu strictu*), from the Peninsula, has diverged greatly in the COI mitochondrial gene from its closest genetic relatives in the *A. capensis* species complex, namely *A. zwicki* (Hottentots Holland Mountains) and *A. cederbergensis* (Cederberg Mountains) (6.3% - uncorrected p-distance; 7.0% and average 7.1% respectively – GTR model with alpha 1.64 corrected distance). *Aphanicercella flabellata* from the Peninsula was not available for mtDNA data, as it has not been found since 1979, so it could not be compared to other populations to the east. Positive assortative mating confirmed the biological species status of *A. flabellata* and *A. clavata* in the *A. barnardi* species complex (Chapter 2; Stevens & Picker 1999). These two populations, in Jonkershoek in the northern Hottentots Holland Mountains and the Cape Peninsula respectively, show genetic divergence of 7.5% uncorrected and 8.4% corrected distances (Appendices 4.5, 4.6). The other mate choice experiment in the same study was between the Peninsula population of *A. clavata* and the Cederberg population of *A. bullata*. These populations were separated by distances of 8.1% (uncorrected) and 9.2% (corrected). These *Aphanicercella* divergence figures between the Peninsula and the Cederberg Mountains, and between the Peninsula and the northern Hottentots Holland Mountains are similar to the aforementioned divergences found for *Aphanicerca*. *Aphanicercella clavata* occurs on the Peninsula and in Bain's Kloof in the northern Hottentots

Holland Mountains (Appendix 4.7AA). In terms of genetic distance, the two populations of *A. clavata* have diverged (average 5.3% p-distance; 5.7% corrected) to a degree similar to *A. capensis* and *A. zwicki*, these two species occurring sympatrically with the two *A. clavata* populations respectively. Whether or not *A. clavata* comprises two biological species has not yet been addressed. A very similar sequence divergence of about 5% also using COI mtDNA was found between Cape Peninsula and northern Hottentots Holland Mountains populations of the midge *Elporia barnardi* (Wishart & Hughes 2002). However, this degree of divergence was not found in the case of *Aphanicercopsis denticulata*, which up to now has only been found at a single locality in the southern Peninsula, namely Kirstenbosch, but was also found to be widespread across the south-western Cape, occurring in the Langeberg, Swartberg, Groot Swartberg, and northern Hottentots Holland Mountains (Appendix 4.7JJ). Genetic divergence between the Peninsula and Bain's Kloof (northern Hottentots Holland) populations was much lower than expected at an average of 0.45% p-distance and 0.46% corrected distance. Given the low probability of successful migration across the Cape Flats, this is suggestive of a very stable mitochondrial genome with very few mutations over the time period since the separation of the mountain groups. Samples from across the entire range of this species and using additional markers will be required to address this question. In a recent study of cryptic speciation in South African Onychophora, Daniels *et al*. (in press) found that a sequence divergence of about 6% in partial COI sequences was appropriate for species delimitation.

Widespread species

Aphanicercella nigra (Appendix 4.7DD) is found in the Cederberg and the northern Hottentots Holland Mountains of the WFM, and in the South Eastern Coastal Belt. However, there are a few minor but clear morphological differences between the two populations, and subject to analysis of further collections, may be found to comprise two distinct species. *Aphanicercella cassida* has a very wide distribution from the Mpumalanga Drakensberg (Northern Escarpment Mountains) down through the EH (*sensu* Stuckenberg 1962) to the central region of the SFM. Although the Mpumalanga Drakensberg population is geographically far more distant from the Outeniqua and Groot Swartberg (both SFM) populations than the two *A. clavata* populations on and off the Peninsula, the average genetic divergence between the northern and southern *A. cassida* populations was lower (3.1% p-distance; 3.3% GTR corrected). To date, morphological differences have not been noted between its various populations. However, Balinsky (1956) remarked that some variation in the epiproct shape of *A. cassida* was noticeable. Clearly this is an important area of follow-up research to determine whether it comprises one or two species. Although both *A. clavata* and *A. cassida* have remained morphologically stable (in both male and female anatomy) across disjunct populations at divergences of up to about 6% and 3% respectively, species of the *Aphanicercella barnardi*

species complex (of which *A. clavata* was one) showed positive assortative mating and morphological differences (Stevens & Picker 1999; Chapter 2 of this thesis) at much lower genetic divergences. Three of the species within the complex, namely *A. bullata*, *A. securata* and *A. spatulata*, were not monophyletic in the molecular and combined data sets (Figs 4.11-4.12; Appendix 4.12). These patterns can be attributed to recent speciation with incomplete lineage sorting or mitochondrial introgression (hybridization). Because these samples were drawn from disjunct populations, the former scenario is more likely, considering low vagility. Introgression may be more likely in sympatric members of the *A. capensis* complex (Chapter 3) where morphologically divergent species showed low mitochondrial genetic divergence, even sharing haplotypes in some instances. It is plausible that in all these cases, there is periodic gene flow between closely related species that are in close proximity to each other, but at low levels since the species retained their morphological identity. Using one widely-used estimate of insect mtDNA divergence rates of 2.3% per million years (Brower 1994), it is possible to obtain estimates of divergence times of about 2.5 million years for *A. capensis* and *A. zwicki* (and others of the species complex) as well as the two *A. clavata* populations, and 190k years for the Peninsula and off-Peninsula *A. denticulata* populations. The divergence time of the former two pairs agrees closely with the estimate of 2.6 mya for the break up of the bridge between the Peninsula and the inland Hottentots Holland Mountains (Walker 1952). The genetic divergence between the New Zealand outgroup, *Notonemoura latipennis*, and the southern African taxa varied between 18.1% (smallest uncorrected p-distance) and 32.2% (largest corrected distance), which at 7.9 and 14 mya respectively, does not correlate with the age of fragmentation of Gondwanaland of 140 mya. These ages of separation based on mtDNA divergence are founded on the assumption of zero divergence between the two localities at the time of existence of the land bridge. Divergence times estimated here assume a linear molecular clock relationship between genetic distance and time, which in reality is usually not the case (Rambaut & Bromham 1998). There was also a single calibration point, which is likely to compound the margin of error (Graur & Martin 2004). For these reasons, these times of divergence are offered merely as very rough estimates until an augmented molecular data set can be provided for further analysis.

Biogeography

The ordination of mountain ranges by species divided the region into two major zones, the Cape Folded Mountains (CFM), which comprise the Western folded Mountains (WFM) and Southern Folded Mountains (SFM), and the Eastern Highlands (EH), and one minor zone, the Namaqua Highlands (Fig. 4.13). Because stoneflies may occur in suitable habitat in the ecoregions seaward of the SFM and WFM, I use those two terms to be inclusive of their respective adjacent ecoregions. SFM therefore includes the Southern Coastal Belt and the South

Eastern Coastal belt ecoregions, and the WFM includes the South Western Coastal Belt ecoregion (Fig. 4.16). This pattern follows the divisions of Stuckenberg (1962) who first recognized two main distributional centres of the African relictual palaeogenic fauna, namely a Cape Centre and the Eastern Highlands. His EH comprised three subgroups, the Lesotho Highlands, the Amatola Range, and the Northern Escarpment Mountains. For the Notonemouridae, the three EH subgroups are better represented as just two subgroups, firstly the Eastern Escarpment Mountains (KwaZulu-Natal and Lesotho Drakensberg, and Maluti Mountains of Lesotho), and secondly a group comprising the South Eastern Uplands (localities in the Amatola Mountains, and southern KwaZulu-Natal), the Northern Escarpment Mountains (Mpumalanga Drakensberg), the North Eastern Uplands (northern KwaZulu-Natal Midlands, KwaZulu-Natal Midlands), and the eastern extremity of the SFM (Fig. 4.16A-F; Table 4.3). In the notonemourid biogeographical context, the CFM and EH overlap in the eastern SFM. This is because three of the seven EH species, namely *Afronemoura amatolae*, *Afronemoura spinulata* and *Aphanicercella cassida*, occur in the eastern extremities of the SFM near Grahamstown (called the Southern Eastern Cape Province Highlands here) (Appendix 4.7A,B,Z; Table 4.3), while *A. cassida* also extends further westwards into the central SFM.

Aphanicercella cassida is the most widespread notonemourid in Southern Africa, being common to both EH subgroups and the SFM. The MDS ordination plot excluding *A. cassida* (Fig. 4.13) clearly showed the two main distributional assemblages of the CFM and the EH. When *A. cassida* was included (Fig. 4.14), this clear distinction was lost. The cluster dendrogram excluding *A. cassida* also clearly shows the CFM and EH groups, with the addition of the Kamiesberg (Namaqualand) species *Aphanicercella namaquaensis* sp. n. as the third biogeographical group (Fig. 4.15). As mentioned, the Kamiesberg is an important centre of other palaeoendemic groups (Colville 2006). The cluster analysis dendrogram (Fig. 4.15) shows the general trend of geographically close mountains ranges having a more similar species composition to one another than they have to more distant mountains, although there are exceptions.

The intersection zone of the SFM and WFM is particularly rich in palaeogenic biota (Stuckenberg 1962), with the complex topography thought to play an important causative role in speciation through the provision of refugia and physical barriers to dispersal. This zone does not have a defined area, but it may be estimated to comprise the following more significant mountain range groups: the northern and southern Hottentots Holland and the Hex River

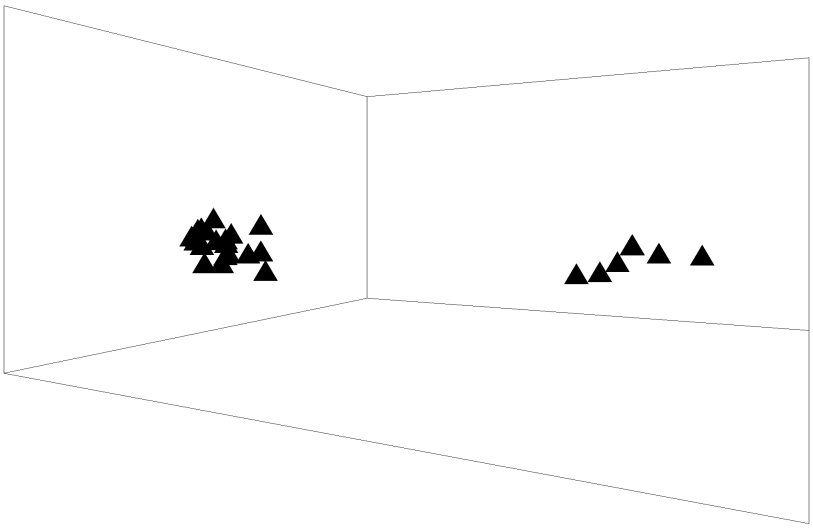


Fig. 4.13. Non-metric multidimensional scaling 3-D ordination of mountain range species composition using presence / absence data of all local notonemourid species; *A. namaquaensis* sp. n. which is a unique species in a unique locality and the widespread *A. cassida* are excluded. The group on the right of the plot comprises the mountains of the Eastern Highlands (*sensu* Stuckenberg 1962), and the group on the left the Cape Folded Mountains.

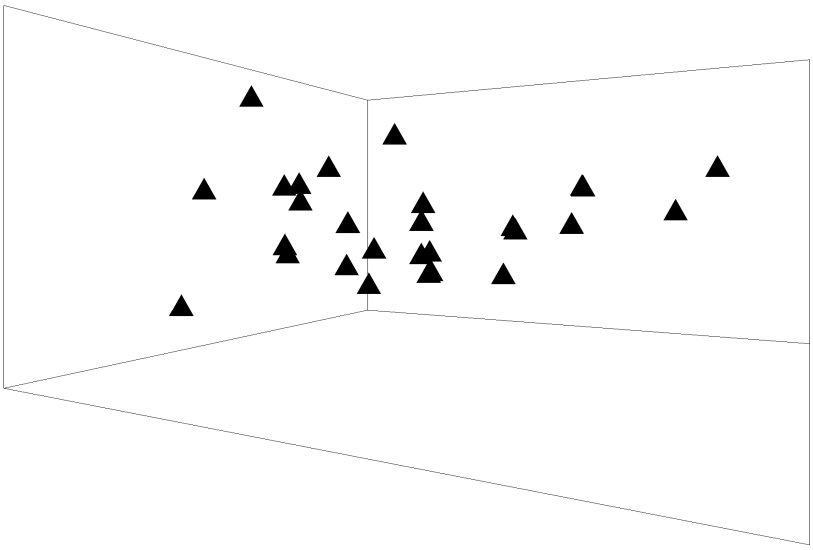


Fig. 4.14. Non-metric multidimensional scaling 3-D ordination of mountain range species composition using presence / absence data of all local notonemourid species. The plot includes the widespread species *A. cassida*. *A. namaquaensis* sp. n. which is a unique species in a unique locality is excluded.

Mountains. Stonefly species richness (and other faunal groups, e.g. hydraenid beetles (Perkins & Balfour-Browne 1994)) supports Stuckenberg's unquantified observations; this intersection zone contains 20 (not all endemic to the area) of the 44 known (13 undescribed) notonemourid species (45.5%) and is certainly the centre of African stonefly diversity. The locality with the highest number of species was the Harold Porter Nature Reserve in Betty's Bay (southern Hottentots Holland Mountains) with nine species in three genera (Appendix 4.1). In general, streams typically supported multiple genera, but multiple congeneric species at one locality was less common (pers. obs.). The most genus-rich mountain ranges (each supporting four genera) were the Groot Swartberg, northern Hottentots Holland, Outeniqua, Langeberg and Cederberg ranges, all within the CFM (Figs 4.15-4.16). The most species-rich mountain group was the northern Hottentots Holland with 14 species (Table 4.3); then the Langeberg with 12 species, the southern Hottentots Holland with 11 species and the Outeniqua with 10 species (Table 4.3).

Endemism

Levels of invertebrate endemism are exceptionally high in the high lying areas of southern Africa e.g. Table Mountain and the Peninsula have more than 111 known endemics (Picker & Samways 1996). The Notonemouridae are no exception, as numerous species are currently only known from single streams or very restricted geographical areas. These include: *Afronemoura stuckenbergi* (single locality), *Aphanicerca breviloba* sp. n. (single stream), *Aphanicerca brevispina* sp. n. (localized, adjacent streams), *Aphanicerca capensis* (Cape Peninsula), *Aphanicerca gnua* (single stream), *Aphanicerca incisura* sp. n. (localized, adjacent streams), *Aphanicerca pickeri* sp. n. (single locality), *Aphanicerca tereta* (single locality), *Aphanicerca uncinata*, *Aphanicerca witsenbergensis* (single stream), *Aphanicercella namaquaensis* sp. n. (single locality), *Aphanicercella pauletteae* sp. n. (single stream), *Aphanicercopsis tabularis* (Cape Peninsula), and *Balinskycercella fontium* (single stream) (Appendix 4.7). The low percentage similarity between community composition of mountains in the cluster analyses indicates that local endemism, at mountain range scale, is widespread (Figs 4.15-4.16), with almost 41% of the species being endemic to a single mountain range group (Table 4.3; Appendix 4.7).

Five of the 31 Level I river ecoregions (Kleynhans *et al*. 2005) were represented by ecoregion endemic notonemourids. These were: the SFM (14 endemics representing 42.4% of the ecoregion's species and 31.8% of the total number of African Notonemouridae species), the WFM (6 endemics, 28.6%, 13.6%), the Eastern Escarpment Mountains (3 endemics, 60%, 6.8%), and one endemic each in the Namaqua Highlands (100%, 2.3%) and Northern Escarpment Mountains (33.3%, 2.3%) (Table 4.3). Endemism within the CFM, at 80% (i.e. 20 of the total of 25 endemic species across all ecoregions), was a striking result. A second

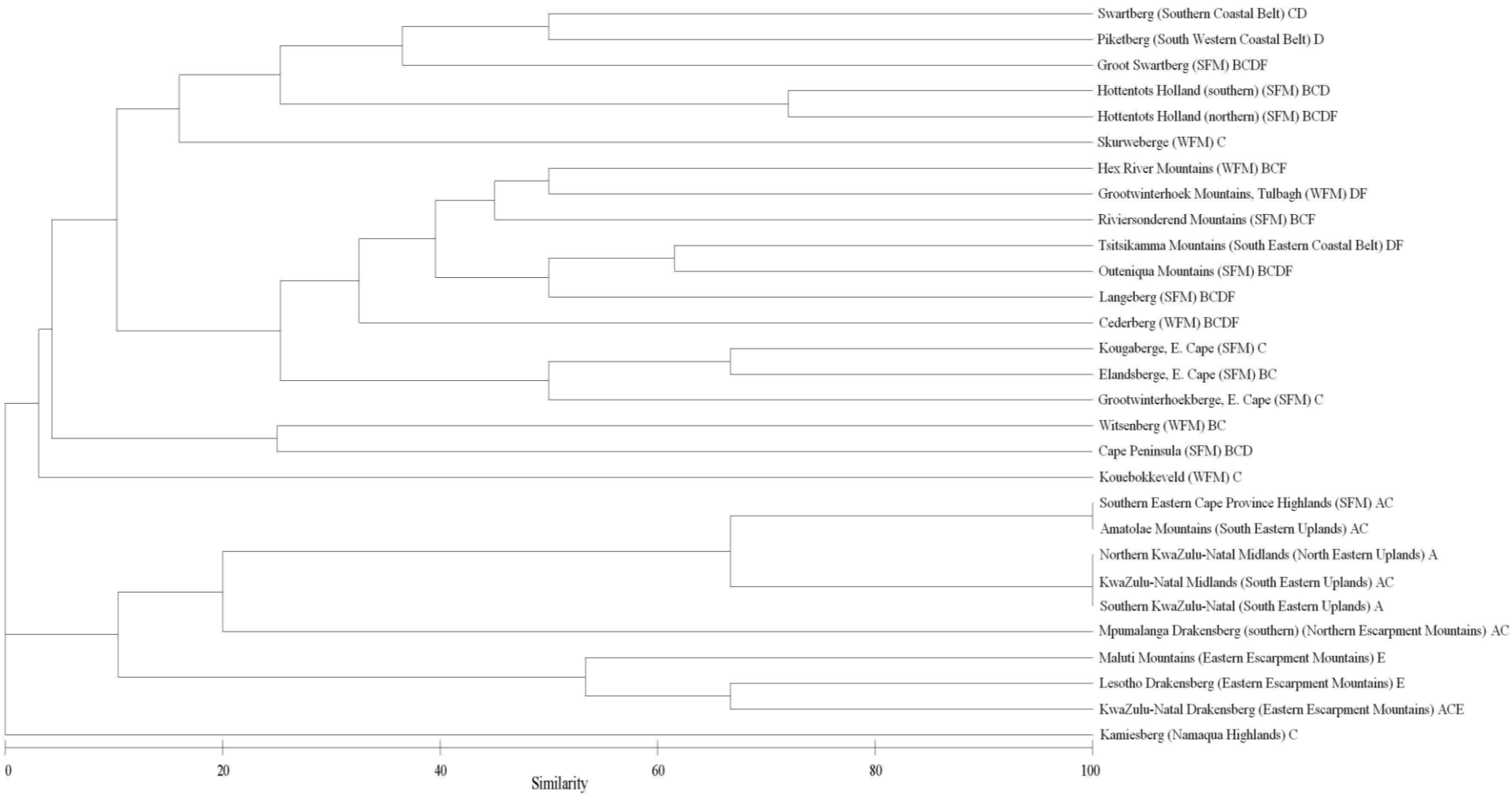


Fig. 4.15. Cluster analysis, using the Bray-Curtis similarity measure and a group average algorithm, of mountain range species composition using presence / absence data of all local notonemourid species excluding *A. cassida* which is widespread. The genera represented at each mountain range are given to the right of the mountain name as follow: A = *Afronemoura*, B = *Aphanicerca*, C = *Aphanicercella*, D = *Aphanicercopsis*, E = *Balinskycercella*, F = *Desmonemoura*. Abbreviations: SFM = Southern Folded Mountains; WFM = Western Folded Mountains.

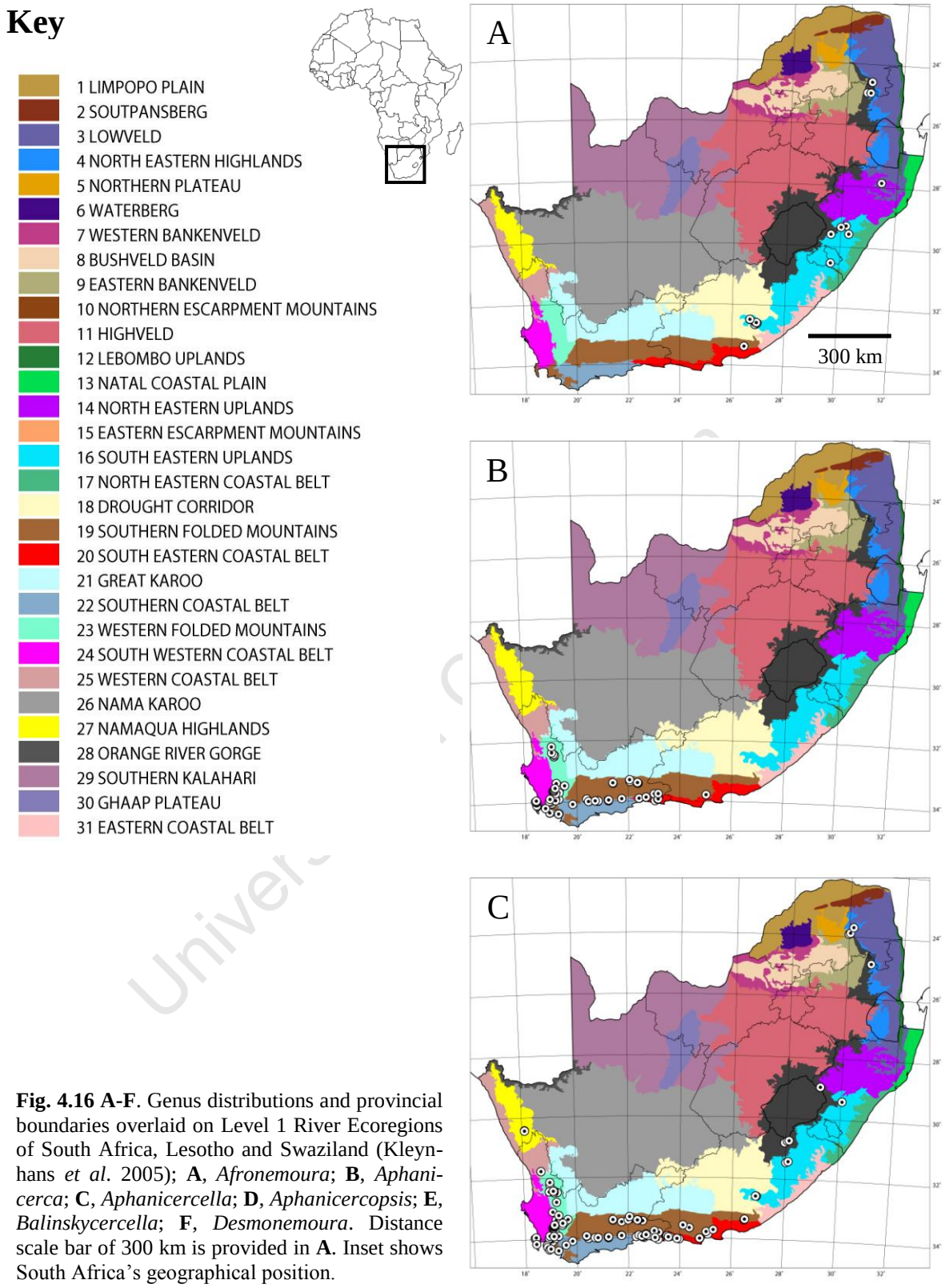


Fig. 4.16 A-F. Genus distributions and provincial boundaries overlaid on Level 1 River Ecoregions of South Africa, Lesotho and Swaziland (Kleynhans *et al*. 2005); **A**, *Afronemoura*; **B**, *Aphanicerca*; **C**, *Aphanicercella*; **D**, *Aphanicercopsis*; **E**, *Balinskycercella*; **F**, *Desmonemoura*. Distance scale bar of 300 km is provided in **A**. Inset shows South Africa's geographical position.

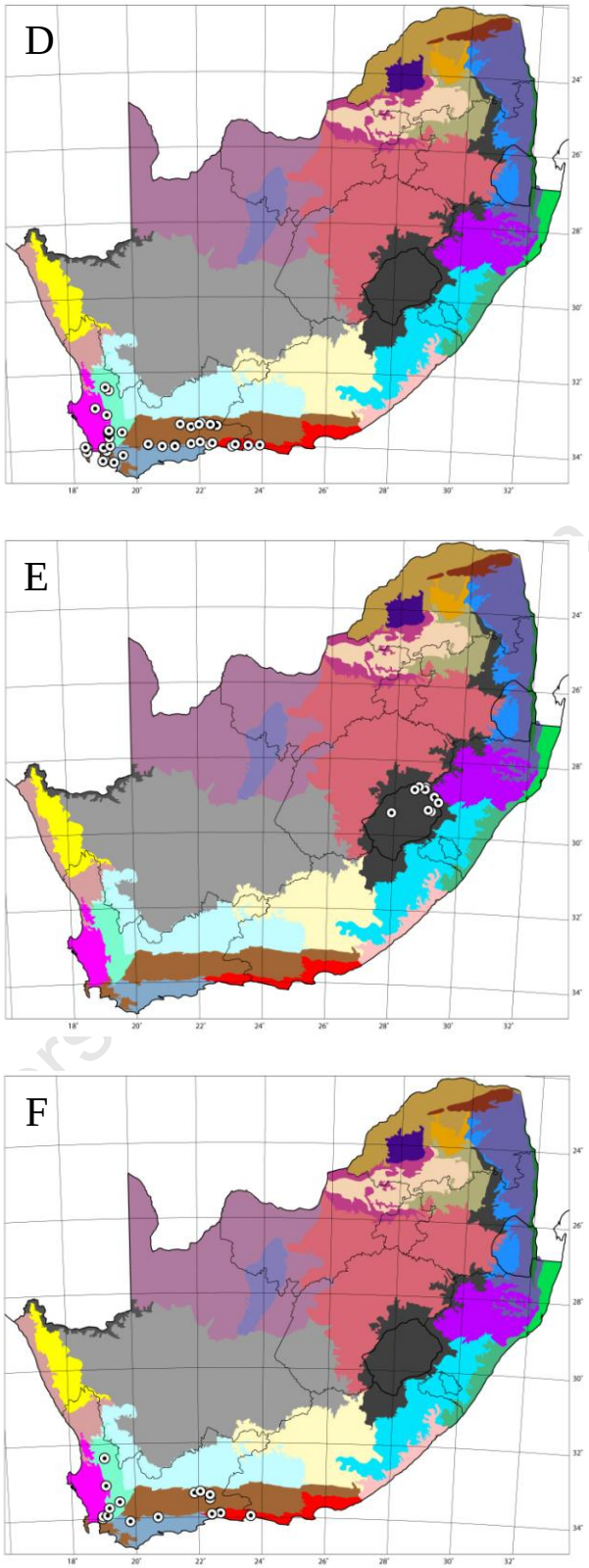


Fig. 4.16. Continued.

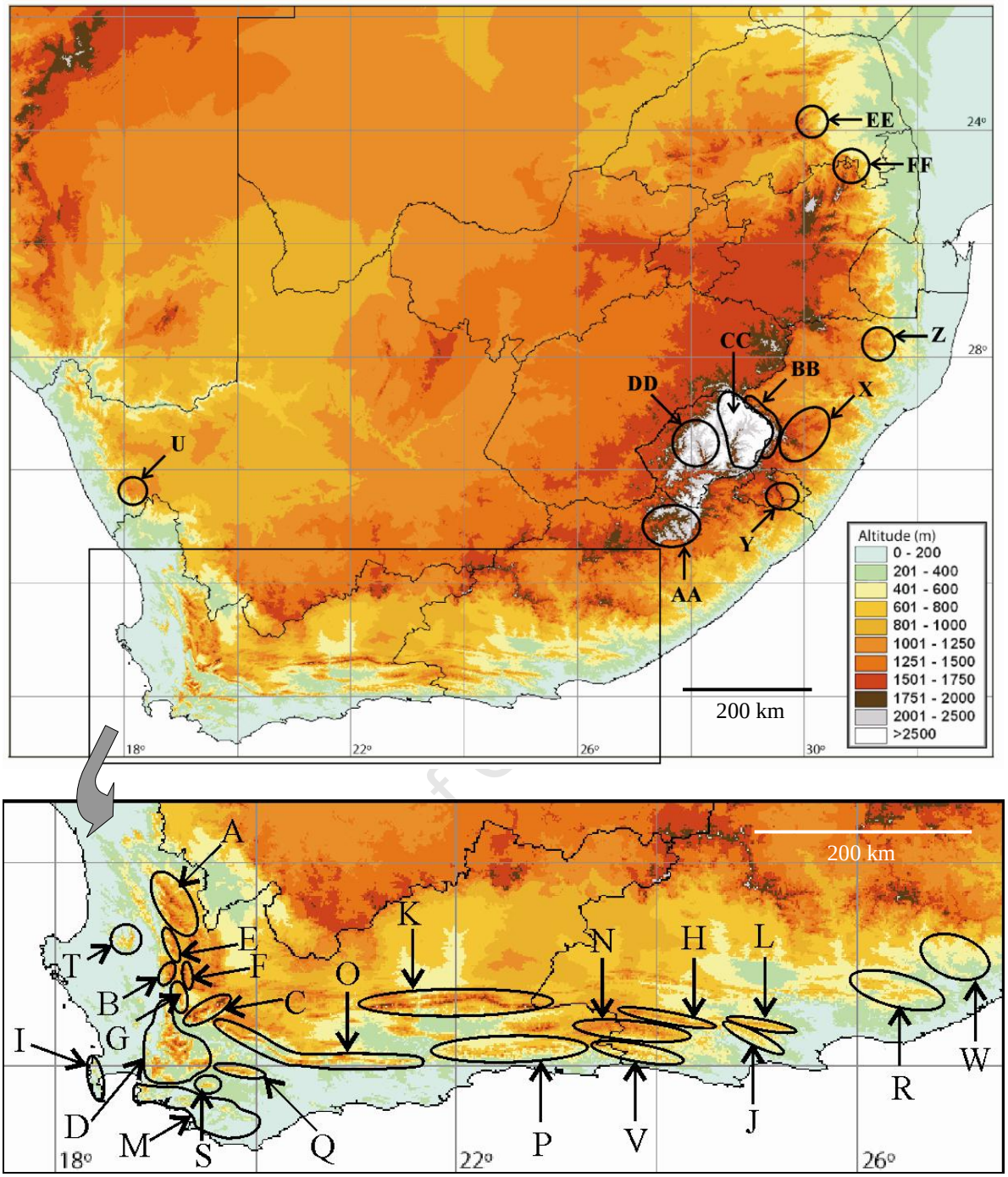


Fig. 4.17 A-FF. Sampled mountain range groups overlaid on a topographical map of South Africa, and neighbouring countries, with provincial and national boundaries shown. **A**, Cederberg; **B**, Grootwinterhoek, Tulbagh; **C**, Hex River; **D**, Hottentots Holland (northern); **E**, Kouebokkeveld; **F**, Skurweberge; **G**, Witsenberg; **H**, Baviaanskloof (E. Cape); **I**, Cape Peninsula; **J**, Elandsberge (E. Cape); **K**, Groot Swartberg; **L**, Groot-Winterhoekberge (E. Cape); **M**, Hottentots Holland (southern); **N**, Kougaberge (E. Cape); **O**, Langeberg; **P**, Outeniqua; **Q**, Riviersonderend; **R**, Southern Eastern Cape Province Highlands; **S**, Swartberg; **T**, Piketberg; **U**, Kamiesberg, Namaqualand; **V**, Tsitsikamma; **W**, Amatolae; **X**, KwaZulu-Natal Midlands; **Y**, Southern KwaZulu-Natal; **Z**, Northern KwaZulu-Natal Midlands; **AA**, Eastern Cape Province Southern Drakensberg; **BB**, KwaZulu-Natal Drakensberg; **CC**, Lesotho Drakensberg; **DD**, Maluti; **EE**, Mpumalanga Drakensberg (northern); **FF**, Mpumalanga Drakensberg (southern).

important endemicity centre was identified by Stuckenberg (1995) for the wormlion clade (*Vermipardus basuto, V. sylphe*) (Diptera: Vermileonidae), namely the northern Lesotho and Mont-aux-Sources area. Interestingly, this region is the only known habitat for the stonefly genus *Balinskycercella* (Fig. 4.16).

Narrow and point endemic localities are of extreme importance in terms of conservation. Creation of farm dams, introduction of livestock or human pollutants, water extraction and toxic run off of fertilizers and pesticides can result in extinction of stonefly species that are restricted to one or two streams, considering their intolerance to perturbations of many water quality variables. Analyses of endemism of other stream fauna are lacking, but it is likely that patterns of endemism observed in the stoneflies will be reflected in other aquatic taxa. Once such data become available, profiles of mountain stream endemicity need to be integrated into conservation policy of the various local conservation authorities.

Historical biogeography

The Notonemouridae are regarded as part of the rich palaeogenic (relictual) fauna of Gondwanan origin (Balinsky 1962) which on the African continent is restricted to southern Africa. It is generally assumed that the common distribution pattern of these taxa in Australia, New Zealand, South America, Madagascar and South Africa suggests an evolutionary origin at the time of the fragmentation of Gondwanaland (Stuckenberg 1962; Day 2005). It has been shown that Australian Gondwanan chironomid larvae retain their ancestral ambient temperature requirements (McKie *et al*. 2004). This finding may apply similarly to other Gondwanan groups such as notonemourid stoneflies and as such they have not been able to invade drier or hotter habitats. Their current distribution therefore reflects refugial pockets that have persisted since the break up of Gondwanaland. Endrödy-Younga (1988) regarded invertebrates of the Cape mountain biome as originating in lower lying regions at a time when the climate was moister and cooler, with subsequent isolation in montane refugia as the southern African climate became warmer and more arid.

It is well known that vagility in Plecoptera is poor and therefore distributions are often most parsimoniously explained by widespread common ancestors on the super continents before drift (Zwick 2000). The existence of a few regionally widespread species e.g. *Aphanicercella cassida, Afronemoura amatolae, Aphanicercopsis outeniquae* and *Desmonemoura pulchellum* in southern Africa suggests that dispersal by slow range expansion is a very real phenomenon, although it may take a while to accomplish across great distances, and presumably only across a hospitable landscape, i.e. short distances between low order streams, unless periodic passive dispersal occurs through natural means such as violent winds or flooding.

There is evidence, from this study, of multiple historical migration patterns. Firstly, *Balinskycercella* has a narrow distribution in the Eastern Escarpment Mountains, and was found to be the sister group to the widespread *Aphanicercella* which has its centre of radiation at the intersection of the WFM and SFM. It may be that the northern Eastern Escarpment Mountains, (Lesotho-Drakensberg Highlands) which were present since the late Jurassic (Stuckenberg 1962), were the centre of origin of *Balinskycercella* which developed from populations of their common ancestor. It is thought that the northern Eastern Escarpment Mountains were also one of the centres of origin and dispersal of *Elporia* species (Stuckenberg 1962) and a *Vermipardus* wormlion clade (Stuckenberg 1995). *Aphanicercella* spread, from its hypothesized origins in the CFM, as far as the Kamiesberg Uplands in the Namaqua Highlands (*A. namaquaensis* sp. n.), and north-easterly as far as the Mpumalanga Drakensberg (*A. cassida*), with most speciation occurring in the CFM. Because *A. cassida* is most closely related to *Aphanicercella pauletteae* sp. n. from the Outeniqua Mountains where the two species are sympatric (Figs 4.11-4.12; Appendix 4.12), their common ancestor may have originated from the Outeniqua area, with subsequent long distance colonization of *A. cassida* of more northern regions.

Secondly, *Afronemoura stuckenbergi* has only been recorded from the southern Mpumalanga Drakensberg, and is presumed therefore to have a very restricted distribution. It is only known from Mariepskop, a fairly isolated forested mountain with a number of endemic taxa including a near insect endemic, the butterfly *Charaxes marieps* (Van Someren & Jackson 1957). *Afronemoura amatolae* was also recorded from that region which is the extreme northern end of its range, but has a much wider distribution than *A. stuckenbergi* with its putative centre in the South Eastern Uplands including the Amatola Mountains which were supposedly completely separated from the KwaZulu-Natal Drakensberg by the mid-Miocene (Stuckenberg 1962) (*ca*. 14 mya). *A. spinulata* also has its main distribution in the South Eastern Uplands, and has extended into the North Eastern Uplands, but not as far north as *A. amatolae*. Taking into account also that the sister group of *Afronemoura* is *Aphanicerca* which is localized to the CFM, it seems likely that *Afronemoura* had its origins in the south, and subsequently spread northwards, and that *A. stuckenbergi* was a more recent migration to the north than *A. cassida* (about 1.3 million years between the northern and southern populations of *A. cassida* at a divergence of 3.1%; no genetic data was available for *A. stuckenbergi*). Equally plausible is the hypothesis that the common ancestor of *Afronemoura* species was widespread in the east following the cladogenic event that also gave rise to *Aphanicerca*, with *A. stuckenbergi* evolving in its current locality. *Afronemoura* and *Aphanicerca* form a stable clade, but their adjacent ranges do not overlap at all (although with more intensive collecting in the contiguous zone it is possible that they may be found to be sympatric in that area). The most likely scenario would be that their common ancestor had a wide distribution from the CFM into the Amatola area with

subsequent allopatric speciation followed by northern migration of *Afronemoura*, and rapid speciation of *Aphanicerca*, a well known phenomenon common to the biota of the Fold belt (Richardson *et al*. 2001).

Thirdly, if indeed *Aphanicercopsis*, which is restricted to the CFM, shared a common ancestor with the rest of the southern African notonemourids (Figs 4.7, 4.10, 4.12; Appendices 4.8-4.10), this ancestor would most likely have had a widespread montane distribution within the south-west, where the greatest diversity and four of the six genera are found, with subsequent migrations following cladogenesis through to the Eastern Escarpment Mountains of Lesotho and the KwaZulu-Natal Drakensberg.

These migration patterns can be unified into the following simple historical biogeographical hypothesis emanating from the cladograms (Figs 4.7, 4.10, 4.12; Appendices 4.8-4.10, 4.16, of which the most accepted one was the *a priori* total evidence cladogram, Fig. 4.12) where *Aphanicercopsis* was the sister group to the remaining five genera. It is illogical to derive a historical biogeographical hypothesis from a very conservative consensus cladogram, because it is certain that the consensus is not the true tree; it is less likely to be the true tree than any one of the most parsimonious trees which are all more parsimonious than the consensus. The *a priori* cladogram though, was fully resolved at generic level, and is therefore reasonable to use for this purpose. Because the model based molecular cladograms recovered some paraphyletic relationships (versus parsimony and morphological monophyly), a detailed time line could not be estimated and will have to be attempted in future studies with a wider genomic sampling.

Because the local notonemourids are a monophyletic group (Terry & Whiting 2003) and are endemic to South Africa and Lesotho, it is likely that the local genera diverged from a common Gondwanan ancestor subsequent to the separation of Africa from the rest of Gondwanaland, which was thought to have occurred about 142-127 mya (Dingle *et al*. 1983). A more recent estimate put the tightest Gondwanan configuration at about 200 mya, with Africa and Antarctica already well separated by 140 mya (Reeves & de Wit 2000). That would place the widespread common ancestor of the six genera across the montane areas of the southern tip of the African continent after the separation from Gondwanaland, and hence the origin of the genera, in the lower Cretaceous period. The current biogeography of the local notonemourids indicates ancient vicariant events resulting in an east-west (or north-east and south-west) separation of the common ancestor/s, resulting in evolution of the current genera, with *Aphanicercella* subsequently able to disperse widely, perhaps due to a combination of a greater tolerance to adverse environmental conditions and superior vagility. These climatic and tectonic events during the early Miocene (*ca*. 20 mya) are thought to have stimulated widespread cladogenesis

(Daniels *et al*. in press) and would account for vicariant isolation of stonefly populations with founder effect and genetic drift resulting in diverging lineages. A mild Miocene uplift with subsequent denudation was one such tectonic event. This was followed by a second more substantial late Pliocene (*ca*. 2.5 mya) neotectonic uplift of about 100m on the west coast and 600-900 metres in the south and east (King 1978; Artyushkov & Hofmann 1998; Partridge 1998). These uplifts that created the Great Escarpment and steeply incised river gorges, of which an example is the Kei River in the Eastern Cape (Moore & Blenkinsop 2006), would have formed affective stream faunal isolating barriers. As highlighted in Chapter 3, vicariant allopatric speciation may not require a specific vicariant event in complex montane landscapes with steep valleys and inhospitable intervening terrain between streams, as steep gorges may provide sufficient physical barriers to dispersal in some taxa (Hughes *et al*. 1999; Wishart & Hughes 2001). These physical barriers may have acted as surrogates for the major geological and climatic events often cited in cases of vicariant allopatric speciation.

Topography affects rainfall spatial patterns and amounts, and these uplifts would have resulted in aridification of the southern African region (Sepulchre *et al*. 2006). Additionally, the development of the cold Benguela current on the west coast due to separation of South America from Antarctica about 11-14 mya is thought to have resulted in aridification of the Cape region (Partridge 1998; Richardson *et al*. 2001). While this ecological factor is thought to have been a major cause of speciation in the Cape flora (Linder & Mann 1998), it has not yet been shown to have stimulated rapid speciation of stream macroinvertebrate fauna, although available data does point to high faunal species turnover during this period (Tolley *et al*. 2008). It is likely though, that shrinking available habitat due to aridification with resultant drying up of streams may have isolated populations, thereby driving speciation. Following this period of aridity, the winter rainfall zone developed along the south west coast, shown by the presence of C_3 grasses to have been in existence since at least the early Pliocene (*ca*. 5 mya) (Franz-Odendaal *et al*. 2002). The winter rainfall is likely to have further increased during the late Quaternary (from 45 kya) due to expansion of Antarctic sea ice (Stuut *et al*. 2004). Although the greatest notonemourid diversity exists within this winter rainfall zone, the role of seasonality *per se* is not clear. However, other Mediterranean-climate regions are also highly biodiverse (Cowling *et al*. 1996; Bonada *et al*. 2008). Climatic factors common to these regions include seasonal flooding and drought, sometimes extreme, in annual cycles (Gasith & Resh 1999). This may have caused local extinctions with resultant isolation of previously contiguous populations. Vicariant events such as aridification, uplift, flooding and drought likely caused refugial habitat fragmentation and local extinctions, with resultant allopatric speciation and emergence of ancestors of the current genera. Pleistocene glaciation cycles (Richardson *et al*. 2001), which are thought to have been more extreme in the south-eastern Cape than in the west (Cowling *et al*.

1996; Barrable *et al*. 2002), and far preceding that in the Cretaceous, climate-affecting volcanism (Partridge 1998), are other potential climatic vicariant influences. The cool conditions of the Last Glacial Maximum (*ca*. 20 kya) in the Antarctic region caused expansion of circumpolar low pressure belts with resultant north-eastwards expansion of the winter rainfall zone (Norström *et al*. 2008). This expansion did not reach as far as north-eastern South Africa which was still in a subtropical belt experiencing summer rains within drier conditions (Norström *et al*. 2008). Because notonemourid species richness (and biodiversity in general) declines east of the Cape Folded Mountain region, it seems logical that winter rainfall on its own was not an important driver of speciation, during this time. If it were, then numerous extinctions must have occurred, but fossil evidence is minimal.

No fossil notonemourids have been found in South Africa, although fossils from other families such as the Palaeonemouridae (Permian) (van Dijk & Geertsema 2004) and the Gripopterygidae (tentatively) (Permian and Upper Triassic) (Riek 1973, 1976a, 1976b) are represented. During these periods Gondwanaland was still united. These families are now extinct in the southern African region. It would appear that vicariance would be the major driver of speciation in Notonemouridae owing to their narrow habitat preferences and very low powers of vagility. On a large scale this took place during continental drift in the Cretaceous, resulting in the current global distribution patterns of the family. On a smaller and more recent scale, evolution of the southern African Notonemouridae would have reflected vicariant events brought about by climate and tectonic changes, with little active dispersal clouding these patterns.

The common ancestor of all six genera likely had its origin in the CFM region. Because allopatric speciation is believed to be far more prevalent than sympatric speciation, and because there are four genera present in the CFM and usually multiple genera within one stream, it is likely that populations of this most recent common ancestor of these genera became separated by vicariant events (or topographical complexity as described earlier, resulting in island-type bottle-necks) within the CFM, allowing the genera to evolve. Species within these genera subsequently underwent cycles of range expansion and speciation in allopatry. Secondary contact would ultimately have occurred resulting in generic sympatry.

The common ancestor of the four *Aphanicercopsis* species is likely to have been restricted to the CFM, considering their current distributions. The single common ancestor of *Aphanicercella*, *Balinskycercella*, *Desmonemoura*, *Afronemoura* and *Aphanicerca* probably had an extended distribution including the CFM, Amatola and Drakensberg regions. The populations of the common ancestor of *Aphanicerca* and *Afronemoura* became isolated from each other

resulting in the evolution of these genera in allopatry. The vicariant event in this case is unknown, but separation of the Amatola Mountains from the Cape Folded Mountains by the formation of the Great Fish River valley during the uplifts and erosions of the mid-Miocene and late-Pliocene (Stuckenberg 1962; Artyushkov & Hofmann 1998; Partridge 1998; Moore & Blenkinsop 2006) is a possible cause. Climatic factors such as increasing aridity followed by expansion of and subsequent contraction of the winter rainfall region may also have been causative. *Afronemoura* would have migrated northwards to the present limit of its range in the northern Mpumalanga Drakensberg. *Aphanicercopsis*, *Aphanicerca*, and *Desmonemoura* remained restricted to the CFM.

The most recent common ancestor of the *Aphanicercella*, *Balinskycercella* clade must have dispersed to become widespread over the entire region from a CFM origin, with isolated populations evolving into present day species and with subsequent range expansions. Most of the current diversity of *Aphanicercella* is centred in the west of the CFM, but is the most widely dispersed genus. *A. cassida* is the only extant *Aphanicercella* species in the EH, and *A. namaquaensis* in the Namaqua Highlands, and may thus be the only survivors of regional extinctions. *Balinskycercella* seems to be adapted to the high altitude of the northern Eastern Escarpment Mountains and may have undergone extinctions elsewhere.

The fact that the genera have speciated, but are nonetheless geographically restricted lends further support for the overwhelming importance of vicariant speciation in this group. In general, it is likely, contrary to Stuckenberg (1962), that migrations occurred from the Cape north-eastwards, rather than the reverse. This pattern has been shown for aspects of the flora of southern Africa (Galley & Linder 2005; Galley *et al*. 2007), chameleons (Tolley *et al*. 2008), and was also thought to apply to mirid bugs due to the greater diversity and morphological specialization of those in the Cape centre (Schuh 1974). There was insufficient data to describe these migration patterns of the Notonemouridae with certainty. This historical biogeographical interpretation can be developed to a more detailed and confident level in future studies by incorporating additional outgroup taxa, especially important in the morphological analysis, and by the addition of more molecular markers with appropriate analyses including a molecular clock.

This study provides one of the few phylogenies for a sample taxon of the rich relictual fauna of southern Africa. It also provides a framework from which to identify areas for further research into stonefly speciation, endemism and morphology. In particular, clarifying the species status of divergent allopatric populations of what are currently considered to be single species, investigating relative rates of genetic versus organismal evolution in cases of closely

related but distinct sympatric species, and accumulating data to further elucidate historical biogeographic patterns and processes. Of prime interest would be establishing estimated dates of divergence of the terminals, and relating this to a range of historical events that would have promoted vicariant cladogenesis.

Appendix 4.1. Collection data used to prepare the distribution maps. Not all information is available for all collections. Numbers of specimens have not been included. DMS = DM Stevens, MDP = MD Picker, KHB = KH Barnard.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Afronemoura amatolae	N/A	25.11.1964	J. Illies	Tyume Cascades, Hogsback	Amatola Mountains	-32.650000	26.883300	Amatolae
Afronemoura amatolae	224	17.05.2003	DMS	Madonna & Child Falls, Hogsback	Amatola Mountains	-32.605300	26.962600	Amatolae
Afronemoura amatolae	N/A	29.11.1979	J. Illies	Seepage area on way to waterfall, Hogsback	Amatola Mountains	-32.605300	26.962600	Amatolae
Afronemoura amatolae	110		T. Branch	39 Steps Waterfall, Hogsback	Amatola Mountains	-32.580222	26.901108	Amatolae
Afronemoura amatolae	N/A	06.11.1985	B. Balinsky	Mt. Sheba, Mpumalanga	Mpumalanga Drakensberg	-24.933331	30.716665	Mpumalanga Drakensberg (southern)
Afronemoura amatolae	N/A	10.04.1976	MDP	Graskop, Mpumalanga	Mpumalanga Drakensberg	-24.933333	30.833333	Mpumalanga Drakensberg (southern)
Afronemoura amatolae	N/A			Palmiet, Grahamstown	Albany area	-33.370850	26.476938	Southern Eastern Cape Province highlands
Afronemoura spinulata	N/A	06.09.1986	S. van Noort	Hogsback	Amatola Mountains	-32.583300	26.933300	Amatolae
Afronemoura spinulata	140	11.12.2000	DMS	Katberg near Hotel	Amatolae	-32.480800	26.682800	Amatolae
Afronemoura spinulata	223	17.05.2003	DMS	Katberg Hotel, Red Trail	Amatolae	-32.488500	26.681200	Amatolae
Afronemoura spinulata	222	17.05.2003	DMS	Katberg Pass	Amatolae	-32.480800	26.682800	Amatolae
Afronemoura spinulata	N/A	11.12.1979	J. Illies	Mkomasi River, Himeville district, KwaZulu-Natal	KwaZulu-Natal Drakensberg	-29.600000	29.633300	KwaZulu-Natal Drakensberg
Afronemoura spinulata	N/A	23.11.-5.12.1970	H. & M. Townes	Karkloof	KwaZulu-Natal Midlands	-29.308743	30.192402	KwaZulu-Natal Midlands
Afronemoura spinulata	N/A	01.05.1974	R. Miller	Town Bush, Pietermaritzburg	KwaZulu-Natal Midlands	-29.566700	30.300000	KwaZulu-Natal Midlands
Afronemoura spinulata	N/A	11.12.1979	J. Illies	Nottingham Road, 50 km from Underberg	KwaZulu-Natal Drakensberg	-29.354989	30.001923	KwaZulu-Natal Midlands
Afronemoura spinulata	N/A	31.10-4.11.1970	H. & M. Townes	Ngome Forest, near Vryheid, Northern KwaZulu-Natal	Northern KwaZulu-Natal Midlands	-27.850000	31.416700	Northern KwaZulu-Natal Midlands
Afronemoura spinulata	N/A			Palmiet, Grahamstown		-33.370850	26.476938	Southern Eastern Cape Province highlands

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Afronemoura spinulata	N/A	01.03.1990	Albany Museum, Grahamstown	Ngele Forest, near Mackton Cottage, near Harding, Southern KwaZulu-Natal (Near Transkei border)	Probably Ngele Mountain, Southern KwaZulu-Natal (KZN Drakensberg foothills)	-30.533215	29.666520	Southern KwaZulu-Natal
Afronemoura stuckenbergi	N/A	04.10.1956	B. Stuckenberg	Mariepskop, Mpumalanga	Mpumalanga Drakensberg	-24.580431	30.865771	Mpumalanga Drakensberg (southern)
Aphanicerca austrocapensis sp. n.	248	16.08.2003	MDP	Garcia's Pass	Langeberg	-33.985800	21.227300	Langeberg
Aphanicerca austrocapensis sp. n.	271	14.06.2004	DMS	Kristalkloof, 17.7 km N of Riversdale on R323	Langeberg	-33.958600	21.230400	Langeberg
Aphanicerca austrocapensis sp. n.	152	19.08.2001	DMS	Gouna pump station, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicerca austrocapensis sp. n.	243	5-Aug-2003	DMS	Keur River Bridge, Montagu Pass, N of George	Outeniqua Mts	-33.907175	22.418134	Outeniqua
Aphanicerca austrocapensis sp. n.	242	5 August 2003	DMS	Bergplaas Forest, on road to Klipplaat, 9km from tar road	Outeniqua Mts	-33.884300	22.689300	Outeniqua
Aphanicerca austrocapensis sp. n.	159	20-Aug-2001	DMS	10 km after Bergplaas turn off on road to Kleinplaat, N of Knysna	Outeniqua Mts	-33.872275	22.687287	Outeniqua
Aphanicerca austrocapensis sp. n.	151	18-Aug-2001	DMS	34.6 km S from start of Uniondale-Knysna Rd, Prince Alfred's Pass	Between Outeniqua and Langkloof mountains	-33.862600	23.178400	Outeniqua
Aphanicerca austrocapensis sp. n.	154	19-Aug-2001	DMS	Prince Alfred's Pass at Thomas Bain Memorial	Outeniqua Mts	-33.860994	23.171860	Outeniqua
Aphanicerca austrocapensis sp. n.	240	4 August 2003	DMS	Prince Alfred's Pass	Outeniqua Mts	-33.860600	23.173000	Outeniqua
Aphanicerca austrocapensis sp. n.	238	4 August 2003	DMS	Road to George from Prince Alfred's Pass. Road crosses stream	Outeniqua Mts	-33.766000	23.005100	Outeniqua
Aphanicerca bicornis	N/A	13-May-1934	R. Anson Cook	Jan Du Toit's Kloof, Waaihoekberge, Hex River Mts	Waaihoekberge, Hex River Mts	-33.546435	19.353113	Hex River Mountains
Aphanicerca bicornis	N/A	17-Apr-1933	KHB	Fouche's Hoek, Mostertshoek, Waaihoek-berge	Waaihoekberge, Hex River Mts	-33.450000	19.283300	Hex River Mountains
Aphanicerca bicornis	N/A	20-Apr-1930	KHB	Sanddrifskloof, Hex River Mts	Hex River Mts	-33.464400	19.529600	Hex River Mountains
Aphanicerca bicornis	136	28-Jun-2000	DMS	Swartboskloof, Stellenbosch	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicerca bicornis	44	8-May-1994	DMS & MDP	"High Noon", 7 km N of Villiersdorp, Elandsrivier	Stetteynsberge	-33.909486	19.293128	Hottentots Holland (northern)
Aphanicerca bicornis	94	15-Aug-1995	DMS & MDP	Assegaaibos, at Nature Conservation offices, Stellenbosch	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicerca bicornis	45	8-May-1994	DMS & MDP	Jan Joubertsgat Bridge, Franschhoek Pass, Du Toit's River	Franschhoekberge	-33.933300	19.166700	Hottentots Holland (northern)
Aphanicerca bicornis	57	16-Jun-1994	DMS & MDP	Molenaars River, Du Toit's Kloof Pass	Kleindrakensteinberge / Dutoitsberge	-33.722389	19.150574	Hottentots Holland (northern)
Aphanicerca bicornis	N/A	1-Jun-1930	KHB	Du Toit's Kloof, Rawsonville	Slanghoekberge, Dutoitsberge	-33.692103	19.313211	Hottentots Holland (northern)
Aphanicerca bicornis	262	4 June 2004	DMS	Bain's Kloof Pass, Eerste Tol, under cement bridge	Limietberge / Slanghoekberge	-33.601800	19.110900	Hottentots Holland (northern)
Aphanicerca bicornis	109	10-Apr-1997	T. Branch	Voelklip Nature Reserve, Hermanus	Kleinriviersberge	-34.380000	19.278000	Hottentots Holland (southern)
Aphanicerca bicornis	130	14-May-2000	DMS & MDP	Harold Porter Botanic Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicerca bicornis	261	16 May 2004	DMS	Leopard's Kloof, Harold Porter Botanic Garden, Betty's Bay	Platberg, Hottentots Holland Mts	-34.346690	18.930410	Hottentots Holland (southern)
Aphanicerca bicornis	260	24 April 2004	DMS	Karmel, Franschhoek Pass	Franschhoekberge	-33.917900	19.161900	Hottentots Holland (northern)
Aphanicerca bovina	37	?	DMS & MDP	Swartboskloof	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicerca bovina	227	3 July 2003	DMS	Jonkershoek Nature Reserve, circular drive on look-out hut side	Jonkershoekberge	-33.989800	18.956900	Hottentots Holland (northern)
Aphanicerca bovina	174	25-Jun-2002	DMS	Jonkershoek Nature Reserve, Stellenbosch, circular drive, river after Jakkalsrivier	Stellenboschberg	-33.989100	18.968400	Hottentots Holland (northern)
Aphanicerca bovina	175	25-Jun-2002	DMS	Jonkershoek Nature Reserve, homeward bound circular drive, small stream under road	Stellenboschberg	-33.979100	18.950100	Hottentots Holland (northern)
Aphanicerca bovina	229	3 July 2003	DMS	Assegaaibos Nature Reserve, Stellenbosch	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)
Aphanicerca bovina	N/A	1-Oct-1932	HG Wood	Franschhoek Pass, East side		-33.917628	19.163056	Hottentots Holland (northern)
Aphanicerca breviloba sp. n.	62	4-Dec-1994	DMS & MDP	Swartberg pass, Boegoekloof, 1886m, between Oudtshoorn and Prince Albert	Groot Swartberg	-33.357400	22.058500	Groot Swartberg
Aphanicerca brevispina sp. n.	284	6 July 2004	DMS	Harold Porter Nature Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicerca brevispina sp. n.	293	15 Sept 2004	DMS	Faerie Glen Picnic Site, Kleinmond	Southern Hottentots Holland Mts	-34.330262	18.991217	Hottentots Holland (southern)
Aphanicerca brevispina sp. n.	7	31-May-1993	DMS & MDP	Clarence Drive, monument site 10 km N of Rooiels	Koeelberg, Hottentots Holland Mts	-34.207200	18.833100	Hottentots Holland (southern)
Aphanicerca capensis	1	25-Apr-1993	DMS & MDP	Boyes Drive, Kalk Bay	Cape Peninsula	-34.123700	18.449500	Cape Peninsula

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicerca capensis	11	5-Jun-1993	DMS & MDP	Silvermine Nature Reserve, Steenberg	Steenberg, Cape Peninsula	-34.100100	18.429300	Cape Peninsula
Aphanicerca capensis	23	25-Jun-1993	DMS & MDP	Cecilia State Forest, near Cecilia Forest Station, Cape Peninsula	Table Mt, Cape Peninsula	-33.997800	18.425700	Cape Peninsula
Aphanicerca capensis	36	21-Sep-1993	MDP	Liesbeeck River, Kirstenbosch	Table Mt., Cape Peninsula	-33.987600	18.434900	Cape Peninsula
Aphanicerca capensis	297	5-Jul-2007	DMS	Below Kirstenbosch, Boschenheuvel Arboretum	Cape Peninsula	-33.987460	18.437190	Cape Peninsula
Aphanicerca capensis	24	31-Oct-1993	DMS & MDP	Skeleton Gorge, Kirstenbosch, Table Mountain, Cape Peninsula	Table Mt, Cape Peninsula	-33.981900	18.424400	Cape Peninsula
Aphanicerca capensis	22	21-Jun-1993	DMS	Slangolie Ravine	Twelve Apostles, Cape Peninsula	-33.977700	18.385100	Cape Peninsula
Aphanicerca capensis	168	16-Jun-2002	DMS	Pipe Track, where pipe visible & crosses stream (before Woody Ravine), Cape Peninsula	Twelve Apostles, Cape Peninsula	-33.970400	18.386000	Cape Peninsula
Aphanicerca capensis	298	10-Jul-2007	DMS	Theresa Avenue, Camps Bay	Twelve Apostles, Cape Peninsula	-33.967920	18.382010	Cape Peninsula
Aphanicerca capensis	144	4-Aug-2001	DMS	Platteklip Gorge, Tafelberg Rd, Table Mountain	Table Mt., Cape Peninsula	-33.955700	18.415900	Cape Peninsula
Aphanicerca capensis	20	16-Jun-1993	DMS & MDP	Gardens, Table Mountain	Table Mt, Cape Peninsula	-33.943300	18.419400	Cape Peninsula
Aphanicerca capensis	133	4-Jul-2000	DMS & MDP	Gardens, Table Mountain	Table Mt, Cape Peninsula	-33.943300	18.419400	Cape Peninsula
Aphanicerca cederbergensis sp. n.	126	21-Sep-1999	DMS	Trib of Driehoekrivier, above Sederhoutkloof, Koerasieberg near Eikeboom, Cederberg	Koerasieberg, Cederberg	-32.475588	19.154878	Cederberg
Aphanicerca cederbergensis sp. n.	143	2-Aug-2001	DMS	Sneeuberg, Cederberg	Cederberg	-32.501528	19.155643	Cederberg
Aphanicerca cederbergensis sp. n.	291	07.Sep.04	DMS	Eikeboom, 16.4 km S of Algeria, Cederberg	Cederberg	-32.454900	19.169600	Cederberg
Aphanicerca cederbergensis sp. n.	251	14 Sept 2003	DMS	Concrete bridge 11.2 km after Algeria on road to Sanddrift	Cederberg	-32.425600	19.131800	Cederberg
Aphanicerca cederbergensis sp. n.	121	18-Sep-1999	DMS	Algeria, Cederberg	Cederberg	-32.374100	19.062000	Cederberg
Aphanicerca cederbergensis sp. n.	125	20-Sep-1999	DMS	Fortyn se Kloof, Jeep track south of Pakhuis Pass	Krakadouwberge, Cederberg	-32.175700	19.063800	Cederberg

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicerca chanae	294	21 March 2005	DMS	Honeywood Farm, near Grootvadersbosch Nature Reserve, near Heidelberg	Langeberg	-33.999546	20.814229	Langeberg
Aphanicerca chanae	258	20 April 2004	DMS	Marloth Nature Reserve, Swellendam	Langeberg	-33.999200	20.456200	Langeberg
Aphanicerca chanae	232	3 August 2003	DMS	Marloth Nature Reserve, Swellendam. First stream on road.	Langeberg	-33.996900	20.445300	Langeberg
Aphanicerca chanae	102	9-Mar-1996	DMS	Grootvadersbosch River, Grootvadersbosch Nature Reserve, near Heidelberg	Langeberg	-33.985891	20.823277	Langeberg Mts
Aphanicerca gnua	16	12-Jun-1994	DMS & MDP	Kleinboontjiesrivier, turn off R46 19 km south of Ceres Nature Reserve, stream under road.	Witsenberg	-33.382737	19.213298	Witsenberg
Aphanicerca incisura sp. n.	171	18-Jun-2002	DMS	Farm "Bergheim", Damsrivier, on R62 between Montagu and Barrydale	Langeberg Mts	-33.932800	20.380900	Langeberg Mts
Aphanicerca incisura sp. n.	209	1-Oct-2002	DMS	Huisrivier, Ravenna Mountain Retreat, on R62 between Montagu and Barrydale	Langeberg Mts	-33.918500	20.378800	Langeberg Mts
Aphanicerca longiloba sp. n.	233	3 August 2003	DMS	Marloth Nature Reserve, Swellendam. Second stream on road.	Langeberg	-33.999000	20.456500	Langeberg
Aphanicerca longiloba sp. n.	232	3 August 2003	DMS	Marloth Nature Reserve, Swellendam. First stream on road.	Langeberg	-33.996900	20.445300	Langeberg
Aphanicerca longiloba sp. n.	78	1-Jul-1995	DMS & MDP	Garcia's Pass, 13.5 km N of Riversdale on R323; road crosses stream, forestry road.	Langeberg	-33.985800	21.227300	Langeberg
Aphanicerca longiloba sp. n.	145	17-Aug-2001	DMS	Tradouw Pass, 24.2 km from N2, 2nd lay-bye	Langeberg	-33.982738	20.708599	Langeberg
Aphanicerca longiloba sp. n.	80	1-Jul-1995	DMS & MDP	Garcia's Pass, 16.2 km N of Riversdale on R323; concrete channel	Langeberg	-33.968000	21.219700	Langeberg
Aphanicerca longiloba sp. n.	259	20 April 2004	DMS	Kristalkloof, 17.7 km N of Riversdale on R323	Langeberg	-33.958600	21.230400	Langeberg
Aphanicerca longiloba sp. n.	192	10-Jul-2002	DMS	Sleeping Beauty Trail, Garcia's Pass, 18.4 km N of Riversdale on R323; 1st stream	Langeberg	-33.956900	21.216100	Langeberg
Aphanicerca lyrata	227	3 July 2003	DMS	Jonkershoek Nature Reserve, circular drive on look-out hut side	Jonkershoekberge	-33.989800	18.956900	Hottentots Holland (northern)
Aphanicerca lyrata	132	30-Jun-2000	DMS & MDP	Assegaaibos Nature Reserve, Stellenbosch	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)
Aphanicerca lyrata	26	27-Apr-1986	MDP	Jonkershoek, Stellenbosch	Stellenboschberg / Jonkershoekberge	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicerca lyrata	42	8-May-1994	DMS & MDP	Swiss Farm Excelsior, Franschhoek	Franschhoekberge	-33.937600	19.110900	Hottentots Holland (northern)
Aphanicerca lyrata	134	9-Jul-2000	DMS & MDP	Start of Bain's Kloof Pass	Limietberge	-33.645100	19.071500	Hottentots Holland (northern)

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicerca lyrata	137	Aug-2000	DMS	Harold Porter Botanic Reserve, Betty's Bay	Platberg, Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicerca mclellani sp. n.	220	Dec-1967	P & B Stuckenberg	Otterford Forestry Station, Hankey district	Elandsberge	-33.783867	25.019421	Elandsberge
Aphanicerca mclellani sp. n.	271	14.Jun.04	DMS	Kristalkloof, 17.7 km N of Riversdale on R323	Langeberg	-33.958600	21.230400	Langeberg
Aphanicerca mclellani sp. n.	196	5-Aug-2002	DMS	Road between R323 and Herbertsdale, near Cloete's Pass	Langeberg	-33.919800	21.742100	Langeberg Mts
Aphanicerca mclellani sp. n.	103	6-Mar-1996	DMS	Terblans Walk, Gouna Forest, N of Knysna	Outeniqua Mts	-33.947500	23.141100	Outeniqua
Aphanicerca mclellani sp. n.	237	4 August 2003	DMS	Kom se Pad, Gouna Forest, N of Knysna	Outeniqua Mts	-33.947500	23.141100	Outeniqua
Aphanicerca mclellani sp. n.	68	3-Dec-1994	DMS & MDP	Ysternek Nature Reserve, Prince Alfred's Pass, Knysna area	Outeniqua Mts	-33.933325	23.163417	Outeniqua
Aphanicerca mclellani sp. n.	219	27-Nov-2002	DMS	Keur River Bridge, Montagu Pass, N of George	Outeniqua Mts	-33.907175	22.418134	Outeniqua
Aphanicerca mclellani sp. n.	159	20-Aug-2001	DMS	10 km after Bergplaas turn off on road to Kleinplaat, N of Knysna	Outeniqua Mts	-33.872275	22.687287	Outeniqua
Aphanicerca mclellani sp. n.	70	3-Dec-1994	DMS & MDP	Prince Alfred's Pass, a few km's S of Avontuur	Between Outeniqua and Langkloof mountains	-33.756219	23.159235	Outeniqua
Aphanicerca pickeri sp. n.	179	2-Jul-2002	DMS	Fernkloof Nature Reserve, Hermanus; second entrance; bridge over stream near gate	Kleinriviersberge	-34.398479	19.273004	Hottentots Holland (southern)
Aphanicerca pickeri sp. n.	180	2-Jul-2002	DMS	Fernkloof Nature Reserve, Hermanus; second entrance; below dam	Kleinriviersberge	-34.393900	19.276100	Hottentots Holland (southern)
Aphanicerca pickeri sp. n.	178	2-Jul-2002	DMS	Fernkloof Nature Reserve, Hermanus, waterfall path, Assegaaibos Waterfall	Kleinriviersberge	-34.390000	19.269100	Hottentots Holland (southern)
Aphanicerca swartbergensis sp. n.	235	3-Aug-2003	DMS	Oudemuragie Road, 14.9 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.413400	22.383000	Groot Swartberg
Aphanicerca swartbergensis sp. n.	185	8-Jul-2002	DMS	Seweweekspoort, 8 km south of Gamkapoortdam turn-off; road crosses stream.	Klein Swartberge	-33.412100	21.408700	Groot Swartberg
Aphanicerca swartbergensis sp. n.	234	3-Aug-2003	DMS	Oudemuragie Road, 11.6 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.411200	22.354100	Groot Swartberg

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicerca swartbergensis sp. n.	184	8-Jul-2002	DMS	Seweweekspoort, 7 km south of Gamkapoortdam turn-off, wooded section of stream.	Klein Swartberg	-33.405500	21.400500	Groot Swartberg
Aphanicerca swartbergensis sp. n.	183	8-Jul-2002	DMS	Seweweekspoort, 6 km south of Gamkapoortdam turn-off, where side stream joins main stream.	Klein Swartberg	-33.394300	21.399200	Groot Swartberg
Aphanicerca swartbergensis sp. n.	217	26-Nov-2002	DMS	Oudemuragie Road, "Rust en Vrede" waterfall.	Groot Swartberg	-33.391800	22.355900	Groot Swartberg
Aphanicerca swartbergensis sp. n.	249	16 Aug 2003	MDP	Swartberg Pass, on Prince Albert side	Groot Swartberg	-33.299600	22.050100	Groot Swartberg
Aphanicerca tereta	N/A	Nov-1928	KHB	Riviersonderend Mts	Riviersonderend Mts	-34.082000	19.829100	Riviersonderend Mts
Aphanicerca uncinata	N/A	Jan-1916	KHB	Hottentots Holland Mts (East side of Sneeukop, Landdroskop, Sugarloaf)	Hottentots Holland Mts	-34.050000	19.016700	Hottentots Holland (northern)
Aphanicerca uncinata	137	22-Aug-2000	DMS	Harold Porter Botanic Reserve, Betty's Bay	Platberg, Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicerca uncinata	295	16 June 2007	DMS	Leopard's Kloof, Harold Porter Nature Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.346690	18.930410	Hottentots Holland (southern)
Aphanicerca witsenbergensis sp. n.	287	18 July 2004	DMS	Kleinboontjiesrivier, turn off R46 19 km south of Ceres Nature Reserve, Witsenberg Game Park	Witsenberg	-33.382737	19.213298	Witsenberg
Aphanicerca zwicki sp. n.	228	3 July 2003	DMS	Jonkershoek Nature Reserve, circular drive at hairpin bend	Jonkershoekberge	-33.993700	18.974900	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	37	5-July-1996 & 18-August-1996	DMS & MDP	Swartboskloof, Stellenbosch	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	227	3 July 2003	DMS	Jonkershoek Nature Reserve, circular drive on look-out hut side	Jonkershoekberge	-33.989800	18.956900	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	254	26 Sept 2003	DMS	Jonkershoek Nature Reserve, Stellenbosch, circular drive, river after Jakkalsrivier	Stellenboschberg	-33.989100	18.968400	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	246	3 Sept 2003	DMS	Assegaaibos Nature Reserve, Stellenbosch	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	132	30-Jun-2000	DMS & MDP	Jonkershoek, at Nature Conservation offices	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	4	23-May-1993	DMS & MDP	Franschhoek Pass, Du Toit's River Bridge	Franschhoekberge	-33.948057	19.168624	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	113	18-Aug-1997	DMS & MDP	Pniel, near Boschendal	Grootdrakensteinberge / Jonkershoekberge	-33.900000	18.950000	Hottentots Holland (northern)

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicerca zwicki sp. n.	215	14-Nov-2002	DMS	Klip River, trib of Molenaars River, Du Toit's Kloof Pass, 7.5 km N of old tunnel	Dutoitsberge	-33.722100	19.182100	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	299	15-Jul-2007	DMS	Bain's Kloof Pass, 1st stream N of Wellington	Limietberge	-33.645158	19.070927	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	204	4-Sep-2002	DMS	Gawie se Water, Bain's Kloof	Limietberge / Slanghoekberge	-33.641300	19.104100	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	301	15-Jul-2007	DMS	Bain's Kloof, stream under where concrete rubbish bin is.	Limietberge / Slanghoekberge	-33.601820	19.110870	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	262	4 June 2004	DMS	Bain's Kloof Pass, Eerste Tol, under cement bridge	Limietberge / Slanghoekberge	-33.601800	19.110900	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	300	15-Jul-2007	DMS	Bain's Kloof, sharp bend with white brick wall	Limietberge / Slanghoekberge	-33.594720	19.121140	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	264	4 June 2004	DMS	Bain's Kloof Pass, cement wall bridge	Limietberge / Slanghoekberge	-33.592800	19.123600	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	206	4-Sep-2002	DMS	Bain's Kloof Pass, concrete channel	Limietberge	-33.592100	19.125000	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	303	15-Jul-2007	DMS	Bain's Kloof, Steenbok Park	Limietberge / Slanghoekberge	-33.555860	19.149920	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	205	4-Sep-2002	DMS	Bain's Kloof Pass, Steenbok Park	Limietberge	-33.555800	19.150000	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	304	15-Jul-2007	DMS	Bain's Kloof, Bastiaanskloof River, crossing road	Limietberge / Slanghoekberge	-33.547060	19.163000	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	207	4-Sep-2002	DMS	Bain's Kloof Pass, road crosses large stream at N end of pass	Limietberge	-33.546100	19.163800	Hottentots Holland (northern)
Aphanicerca zwicki sp. n.	119	13-Feb-1999	DMS & MDP	Oubos, Riviersonderend	Riviersonderend Mts	-34.082000	19.829100	Riviersonderend Mts
Aphanicerca zwicki sp. n.	10	31-May-1993	DMS & MDP	Harold Porter Botanic Reserve, Betty's Bay	Platberg, Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicerca zwicki sp. n.	261	16 May 2004	DMS	Leopard's Kloof, Harold Porter Botanic Garden, Betty's Bay	Platberg, Hottentots Holland Mts	-34.346690	18.930410	Hottentots Holland (southern)
Aphanicerca zwicki sp. n.	8	31-May-1993	DMS & MDP	Clarence Drive, N of Rooiels	Koeelberg, Hottentots Holland Mts	-34.200000	18.766700	Hottentots Holland (southern)
Aphanicercella barnardi	270	8 June 2004	DMS	Eikeboom, 16.4 km S of Algeria, Cederberg	Cederberg	-32.454900	19.169600	Cederberg
Aphanicercella barnardi	266	7 June 2004	DMS	Hex River, 10.2 km S of Algeria road on old road between Citrusdal and Clanwilliam	Cederberg	-32.445300	18.972600	Cederberg

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella barnardi	143	2-Aug-2001	DMS	Sneeuberg, Cederberg	Cederberg	-32.427900	19.160500	Cederberg
Aphanicercella barnardi	290	07.Sep.04	DMS	11.2 km S of Algeria, Cederberg	Cederberg	-32.425600	19.131800	Cederberg
Aphanicercella barnardi	268	8 June 2004	DMS	Algeria, Cederberg. Tributary of Rondegat River alongside camp site	Cederberg	-32.374100	19.062000	Cederberg
Aphanicercella barnardi	289	07.Sep.04	DMS	Algeria, Cederberg	Cederberg	-32.374100	19.062000	Cederberg
Aphanicercella barnardi	267	8 June 2004	DMS	Rondegat River under road near Algeria on lower Clanwilliam Algeria road	Cederberg	-32.370100	19.053800	Cederberg
Aphanicercella barnardi	256	1 Oct 2003	DMS	Dwarsrivier, on Dwarsrivier Farm, Clanwilliam, at foot of Krakadouw Peak	Cederberg	-32.220800	19.004200	Cederberg
Aphanicercella barnardi	292	07.Sep.04	DMS	Pakhuis Pass, Kliphuis Camp Site	Cederberg	-32.135800	19.002500	Cederberg
Aphanicercella barnardi	56	10-Sep-1994	MDP	Pakhuispad, 27 E Clanwilliam, near Louis Leipold's grave	Pakhuisberg, Cederberg	-32.135800	19.002500	Cederberg
Aphanicercella barnardi	N/A	4-Jun-1929	KHB	Fairy Glen, Brandwacht, Worcester	Hex River Mountains	-33.550000	19.450000	Hex River Mountains
Aphanicercella barnardi	195	4-Aug-2002	Julia Wakeling	Hex River Mountain & Ski Club Hut, below Milner Ridge Peak	Hex River Mountains	-33.487600	19.465000	Hex River Mountains
Aphanicercella barnardi	285	13 July 2004	DMS	Hex River Valley; 1st tributary of Sanddrift River after gate at bridge	Hex River Mountains	-33.464400	19.529600	Hex River Mountains
Aphanicercella barnardi	286	13 July 2004	DMS	Hex River Valley; 2nd tributary of Sanddrift River after gate at bridge	Hex River Mountains	-33.450400	19.549900	Hex River Mountains
Aphanicercella barnardi	18	13-Jun-1993	DMS & MDP	61.5 km N of Witsenberg Valley turn off, on the R303 where R303 crosses river	Kouebokkeveldberge	-32.790723	19.245105	Kouebokkeveld
Aphanicercella bifurcata	N/A	Sep-1932	KHB	Grootwinterhoek Mts, 4000-5000 feet	Groot Winterhoekberge	-33.113241	19.086303	Grootwinterhoek
Aphanicercella bifurcata	N/A	Sep-1933	KHB	Matroosberg, Hex River Mts	Hex River Mountains	-33.366700	19.666700	Hex River Mountains
Aphanicercella bifurcata	N/A			Humansdorp	Kougaberge	-34.000000	24.770000	Kougaberge
Aphanicercella bifurcata	294	21 March 2005	DMS	Honeywood Farm, near Grootvadersbosch Nature Reserve, near Heidelberg	Langeberg	-33.999546	20.814229	Langeberg
Aphanicercella bifurcata	258	20 April 2004	DMS	Marloth Nature Reserve, Swellendam	Langeberg	-33.999200	20.456200	Langeberg

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella bifurcata	118	4-Apr-1998	DMS	Marloth Nature Reserve, Swellendam	Langeberg	-33.996900	20.445300	Langeberg
Aphanicercella bifurcata	145	17-Aug-2001	DMS	Tradouw Pass, 24.2 km from N2, 2nd lay-bye	Langeberg	-33.982738	20.708599	Langeberg
Aphanicercella bifurcata	259	20 April 2004	DMS	Kristalkloof, Garcia's Pass, N of Riversdale on R323	Langeberg	-33.958600	21.230600	Langeberg
Aphanicercella bifurcata	192	11-Jul-2002	DMS	Sleeping Beauty Trail, Garcia's Pass, 18.4 km N of Riversdale on R323; 1st stream	Langeberg	-33.956900	21.216100	Langeberg
Aphanicercella bifurcata	193	11-Jul-2002	DMS	Sleeping Beauty Trail, Garcia's Pass, 18.4 km N of Riversdale on R323; 2nd stream	Langeberg	-33.956400	21.211800	Langeberg
Aphanicercella bifurcata	102	9-Mar-1996	DMS	Grootvadersbosch River, Grootvadersbosch Nature Reserve, near Heidelberg	Langeberg	-33.985891	20.823277	Langeberg Mts
Aphanicercella bifurcata	101	3-Mar-1996	DMS	Jubilee Creek, Knysna	Outeniqua Mts			Outeniqua
Aphanicercella bifurcata	241	4 August 2003	DMS	Gouna pump station, Gouna Forest, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicercella bifurcata	67	3-Dec-1994	DMS & MDP	3 km W of Hoekwil, on road to Saasveld, George district, Outeniqua	Outeniqua Mts	-33.983300	22.616700	Outeniqua
Aphanicercella bifurcata	100	7-Mar-1996	DMS	Touw River Waterfall, Giant Kingfisher Trail, Wilderness	Outeniqua Mts	-33.966159	22.552961	Outeniqua
Aphanicercella bifurcata	74	3-Dec-1994	DMS & MDP	"Big Tree" picnic site, en route to Kleinplaat from Woodville; 2 km of Bergplaas turn off; near Wilderness	Outeniqua Mts	-33.924308	22.671759	Outeniqua
Aphanicercella bifurcata	96	6-Mar-1996	DMS	Kom se Pad, 9 km E of Terblans Walk, Brak River, Gouna Forest, N of Knysna	Outeniqua Mts	-33.947500	23.141100	Outeniqua
Aphanicercella bifurcata	141	12-Dec-2000	DMS	Homtini River, Knysna area	Outeniqua	-33.948234	22.919236	Outeniqua
Aphanicercella bifurcata	68	3-Dec-1994	DMS & MDP	Ysternek Nature Reserve, Prince Alfred's Pass, Knysna area	Outeniqua Mts	-33.933325	23.163417	Outeniqua
Aphanicercella bifurcata	104	4-Mar-1996	DMS	Bloukrans Forest, Stinkhoutkloof Forest Trail, Klip River, Knysna	Outeniqua Mts	-33.959545	23.615247	Outeniqua
Aphanicercella bifurcata	156	20-Aug-2001	DMS	Malgas River, near George	Outeniqua Mts	-33.958298	22.311839	Outeniqua
Aphanicercella bifurcata	97	6-Mar-1996	DMS	Karatara River, Seven Passes Road, near Barrington	Outeniqua Mts	-33.933300	22.766700	Outeniqua

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella bifurcata	277	16 June 2004	DMS	Loredo-North road near Nature's Valley	Outeniqua Mts	-33.932500	23.502000	Outeniqua
Aphanicercella bifurcata	243	5 August 2003	DMS	Keur River Bridge, Montagu Pass, N of George	Outeniqua Mts	-33.907175	22.418134	Outeniqua
Aphanicercella bifurcata	159	20-Aug-2001	DMS	10 km after Bergplaas turn off on road to Kleinplaat, N of Knysna	Outeniqua Mts	-33.872275	22.687287	Outeniqua
Aphanicercella bifurcata	N/A	14-Apr-1933	HG Wood	Montagu Pass, Outeniqua Mts	Outeniqua Mts	-33.907175	22.418134	Outeniqua
Aphanicercella bifurcata	105	6-Mar-1996	DMS	Kaaimans River, trib on Forest Road to George off Seven passes road	Outeniqua Mts	-33.964978	22.561614	Outeniqua
Aphanicercella bifurcata	N/A			Oudebosch, Riviersonderend Mts, 1500-3500 feet	Riviersonderend Mts	-34.082000	19.829100	Riviersonderend Mts
Aphanicercella bifurcata	119	13-Feb-1999	DMS & MDP	Oubos, Riviersonderend Mts	Riviersonderend Mts	-34.082000	19.829100	Riviersonderend Mts
Aphanicercella bifurcata	N/A	Aug-Nov-2000	Albany Museum Survey	Salt River, Hol River and Wit River, Nature's Valley	Tsitsikamma Mts	-33.968207	23.558304	Tsitsikamma Mts
Aphanicercella bifurcata	N/A	Aug-2000	Albany Museum Survey	Groot River, Nature's Valley	Tsitsikamma Mts	-33.968207	23.558304	Tsitsikamma Mts
Aphanicercella bullata	253	14 Sept 2003	DMS	Stream 19.5 km after Algeria on road to Sanddrift	Cederberg	-32.463600	19.195900	Cederberg
Aphanicercella bullata	90	3-Jul-1995	DMS & MDP	Longmore Forest, near Loerie (NE of Jeffreys Bay)	Elandsberge	-33.862265	25.085697	Elandsberge
Aphanicercella bullata	84	2-Jul-1995	DMS & MDP	Herrie Drif, Meiringspoort on R29, Groot Swartberg	Groot Swartberg	-33.439479	22.559342	Groot Swartberg
Aphanicercella bullata	235	3-Aug-2003	DMS	Oudemuragie Road, 14.9 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.413400	22.383000	Groot Swartberg
Aphanicercella bullata	187	8-Jul-2002	DMS	Swartberg pass, Die Stalletjie	Groot Swartberg	-33.365400	22.098900	Groot Swartberg
Aphanicercella bullata	198	6-Aug-2002	DMS	Swartberg pass, Boegoekloof, 1886m, between Oudsthoorn and Prince Albert	Groot Swartberg	-33.357400	22.058500	Groot Swartberg
Aphanicercella bullata	N/A			Humansdorp	Kougaberge	-34.000000	24.770000	Kougaberge
Aphanicercella bullata	78	1-Jul-1995	DMS & MDP	Garcia's Pass, 13.5 km N of Riversdale on R323; road crosses stream, forestry road.	Langeberg	-33.985800	21.227300	Langeberg
Aphanicercella bullata	79 80	1-Jul-1995	DMS & MDP	Garcia's Pass, 16.2 km N of Riversdale on R323; concrete channel	Langeberg	-33.968000	21.219700	Langeberg

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella bullata	81	1-Jul-1995	DMS & MDP	Kristalkloof, 17.7 km N of Riversdale on R323	Langeberg Mountains	-33.958600	21.230400	Langeberg
Aphanicercella bullata	171	18-Jun-2002	DMS	Damsrivier, Farm "Bergheim" on R62 between Montagu and Barrydale	Langeberg Mountains	-33.932800	20.380900	Langeberg Mts
Aphanicercella bullata	196	5-Aug-2002	DMS	Road between R323 and Herbertsdale, near Cloete's Pass	Langeberg Mountains	-33.919800	21.742100	Langeberg Mts
Aphanicercella bullata	170	18-Jun-2002	DMS	Huisrivier, Ravenna Mountain Retreat, on R62 between Montagu and Barrydale	Langeberg Mountains	-33.918500	20.378800	Langeberg Mts
Aphanicercella bullata	274	15-Jun-2004	DMS	2nd stream under road on R327 to Herbertsdale (after large river), near Cloete's Pass	Langeberg Mountains	-33.917900	21.736900	Langeberg Mts
Aphanicercella bullata	273	15-Jun-2004	DMS	1st stream under road on R327 to Herbertsdale (after large river), near Cloete's Pass	Langeberg Mountains	-33.909800	21.720700	Langeberg Mts
Aphanicercella bullata	276	16.Jun.04	DMS	Brackenhill Waterfall, E of Knysna, stream under side road	Outeniqua Mts	-34.046000	23.163100	Outeniqua
Aphanicercella bullata	275	15-Jun-2004	DMS	Gouna pump station, Gouna Forest, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicercella bullata	278	16 June 2004	DMS	Road to Bergplaas. Road crosses stream at hairpin bend	Outeniqua Mts	-33.902900	22.673900	Outeniqua
Aphanicercella bullata	242	5 August 2003	DMS	Bergplaas Forest, on road to Klipplaat, 9km from tar road	Outeniqua Mts	-33.884300	22.689300	Outeniqua
Aphanicercella bullata	244	5 Aug 2003	DMS	Oubos, Riviersonderend	Riviersonderend Mts	-34.082000	19.829100	Riviersonderend Mts
Aphanicercella cassida	N/A			Hogsback Inn	Amatolae	-32.583300	26.933300	Amatolae
Aphanicercella cassida	N/A			Hogsback	Amatolae	-32.580222	26.901108	Amatolae
Aphanicercella cassida	86 & 87	2-Jul-1995	DMS & MDP	Enkeldoorn, Baviaanskloof	Baviaanskloofberge / Kougaberge	-33.656946	24.365694	Baviaanskloof
Aphanicercella cassida	88	2-Jul-1995	DMS & MDP	Poortjie, Baviaanskloof	Baviaanskloofberge / Kougaberge	-33.571939	24.118545	Baviaanskloof
Aphanicercella cassida	N/A			Xuka river trib	SE of Elliot, Eastern Cape	-31.450000	28.016700	Eastern Cape Province Southern Drakensberg
Aphanicercella cassida	N/A			Kudidwayo river, Marinus	SE of Elliot, Eastern Cape	-31.416700	28.100000	Eastern Cape Province Southern Drakensberg

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella cassida	N/A			Rhodes	Near Barkly East, near Lesotho	-30.800000	27.950000	Eastern Cape Province Southern Drakensberg
Aphanicercella cassida	N/A			Bell river	Near Barkly East, near Lesotho	-30.750000	28.050000	Eastern Cape Province Southern Drakensberg
Aphanicercella cassida	N/A			Naude's neck pass	Near Barkly East, near Lesotho	-30.733300	28.116700	Eastern Cape Province Southern Drakensberg
Aphanicercella cassida	91	3-Jul-1995	DMS & MDP	Longmore Forest near Loerie	Elandsberge	-33.862265	25.085697	Elandsberge
Aphanicercella cassida	85	2-Jul-1995	DMS & MDP	Near Uitspan Drif, Meiringspoort on R29, Groot Swartberg	Groot Swartberg	-33.439479	22.559342	Groot Swartberg
Aphanicercella cassida	147	18-Aug-2001	DMS	24.7 km W of R328 on road to Calitzdorp (near Swartberg Pass)	Groot Swartberg	-33.465052	21.741220	Groot Swartberg
Aphanicercella cassida	235	3-Aug-2003	DMS	Oudemuragie Road, 14.9 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.413400	22.383000	Groot Swartberg
Aphanicercella cassida	185	8-Jul-2002	DMS	Seweweekspoort, 8 km south of Gamkapoortdam turn-off; road crosses stream.	Klein Swartberg	-33.412100	21.408700	Groot Swartberg
Aphanicercella cassida	234	3-Aug-2003	DMS	Oudemuragie Road, 11.6 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.411200	22.354100	Groot Swartberg
Aphanicercella cassida	148	18-Aug-2001	DMS	12.7 km W of R328 on road to Calitzdorp (near Swartberg Pass)	Groot Swartberg	-33.405228	21.995098	Groot Swartberg
Aphanicercella cassida	184	8-Jul-2002	DMS	Seweweekspoort, 7 km south of Gamkapoortdam turn-off, wooded section of stream.	Klein Swartberg	-33.405500	21.400500	Groot Swartberg
Aphanicercella cassida	186	8-Jul-2002	DMS	Road to Prince Albert from Calitzdorp where gravel road meets tar road at bridge over river.	Groot Swartberg	-33.405400	21.995800	Groot Swartberg
Aphanicercella cassida	183	8-Jul-2002	DMS	Seweweekspoort, 6 km south of Gamkapoortdam turn-off, where side stream joins main stream.	Klein Swartberg	-33.394300	21.399200	Groot Swartberg
Aphanicercella cassida	182	8-Jul-2002	DMS	Seweweekspoort, 5 km south of Gamkapoortdam turn-off.	Klein Swartberg	-33.390900	21.406000	Groot Swartberg
Aphanicercella cassida	150	18-Aug-2001	DMS	Malvadraai, Swartberg Pass	Groot Swartberg	-33.299600	22.050100	Groot Swartberg
Aphanicercella cassida	92	3-Jul-1995	DMS & MDP	Groendal Nature Reserve (Forest station), NW Uitenhage	Groot-Winterhoekberge	-33.714354	25.289761	Groot-Winterhoekberge

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella cassida	N/A	24-Apr-1957	B. Stuckenberg	Cathedral Peak	KwaZulu-Natal Drakensberg	-28.945390	29.202504	KwaZulu-Natal Drakensberg
Aphanicercella cassida	N/A	21-Jul-1965	B. Stuckenberg	Balgowan, KwaZulu-Natal midlands	KwaZulu-Natal midlands	-29.396870	30.037772	KwaZulu-Natal Midlands
Aphanicercella cassida	196	5-Aug-2002	DMS	Road between R323 and Herbertsdale, near Cloete's Pass	Langeberg Mountains	-33.919800	21.742100	Langeberg Mts
Aphanicercella cassida	274	15-Jun-2004	DMS	2nd stream under road on R327 to Herbertsdale (after large river), near Cloete's Pass	Langeberg Mountains	-33.917900	21.736900	Langeberg Mts
Aphanicercella cassida	N/A			Metlapetsi R, Haenertsburg	Mpumalanga Drakensberg (northern)	-23.916700	29.983300	Mpumalanga Drakensberg (northern)
Aphanicercella cassida	N/A			Magoebaskloof	Mpumalanga Drakensberg (northern)	-23.881086	30.031639	Mpumalanga Drakensberg (northern)
Aphanicercella cassida	N/A			Duiwelskloof	Mpumalanga Drakensberg (northern)	-23.695750	30.141012	Mpumalanga Drakensberg (northern)
Aphanicercella cassida	N/A	23-May-1996	H. Dallas	Sabie area – Mac Mac tributary; Lisbon River at bridge; Forest stream	Mpumalanga Drakensberg	-24.862001	30.835997	Mpumalanga Drakensberg (southern)
Aphanicercella cassida	N/A	22.06.2002	J. van Alphen-Stahl	Lisbon River	Mpumalanga Drakensberg	-24.862001	30.835997	Mpumalanga Drakensberg (southern)
Aphanicercella cassida	276	16.Jun.04	DMS	Brackenhill Waterfall, E of Knysna, stream under side road	Outeniqua Mts	-34.046000	23.163100	Outeniqua
Aphanicercella cassida	N/A	16-Apr-1933	HG Wood	Kaaimansgat, Wilderness	Outeniqua Mts	-33.942968	22.528532	Outeniqua
Aphanicercella cassida	158	20-Aug-2001	DMS	5 km after turn off on road to Bergplaas, N of Knysna	Outeniqua Mts	-33.897612	22.687992	Outeniqua
Aphanicercella cassida	242	5 August 2003	DMS	Bergplaas Forest, on road to Klipplaat, 9km from tar road	Outeniqua Mts	-33.884300	22.689300	Outeniqua
Aphanicercella cassida	159	20-Aug-2001	DMS	10 km after Bergplaas turn off on road to Kleinplaat, N of Knysna	Outeniqua Mts	-33.872275	22.687287	Outeniqua
Aphanicercella cassida	151	18-Aug-2001	DMS	34.6 km S from start of Uniondale-Knysna Rd, Prince Alfred’s Pass	Between Outeniqua and Langkloof mountains	-33.862600	23.178400	Outeniqua
Aphanicercella cassida	240	4 August 2003	DMS	Prince Alfred's Pass	Outeniqua Mts	-33.860600	23.173000	Outeniqua
Aphanicercella cassida	154	19-Aug-2001	DMS	Prince Alfred’s Pass at Thomas Bain Memorial	Outeniqua Mts	-33.860994	23.171860	Outeniqua

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella cassida	239	4 August 2003	DMS	Prince Alfred's Pass at turn-off to George (N9)	Outeniqua Mts	-33.811800	23.175000	Outeniqua
Aphanicercella cassida	238	4 August 2003	DMS	Road to George from Prince Alfred's Pass. Road crosses stream	Outeniqua Mts	-33.766000	23.005100	Outeniqua
Aphanicercella cassida	93	3-Jul-1995	DMS & MDP	Palmiet River, Grahamstown	Albany area	-33.370850	26.476938	Southern Eastern Cape Province highlands
Aphanicercella cassida	N/A			Howisons poort	Near Grahamstown	-33.350000	26.500000	Southern Eastern Cape Province highlands
Aphanicercella clavata	169	16-Jun-2002	DMS	Cecilia State Forest, near Cecilia Forest Station, Cape Peninsula	Table Mt, Cape Peninsula	-33.997800	18.425700	Cape Peninsula
Aphanicercella clavata	297	5-Jul-2007	DMS	Below Kirstenbosch, Boschenheuvel Arboretum	Cape Peninsula	-33.987460	18.437190	Cape Peninsula
Aphanicercella clavata	167	16-Jun-2002	DMS	Victoria Rd, 1 km before kamat, Slangolie Stream, Cape Peninsula	Twelve Apostles, Cape Peninsula	-33.970600	18.371300	Cape Peninsula
Aphanicercella clavata	21	21-Jun-1993	DMS	Pipe track, Twelve Apostles	Twelve Apostles, Cape Peninsula	-33.970400	18.386000	Cape Peninsula
Aphanicercella clavata	298	10-Jul-2007	DMS	Theresa Avenue, Camps Bay	Twelve Apostles, Cape Peninsula	-33.967920	18.382010	Cape Peninsula
Aphanicercella clavata	20	16-Jun-1993	DMS & MDP	Gardens, Table Mountain	Table Mt, Cape Peninsula	-33.943300	18.419400	Cape Peninsula
Aphanicercella clavata	299	15-Jul-2007	DMS	Bain's Kloof Pass, 1st stream N of Wellington	Limietberge	-33.644950	19.071020	Hottentots Holland (northern)
Aphanicercella flabellata	N/A		MDP	Glencairn; Cape Peninsula	Cape Peninsula	-34.158738	18.428162	Cape Peninsula
Aphanicercella flabellata	131	1-Jun-2000	DMS & MDP	Headwaters of Lourens River, Lourensford Farm, Somerset West	Stellenboschberg, Hottentots Holland	-34.040700	18.924300	Hottentots Holland (northern)
Aphanicercella flabellata	228	3 July 2003	DMS	Jonkershoek Nature Reserve, circular drive at hairpin bend	Jonkershoekberge	-33.993700	18.974900	Hottentots Holland (northern)
Aphanicercella flabellata	173	25-Jun-2002	DMS	Swartboskloof, Stellenbosch	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicercella flabellata	227	3 July 2003	DMS	Jonkershoek Nature Reserve, circular drive on look-out hut side	Jonkershoekberge	-33.989800	18.956900	Hottentots Holland (northern)
Aphanicercella flabellata	254	26 Sept 2003	DMS	Jonkershoek Nature Reserve, Stellenbosch, circular drive, river after Jakkalsrivier	Stellenboschberg	-33.989100	18.968400	Hottentots Holland (northern)

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella flabellata	175	25-Jun-2002	DMS	Jonkershoek Nature Reserve, homeward bound circular drive, small stream under road	Stellenboschberg	-33.979100	18.950100	Hottentots Holland (northern)
Aphanicercella flabellata	246	3 Sept 2003	DMS	Assegaaibos Nature Reserve, Stellenbosch	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)
Aphanicercella flabellata	43	8-May-1994	DMS & MDP	Karmel campsite, East side of Franschhoek Pass, 4 km E Franschhoek	Franschhoekberge	-33.917628	19.163056	Hottentots Holland (northern)
Aphanicercella flabellata	42	8-May-1994	DMS & MDP	Swiss Farm Excelsior, Franschhoek	Franschhoekberge	-33.937600	19.110900	Hottentots Holland (northern)
Aphanicercella flabellata	113	18-Aug-1997	DMS & MDP	Pniel, near Boschendal	Grootdrakensteinberge / Jonkershoekberge	-33.900000	18.950000	Hottentots Holland (northern)
Aphanicercella flabellata	29	15-Jun-1981	MDP	Du Toits Kloof Pass, 2 km after tunnel, Molenaars River	Du Toitsberge	-33.722389	19.150574	Hottentots Holland (northern)
Aphanicercella flabellata	203	4-Sep-2002	DMS	Bain's Kloof Pass, 1st stream N of Wellington	Limietberge	-33.645300	19.071500	Hottentots Holland (northern)
Aphanicercella flabellata	49	10-Jul-1994	DMS & MDP	Base of Bain's Kloof Pass, 4 km out of Wellington, crossing road	Limietberge	-33.645100	19.071500	Hottentots Holland (northern)
Aphanicercella flabellata	299	15-Jul-2007	DMS	Bain's Kloof Pass, 1st stream N of Wellington	Limietberge	-33.644950	19.071020	Hottentots Holland (northern)
Aphanicercella flabellata	50	10-Jul-1994	DMS & MDP	Gawie se Water, Bain's Kloof	Limietberge / Slanghoekberge	-33.641300	19.104100	Hottentots Holland (northern)
Aphanicercella flabellata	287	18 July 2004	DMS	Kleinboontjiesrivier, turn off R46 19 km south of Ceres Nature Reserve, Witsenberg Game Park	Witsenberg	-33.382737	19.213298	Witsenberg
Aphanicercella namaquaensis sp. n.	JC186	21-Aug-2005	J.F. Colville & A. Roberts	Damsland farm, Rooiberg Mountain, Kamiesberg, Northern Cape	Kamiesberg	-30.411312	18.106022	Kamiesberg
Aphanicercella namaquaensis sp. n.	JC189	21-Aug-2005	J.F. Colville & A. Roberts	Damsland farm, Rooiberg Mountain, Kamiesberg, Northern Cape	Kamiesberg	-30.429541	18.104700	Kamiesberg
Aphanicercella nigra	115	9-Sep-1997	MDP	Hex River, Uitsig, 17 km N Citrusdal	Cederberg	-32.445300	18.972600	Cederberg
Aphanicercella nigra	111	?	L. Prendini	Wolfberg, Cederberg	Cederberg	-32.472851	19.276274	Cederberg
Aphanicercella nigra	220	Dec-1967	P & B Stuckenberg	Otterford Forestry Station, Hankey district	Elandsberge	-33.783867	25.019421	Elandsberge
Aphanicercella nigra	N/A	1-Oct-1933	KHB	Franschhoek Pass (East side)	Wemmershoekberge / Franschhoekberge	-33.917628	19.163056	Hottentots Holland (northern)

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella nigra	92	3-Jul-1995	DMS & MDP	Groendal Nature Reserve (Forest station), NW Uitenhage	Groot-Winterhoekberge	-33.714354	25.289761	Groot-Winterhoekberge
Aphanicercella nigra	N/A			Humansdorp	Kougaberge	-34.000000	24.770000	Kougaberge
Aphanicercella nigra	241	4 August 2003	DMS	Gouna pump station, Gouna Forest, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicercella nigra	152	19-Aug-2001	DMS	Gouna pump station, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicercella nigra	158	20-Aug-2001	DMS	5 km after turn off on road to Bergplaas, N of Knysna	Outeniqua Mts	-33.897612	22.687992	Outeniqua
Aphanicercella nigra	306	8 Nov 2007	DMS	E of Tsitsikamma National Park, stream feeding into ocean rock pool en route to The Fernery on the Dolphin Trail	Tsitsikamma Mts	-34.032580	23.973730	Tsitsikamma Mts
Aphanicercella nigra	305	7 Nov 2007	DMS	Tsitsikamma National Park, Blue Duiker Trail	Tsitsikamma Mts	-34.018040	23.889230	Tsitsikamma Mts
Aphanicercella nigra	N/A	Aug-2000	Albany Museum Survey	Salt River, Nature's Valley	Tsitsikamma Mts	-33.968207	23.558304	Tsitsikamma Mts
Aphanicercella nigra	N/A	Aug-2000	Albany Museum Survey	Groot River, Nature's Valley	Tsitsikamma Mts	-33.968207	23.558304	Tsitsikamma Mts
Aphanicercella pauletteae	275	15-Jun-2004	DMS	Gouna pump station, Gouna Forest, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicercella quadrata	126	21-Sep-1999	DMS	Trib of Driehoekrivier, above Sederhoutkloof, Koerasieberg near Eikeboom, Cederberg	Koerasieberg, Cederberg	-32.475588	19.154878	Cederberg
Aphanicercella quadrata	143	2-Aug-2001	DMS	Sneeuberg, Cederberg	Cederberg	-32.427900	19.160500	Cederberg
Aphanicercella quadrata	111	?	L. Prendini	Wolfberg, Cederberg	Cederberg	-32.472851	19.276274	Cederberg
Aphanicercella quadrata	56	10-Sep-1994	MDP	Pakhuispad, 27 E Clanwilliam, near Louis Leipold's grave	Pakhuisberg, Cederberg	-32.135800	19.002500	Cederberg
Aphanicercella quadrata	195	4-Aug-2002	Julia Wakeling	Hex River Mountain & Ski Club Hut, below Milner Ridge Peak	Hex River Mts	-33.487600	19.465000	Hex River Mountains
Aphanicercella scutata	126	21-Sep-1999	DMS	Trib of Driehoekrivier, above Sederhoutkloof, Koerasieberg near Eikeboom, Cederberg	Koerasieberg, Cederberg	-32.475588	19.154878	Cederberg
Aphanicercella scutata	143	2-Aug-2001	DMS	Sneeuberg, Cederberg	Cederberg	-32.427900	19.160500	Cederberg

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella scutata	251	14 Sept 2003	DMS	Concrete bridge 11.2 km after Algeria on road to Sanddrift	Cederberg	-32.425600	19.131800	Cederberg
Aphanicercella scutata	31	11-Aug-1992	MDP	Viljoen's Pass, East side of Landdroskop, Palmiet River near source, Hottentots Holland Mts	Hottentots Holland Mts	-34.078286	19.057226	Hottentots Holland (northern)
Aphanicercella scutata	37	5-Jul-1996	DMS & MDP	Swartboskloof, Stellenbosch	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicercella scutata	N/A			Assegaaibos		-33.966464	18.926315	Hottentots Holland (northern)
Aphanicercella scutata	172	25-Jun-2002	DMS	Jonkershoek; outward bound on circular drive	Jonkershoek Mountains	-33.979800	18.945400	Hottentots Holland (northern)
Aphanicercella scutata	202	25-Aug-2002	MDP	Franschhoek Pass, Villiersdorp side (sweep)	Franschhoek Mountains	-33.973000	19.175700	Hottentots Holland (northern)
Aphanicercella scutata	229	3 July 2003	DMS	Assegaaibos Nature Reserve, Stellenbosch	Jonkershoekberge	-33.966464	18.926315	Hottentots Holland (northern)
Aphanicercella scutata	247	3 Sept 2003	DMS	Theewaters Nature Reserve, road crosses stream, Road to Franschhoek from R43)	Stettynsberge	-33.938600	19.161400	Hottentots Holland (northern)
Aphanicercella scutata	5	23-May-1993	DMS & MDP	Franschhoek Pass, Du Toit's River Bridge, 3 km N of site 4	Franschhoekberge	-33.948057	19.168624	Hottentots Holland (northern)
Aphanicercella scutata	113	18-Aug-1997	DMS & MDP	Pniel, near Boschendal	Grootdrakensteinberge / Jonkershoekberge	-33.900000	18.950000	Hottentots Holland (northern)
Aphanicercella scutata	112	21-Aug-1997	H. Dallas	Berg River	Franschhoekberge / Grootdrakensteinberge	-33.912326	19.111695	Hottentots Holland (northern)
Aphanicercella scutata	76	6-Oct-1994	G. Ractliffe	Du Toit's Kloof, Molenaars River	Du Toitsberge	-33.722389	19.150574	Hottentots Holland (northern)
Aphanicercella scutata	204	4-Sep-2002	DMS	Gawie se Water, Bain's Kloof	Limietberge / Slanghoekberge	-33.641300	19.104100	Hottentots Holland (northern)
Aphanicercella scutata	301	15-Jul-2007	DMS	Bain's Kloof, stream under where concrete rubbish bin is.	Limietberge / Slanghoekberge	-33.601820	19.110870	Hottentots Holland (northern)
Aphanicercella scutata	300	15-Jul-2007	DMS	Bain's Kloof, sharp bend with white brick wall	Limietberge / Slanghoekberge	-33.594720	19.121140	Hottentots Holland (northern)
Aphanicercella scutata	282	28 June 2004	DMS	Bain's Kloof Pass, cement wall bridge	Limietberge / Slanghoekberge	-33.592800	19.123600	Hottentots Holland (northern)

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella scutata	302	15-Jul-2007	DMS	Bain's Kloof, concrete channel.	Limietberge / Slanghoekberge	-33.592280	19.124610	Hottentots Holland (northern)
Aphanicercella scutata	280	28 June 2004	DMS	Bain's Kloof Pass, Tweede Tol	Limietberge / Slanghoekberge	-33.569600	19.138500	Hottentots Holland (northern)
Aphanicercella scutata	303	15-Jul-2007	DMS	Bain's Kloof, Steenbok Park	Limietberge / Slanghoekberge	-33.555860	19.149920	Hottentots Holland (northern)
Aphanicercella scutata	288	18 July 2004	DMS	Bain's Kloof Pass, bridge near Limietberg Nature Reserve sign	Limietberge / Slanghoekberge	-33.547000	19.163000	Hottentots Holland (northern)
Aphanicercella scutata	117	Dec-1998 ?	DMS	Fernkloof Nature Reserve, Hermanus	Kleinriviersberge	-34.399800	19.272500	Hottentots Holland (southern)
Aphanicercella scutata	180	2-Jul-2002	DMS	Fernkloof Nature Reserve, Hermanus; second entrance; below dam	Kleinriviersberge	-34.393900	19.276100	Hottentots Holland (southern)
Aphanicercella scutata	10	31-May-1993	DMS & MDP	Harold Porter Botanic Reserve, Betty's Bay	Platberg, Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicercella scutata	7	25-Aug-1994	MDP	Clarence Drive, monument site 10 km N of Rooiels	Koeelberg, Hottentots Holland Mts	-34.207200	18.833100	Hottentots Holland (southern)
Aphanicercella scutata	9	31-May-1993	DMS & MDP	Clarence Drive, 7 km E of monument	Koeelberg, Hottentots Holland Mts	-34.267993	18.850946	Hottentots Holland (southern)
Aphanicercella scutata	17	13-Jun-1993	DMS & MDP	Gydo Pass 1.5 km down Witsenberg Valley turn off	Skurweberge	-33.230911	19.312701	Skurweberge
Aphanicercella scutata	77	1-Jul-1995	DMS & MDP	26.4 km East of Caledon, 1.3 km West of Krige Station turn off	Swartberg, between Riviersonderend Mts and Kleinriviersberge	-34.206356	19.573599	Swartberg
Aphanicercella securata	202	25-Aug-2002	MDP	Franschhoek Pass, Villiersdorp side (sweep)	Franschhoek Mountains	-33.973000	19.175700	Hottentots Holland (northern)
Aphanicercella securata	44	8-May-1994	DMS & MDP	"High Noon", 7 km N of Villiersdorp, Elandsrivier	Stetteynsberge	-33.909486	19.293128	Hottentots Holland (northern)
Aphanicercella securata	257	13 August 2003	MDP	Harold Porter Botanic Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicercella securata	296	16 June 2007	DMS	Disa Kloof, Harold Porter Botanic Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.349920	18.926530	Hottentots Holland (southern)
Aphanicercella spatulata	N/A	22-May-1962	Unknown	Driefontein Bridge, Greater Berg River	Grootdrakensteinberge	-33.912326	19.111695	Hottentots Holland (northern)

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercella spatulata	280	28 June 2004	DMS	Bain's Kloof Pass, Tweede Tol	Limietberge / Slanghoekberge	-33.569600	19.138500	Hottentots Holland (northern)
Aphanicercella spatulata	303	15-Jul-2007	DMS	Bain's Kloof, Steenbok Park	Limietberge / Slanghoekberge	-33.555860	19.149920	Hottentots Holland (northern)
Aphanicercella spatulata	304	15-Jul-2007	DMS	Bain's Kloof, Bastiaanskloof River, crossing road	Limietberge / Slanghoekberge	-33.547060	19.163000	Hottentots Holland (northern)
Aphanicercella spatulata	265	4 June 2004	DMS	Bastiaanskloof, Bain's Kloof Pass	Limietberge / Slanghoekberge	-33.537700	19.144600	Hottentots Holland (northern)
Aphanicercella spatulata	10	31 May 1993	DMS & MDP	Harold Porter Nature Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicercella spatulata	N/A	20-Sep-1952	A. Harrison	Palmiet River	Southern Hottentots Holland Mts	-34.326145	18.979920	Hottentots Holland (southern)
Aphanicercopsis denticulata	297	5-Jul-2007	DMS	Below Kirstenbosch, Boschenheuvel Arboretum	Cape Peninsula	-33.987460	18.437190	Cape Peninsula
Aphanicercopsis denticulata	198	6-Aug-2002	DMS	Swartberg pass, Boegoekloof, 1886m, between Oudtshoorn and Prince Albert	Groot Swartberg	-33.357400	22.058500	Groot Swartberg
Aphanicercopsis denticulata	N/A	Aug-1929	KHB	Grootwinterhoek Mts, Tulbagh	Groot Winterhoekberge	-33.113241	19.086303	Grootwinterhoek
Aphanicercopsis denticulata	300	15-Jul-2007	DMS	Bain's Kloof, sharp bend with white brick wall	Limietberge / Slanghoekberge	-33.594720	19.121140	Hottentots Holland (northern)
Aphanicercopsis denticulata	302	15-Jul-2007	DMS	Bain's Kloof, concrete channel.	Limietberge / Slanghoekberge	-33.592280	19.124610	Hottentots Holland (northern)
Aphanicercopsis denticulata	304	15-Jul-2007	DMS	Bain's Kloof, Bastiaanskloof River, crossing road	Limietberge / Slanghoekberge	-33.547060	19.163000	Hottentots Holland (northern)
Aphanicercopsis denticulata	N/A	Jul-1932	HG Wood	Palmiet River, near Kleinmond	Hottentots Holland Mts	-34.330262	18.991217	Hottentots Holland (southern)
Aphanicercopsis denticulata	77	1-Jul-1995	DMS & MDP	26.4 km East of Caledon, 1.3 km West of Krige Station turn off	Swartberg, between Riviersonderend Mts and Kleinriviersberge	-34.206356	19.573599	Swartberg
Aphanicercopsis denticulata	N/A	Sep-1930	KHB	Nonna Kloof, Worcester	Langeberg Mts (western extremity)	-33.588971	19.561776	Langeberg
Aphanicercopsis hawaquae	270	8 June 2004	DMS	Eikeboom, 16.4 km S of Algeria, Cederberg	Cederberg	-32.454900	19.169600	Cederberg
Aphanicercopsis hawaquae	268	8 June 2004	DMS	Algeria, Cederberg. Tributary of Rondegat River alongside camp site	Cederberg	-32.374100	19.062000	Cederberg

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercopsis hawaquae	85	2-Jul-1995	DMS & MDP	Near Uitspan Drif, Meiringspoort on R29, Groot Swartberg	Groot Swartberg	-33.439479	22.559342	Groot Swartberg
Aphanicercopsis hawaquae	147	18-Aug-2001	DMS	24.7 km W of R328 on road to Calitzdorp (near Swartberg Pass)	Groot Swartberg	-33.465052	21.741220	Groot Swartberg
Aphanicercopsis hawaquae	235	3-Aug-2003	DMS	Oudemuragie Road, 14.9 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.413400	22.383000	Groot Swartberg
Aphanicercopsis hawaquae	234	3-Aug-2003	DMS	Oudemuragie Road, 11.6 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.411200	22.354100	Groot Swartberg
Aphanicercopsis hawaquae	148	18-Aug-2001	DMS	12.7 km W of R328 on road to Calitzdorp (near Swartberg Pass)	Groot Swartberg	-33.405228	21.995098	Groot Swartberg
Aphanicercopsis hawaquae	183	8-Jul-2002	DMS	Seweweekspoort, 6 km south of Gamkapoortdam turn-off, where side stream joins main stream.	Klein Swartberg	-33.394300	21.399200	Groot Swartberg
Aphanicercopsis hawaquae	N/A			Palmiet River, Elgin-Grabouw	Hottentots Holland (northern)	-34.078286	19.057226	Hottentots Holland (northern)
Aphanicercopsis hawaquae	N/A	10-Jun-1932	KHB & HG Wood	Jonkershoek, Stellenbosch	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Aphanicercopsis hawaquae	5	23-May-1993	DMS & MDP	Franschhoek Pass, Du Toit's River Bridge, 3 km N of site 4	Franschhoekberge	-33.948057	19.168624	Hottentots Holland (northern)
Aphanicercopsis hawaquae	46	24-May-1994	G. Ractliffe	Du Toit's Kloof Pass, Molenaars River, station 1A#26	Du Toitsberge, Slanghoekberge	-33.722389	19.150574	Hottentots Holland (northern)
Aphanicercopsis hawaquae	282	28 June 2004	DMS	Bain's Kloof Pass, cement wall bridge	Limietberge / Slanghoekberge	-33.592800	19.123600	Hottentots Holland (northern)
Aphanicercopsis hawaquae	230	4 July 2003	DMS	Tweede Tol, Bain's Kloof Pass	Limietberge / Slanghoekberge	-33.569600	19.138500	Hottentots Holland (northern)
Aphanicercopsis hawaquae	55	10-Jul-1994	DMS & MDP	Bain's Kloof, Steenbok Nature Park, 1 km N of Tweede Tol	Limietberge / Slanghoekberge	-33.555800	19.150000	Hottentots Holland (northern)
Aphanicercopsis hawaquae	265	4 June 2004	DMS	Bastiaanskloof, Bain's Kloof Pass	Limietberge / Slanghoekberge	-33.537700	19.144600	Hottentots Holland (northern)
Aphanicercopsis hawaquae	271	14 June 2004	DMS	Kristalkloof, 17.7 km N of Riversdale on R323	Langeberg Mountains	-33.958600	21.230400	Langeberg
Aphanicercopsis hawaquae	171	18-Jun-2002	DMS	Damsrivier, Farm "Bergheim" on R62 between Montagu and Barrydale	Langeberg Mountains	-33.932800	20.380900	Langeberg Mts
Aphanicercopsis hawaquae	196	5-Aug-2002	DMS	Road between R323 and Herbertsdale, near Cloete's Pass	Langeberg Mountains	-33.919800	21.742100	Langeberg Mts

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercopsis hawaquae	170	18-Jun-2002	DMS	Huisrivier, Ravenna Mountain Retreat, on R62 between Montagu and Barrydale	Langeberg Mountains	-33.918500	20.378800	Langeberg Mts
Aphanicercopsis hawaquae	N/A			Piketberg	Piketberg	-32.931314	18.729607	Piketberg
Aphanicercopsis hawaquae	179	2-Jul-2002	DMS	Fernkloof Nature Reserve, Hermanus; second entrance; bridge over stream near gate	Kleinriviersberge	-34.399800	19.272500	Hottentots Holland (southern)
Aphanicercopsis hawaquae	180	2-Jul-2002	DMS	Fernkloof Nature Reserve, Hermanus; second entrance; below dam	Kleinriviersberge	-34.393900	19.276100	Hottentots Holland (southern)
Aphanicercopsis hawaquae	284	6 July 2004	DMS	Harold Porter Nature Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.352300	18.927000	Hottentots Holland (southern)
Aphanicercopsis hawaquae	296	16 June 2007	DMS	Disa Kloof, Harold Porter Botanic Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.349920	18.926530	Hottentots Holland (southern)
Aphanicercopsis hawaquae	77	1-Jul-1995	DMS & MDP	26.4 km East of Caledon, 1.3 km West of Krige Station turn off	Swartberg, between Riviersonderend Mts and Kleinriviersberge	-34.206356	19.573599	Swartberg
Aphanicercopsis hawaquae	295	16 June 2007	DMS	Leopard's Kloof, Harold Porter Nature Reserve, Betty's Bay	Platberg, Southern Hottentots Holland Mts	-34.346690	18.930410	Hottentots Holland (southern)
Aphanicercopsis outeniquae	78	1-Jul-1995	DMS & MDP	Garcia's Pass, 13.5 km N of Riversdale on R323; road crosses stream, forestry road.	Langeberg Mountains	-33.985800	21.227300	Langeberg
Aphanicercopsis outeniquae	102	9-Mar-1996	DMS	Grootvadersbosch River, Grootvadersbosch Nature Reserve, Langeberg Mountains	Langeberg Mountains	-33.985891	20.823277	Langeberg Mts
Aphanicercopsis outeniquae	152	19-Aug-2001	DMS	Gouna pump station, Knysna	Outeniqua Mts	-33.991500	23.040000	Outeniqua
Aphanicercopsis outeniquae	275	15-Jun-2004	DMS	Gouna pump station, Gouna Forest, Knysna	Outeniqua Mts	-33.990700	23.040700	Outeniqua
Aphanicercopsis outeniquae	103	2-Mar-1996	DMS	Terblans Walk, Gouna Forest, Knysna	Outeniqua Mts	-33.947500	23.141100	Outeniqua
Aphanicercopsis outeniquae	68	3-Dec-1994	DMS & MDP	Ysternek Nature Reserve, Prince Alfred's Pass, Knysna area	Outeniqua Mts	-33.933325	23.163417	Outeniqua
Aphanicercopsis outeniquae	237	4 August 2003	DMS	Kom se Pad, Gouna Forest, N of Knysna	Outeniqua Mts	-33.947500	23.141100	Outeniqua
Aphanicercopsis outeniquae	156	20-Aug-2001	DMS	Malgas River, near George	Outeniqua Mts	-33.958298	22.311839	Outeniqua
Aphanicercopsis outeniquae	243	5 August 2003	DMS	Keur River Bridge, Montagu Pass, N of George	Outeniqua Mts	-33.907175	22.418134	Outeniqua

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Aphanicercopsis outeniquae	N/A	Feb-1932	KHB	Robinson Pass, George	Outeniqua Mts	-33.880607	22.028591	Outeniqua
Aphanicercopsis outeniquae	N/A	Apr-1933	HG Wood	Montagu Pass, George	Outeniqua Mts	-33.907175	22.418134	Outeniqua
Aphanicercopsis outeniquae	N/A	Nov-2000	Albany Museum Survey	Salt River, Nature's Valley	Tsitsikamma Mts	-33.968207	23.558304	Tsitsikamma Mts
Aphanicercopsis outeniquae	N/A	7-Dec-1979	J. Londt	Tsitsikamma Coastal Park, Storms River	Tsitsikamma Mts	-33.958504	23.924402	Tsitsikamma Mts
Aphanicercopsis tabularis	11	5-Jun-1993	DMS & MDP	Steenberg Peak, Silvermine Nature Reserve	Steenberg Peak, Cape Peninsula	-34.100100	18.429300	Cape Peninsula
Aphanicercopsis tabularis	22	21-Jun-1993	DMS	Slangolie Ravine, Twelve Apostles, Cape Peninsula	Twelve Apostles, Cape Peninsula	-33.977700	18.385100	Cape Peninsula
Aphanicercopsis tabularis	177	30-Jun-2002	DMS	Pipe Track, where pipe visible & crosses stream (before Woody Ravine), Cape Peninsula	Twelve Apostles, Cape Peninsula	-33.970400	18.386000	Cape Peninsula
Aphanicercopsis tabularis	298	10-Jul-2007	DMS	Theresa Avenue, Camps Bay	Twelve Apostles, Cape Peninsula	-33.967920	18.382010	Cape Peninsula
Balinskycercella fontium	N/A	25-Jan-1954	B. Balinsky	Tugela River, Mont-aux-Sources, KwaZulu Natal	KwaZulu-Natal Drakensberg	-28.745700	28.913500	KwaZulu-Natal Drakensberg
Balinskycercella gudu	162	13-Mar-2002	DMS	Gudu Falls, Royal Natal National Park, KWZ/Natal Drakensberg	KwaZulu-Natal Drakensberg	-28.684997	28.930619	KwaZulu-Natal Drakensberg
Balinskycercella gudu	161	14 & 17-Mar-2002	DMS	Tugela Gorge, Royal Natal National Park, KWZ/Natal Drakensberg	KwaZulu-Natal Drakensberg	-28.745700	28.913500	KwaZulu-Natal Drakensberg
Balinskycercella gudu	N/A	Mar-1959	B. Stuckenberg	Indumeni River, Cathedral Peak district, KwaZulu-Natal Drakensberg	KwaZulu-Natal Drakensberg	-28.945390	29.202504	KwaZulu-Natal Drakensberg
Balinskycercella gudu	N/A	25-Jan-1990	L. Minter	12 km S of Thabang, Lesotho	Lesotho Drakensberg	-29.300000	29.033300	Lesotho Drakensberg
Balinskycercella gudu	N/A	21-Jan-1990	L. Minter	Oxbow, Lesotho	Lesotho Drakensberg	-28.766700	28.600000	Lesotho Drakensberg
Balinskycercella gudu	72	7-Jan-1995	DMS	Tributary at Qiloane Falls, Makheleng River, Maluti Mts, Lesotho	Maluti Mts	-29.400000	27.916700	Maluti Mts
Balinskycercella tugelae	N/A	6-Jan-1958	B. Balinsky	Streams near Crystal Falls, Champagne Castle	KwaZulu-Natal Drakensberg	-29.083300	29.333300	KwaZulu-Natal Drakensberg
Balinskycercella tugelae	161	14 & 17-Mar-2002	DMS	Tugela Gorge, Royal Natal National Park, KWZ/Natal Drakensberg	KwaZulu-Natal Drakensberg	-28.745700	28.913500	KwaZulu-Natal Drakensberg

Appendix 4.1. Continued**.**

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Balinskycercella tugelae	N/A	28-Dec-1958	B. Balinsky	Gudu Falls, Mont-aux-Sources, Royal Natal National Park	KwaZulu-Natal Drakensberg	-28.683300	28.733300	KwaZulu-Natal Drakensberg
Balinskycercella tugelae	N/A	25-Nov-1988	L. Minter	Mangaung River, Lesotho	Lesotho Drakensberg	-29.330000	29.130000	Lesotho Drakensberg
Balinskycercella tugelae	N/A	25-Jan-1990	L. Minter	12 km S of Thabang, Lesotho	Lesotho Drakensberg	-29.300000	29.033300	Lesotho Drakensberg
Balinskycercella tugelae	N/A	21-Jan-1990	L. Minter	Oxbow, Lesotho	Lesotho Drakensberg	-28.766700	28.600000	Lesotho Drakensberg
Desmonemoura brevis	65	4-Dec-1994	DMS & MDP	N toward Kango Caves from Oudtshoorn. 10km E. Kangorivier (Road to Vergelegen).	Groot Swartberg	-33.501211	22.356122	Groot Swartberg
Desmonemoura brevis	218	26-Nov-2002	DMS	Oudemuragie Road, 11.6 km E off R328 from "Rust en Vrede" signboard	Groot Swartberg	-33.411200	22.354100	Groot Swartberg
Desmonemoura brevis	217	26-Nov-2002	DMS	Oudemuragie Road, "Rust en Vrede" waterfall.	Groot Swartberg	-33.391800	22.355900	Groot Swartberg
Desmonemoura brevis	61	4-Dec-1994	DMS & MDP	Swartberg Pass; 12km on road to Die Hel	Groot Swartberg	-33.341038	21.862411	Groot Swartberg
Desmonemoura brevis	64	4-Dec-1994	DMS & MDP	Swartberg Pass; Tweede River, near Prince Albert	Groot Swartberg	-33.300000	22.050000	Groot Swartberg
Desmonemoura brevis	N/A	4-Dec-1979	J. Illies	Bergplaas	Outeniqua	-33.883300	22.666700	Outeniqua
Desmonemoura brevis	N/A	4-Dec-1979	J.Illies	Between Bergplaas & Kleinplaat, near George	Outeniqua	-33.884300	22.689300	Outeniqua
Desmonemoura pulchellum	138	31-Oct-2000	DMS	Algeria, Cederberg	Cederberg	-32.374100	19.062000	Cederberg
Desmonemoura pulchellum	N/A	11.1916& 1932	KHB / KHB & HG Wood	Grootwinterhoek, Tulbagh	Groot Winterhoekberge	-33.113241	19.086303	Grootwinterhoek
Desmonemoura pulchellum	N/A	5-Oct-1931	A.C. Harrison	Hex River, Worcester	Hex River Mts ?	-33.556320	19.512179	Hex River Mountains
Desmonemoura pulchellum	35	24-Nov-1986	MDP	Swartboskloof, Stellenbosch	Stellenboschberg	-33.991700	18.954200	Hottentots Holland (northern)
Desmonemoura pulchellum	N/A	Oct-1933	KHB & HG Wood	Groot Drakenstein (Stellenbosch)	Groot Drakenstein	-33.933300	18.966700	Hottentots Holland (northern)
Desmonemoura pulchellum	59	5-Nov-1994	K. Snaddon	Upper Berg, Franschhoek	Franschhoekberge / Groot Drakensteinberge ??	-33.912326	19.111695	Hottentots Holland (northern)

Appendix 4.1. Continued.

Species	Coll. #	Date	Collector	Locality	Mountain range	Latitude	Longitude	Mountain range group
Desmonemoura pulchellum	215	14-Nov-2002	DMS	Klip River, trib of Molenaars River, Du Toit's Kloof Pass, 7.5 km N of old tunnel	Dutoitsberge	-33.722100	19.182100	Hottentots Holland (northern)
Desmonemoura pulchellum	N/A	Oct-1925	KHB	Tradouw Pass	Langeberg	-33.982738	20.708599	Langeberg
Desmonemoura pulchellum	219	27-Nov-2002	DMS	Keur River Bridge, Montagu Pass, N of George	Outeniqua Mts	-33.907175	22.418134	Outeniqua
Desmonemoura pulchellum	N/A	Dec-1931 & Jan-1933 / Jan-1934	HG Wood / KHB & HG Wood	Oudebosch, Riviersonderend Mts.	Riviersonderend Mts	-34.082000	19.829100	Riviersonderend Mts

Appendix 4.2. Southern African Notonemouridae morphological character descriptions. Where reference is made to figures in the thesis, this is given in separate parentheses to those of illustrations in other works).

Larva

1. Hairs on proxomedial aspect of larval antennae: not longer than other antennal hairs = 0; longer than other antennal hairs = 1 (Fig. 2.10).
2. Group of thick setal tufts positioned about one-third antennal length from base of larval antennae: absent = 0; present = 1 (Fig. 2.5) (Illies 1980, Fig. 2).
3. Complete whorls of long setae on all abdominal segments, save for mid-ventrally: absent = 0; present = 1 (Figs 2.3E, 2.4).
4. Pronotum appears rounded to oval in dorsal view, with rounded corners and equal or subequal width and length: no = 0; yes = 1 (Fig. 2.20).

Adult male

5. Male tergite 9 bears posteriorly directed dorsal lobate processes: absent = 0; present = 1 (Figs 2.11, 2.14, 2.21, 2.26A,F, 3.3A-AA) (Barnard 1934, Figs 7a-f, 9a,b, 10a,b, 11a,b, 12a,b, 13a,b, 21a,b).
6. Male tergite 9 posteriorly directed dorsal lobate process: bilobate process = 0 (Figs 2.11, 2.14, 2.26A, 3.3A-AA) (Barnard 1934, Figs 7a-f, 9a,b, 10a,b, 11a,b, 12a,b, 13a,b); two separate processes with widely separated bases = 1 (Figs 2.21, 2.26F) (Barnard 1934, Fig. 21a,b).
7. Distal margins of bilobate process of male tergite 9: smooth = 0 (Fig. 2.11) (Barnard 1934, Figs 9b, 10b, 11b, 12b); ridge of many spinules = 1 (Figs 2.26A, 3.3A-AA) (Barnard 1934, Fig. 7c-e); ridge of few large spinules = 2 (Fig. 2.14). Character state 2 which is an autapomorphy of *Aphanicerca chanae* in this analysis would also apply to *A. tereta*, which was not included in the analysis.
8. Male tergite 9 bears posteriorly directed stout sclerotized spines on posterior margin: absent = 0; present = 1 (Fig. 2.26C) (Balinsky 1956, Fig. 1b; Balinsky 1967, Fig. 1C). Character state 1 is found in the two *Afronemoura* species included in the study, as well as in *A. stuckenbergi* (Fig. 2.6) which was excluded, i.e. it is a synapomorphy for the *Afronemoura* species.
9. Male tergite 9 posteriorly directed stout sclerotized spines on posterior margin: one = 0 (Fig. 2.26C) (Balinsky 1967, Fig. 1C); two = 1 (Balinsky 1956, Fig. 1b). In *A. stuckenbergi*, which was excluded from this study, there are two spines (Fig. 2.6), but topologically the tergite and spines are more similar to *A. spinulata* (character state 0 with one spine). Therefore, future studies which include *A. stuckenbergi* would require this character to be reformulated to include structural detail rather than simply the number of spines.

10. Male sternite 9: short = 0 (Barnard 1934, Figs 15a, 17a, 20c; Balinsky 1956, Figs 2a,c, 3a,c); broadly elongated = 1 (Balinsky 1956, Fig. 1a,c; Balinsky 1967, Fig. 1A,B; Barnard 1934, Figs 7a,b, 9a, 10a, 11a, 12a, 13a, 14a, 21a); short but with a highly elongated upcurved ventral process = 2.
11. Male epiproct denticulation: absent = 0; present = 1 (Barnard 1934, Figs 7a,b, 9a, 10a, 11a,c, 12a,d, 13a, 14a,e,f,i,j,m,n,q,r, 21a,b).
12. Position of male epiproct denticulation: on flat anterior (ventral) surface of lateral margins = 0 (Barnard 1934, Figs 9a, 21a); on sclerotized very slight convexity of anterior (ventral) margin = 1 (Barnard 1934, Figs 11a,c, 12a); on sclerotized pronounced convexity of anterior (ventral) margin = 2 (Barnard 1934, Figs 7a,b, 10a); protruding laterally on lateral margins = 3 (Barnard 1934, Fig. 14e,i,m,q).
13. Male epiproct sclerotization in taxa in which epiproct denticulation is situated on slight or pronounced convexity: entirely sclerotized = 0; not or lightly sclerotized between lateral margins = 1.
14. Male epiproct base width in taxa where epiproct denticulation is situated on a slight or pronounced convexity: narrow = 0 (Barnard 1934, Figs 9b, 11b, 12d); very broad = 1.
15. Male epiproct apically incised: no = 0; yes = 1 (Fig. 2.18I1-K1) (Barnard 1934, Figs 18a, 19b, 20a).
16. Apex of male epiproct has minute ventral ventrally-directed projection: no = 0; yes = 1.
17. Base of male epiproct: anteriorly directed subtriangular sclerites as continuation of epiproct lateral margins = 0; posteriorly directed semi-circular sclerites as continuation of epiproct lateral margins = 1; curved sclerites as continuations of epiproct lateral sclerites = 2; curved sclerites as continuations of lateral sclerites - one anterad and one mediad = 3; very small suboval plate = 4.
18. Male pleurites 10 elongated posterad: no = 0; yes = 1 (Figs 2.18A1-K1, 2.21, 2.26D-F); (Barnard 1934, Figs 15b, 16a, 21a,b; Balinsky 1956, Figs 2b,c, 3b,c).
19. Male pleurites 10, if elongated: short elongation of posterodorsal margin without articulation = 0 (Fig. 2.26E) (Balinsky 1956, Figs 2b,c, 3b,c); arm-like extension of posterodorsal margin with articulation = 1 (Figs 2.18A1-K1, 2.26D) (Barnard 1934, Figs 15b, 16a); long appendage with circular base = 2 (Figs 2.21, 2.26F) (Barnard 1934, Fig. 21a,b).
20. Male pleurites 10: large and mobile relative to lateral dorsal plates = 0; reduced and fused over whole width to lateral dorsal plates = 1; attached anteriorly to lateral dorsal plates by a thin neck = 2; fused to each other anterior to the lateral dorsal plates = 3.
21. Median dorsal plate of male tergite 10: fused to lateral dorsal plates = 0 (Barnard 1934, Figs 9b, 11b, 12b, 21c); subtriangular (crescentic) = 1 (Fig. 2.18A2-K2) (Barnard 1934, Figs 15c,

16b, 17b, 19a, 20b); broad anteriorly and deeply excised posteriorly = 2 (Barnard 1934, Fig. 14d,h,i,p); membranous = 3.

22. Median dorsal plate of male tergite 10 elongated anterad with a prominent hook: no = 0; yes = 1 (Fig. 2.26E) (Balinsky 1956, Figs 2b,c, 3b,c). This character is a key synapomorphy for *Balinskycercella* species.
23. Male tergite 10 lateral dorsal plates arise from posterior margin of tergite: no = 0; yes = 1.
24. Male tergite 10 lateral dorsal plates sclerotized posteromedially with a spine-like acute apex: no = 0; yes = 1.
25. Male tergite 10 lateral dorsal plates elongated anteriorly: no = 0; yes = 1 (Fig. 2.11) (Barnard 1934, Figs 9a, 11a, 12a).
26. Lateral supporting sclerite of male paraproct: long, thin sclerite/s = 0 (Fig. 2.18A3-K3) (Barnard 1934, Figs 14g,k,o,s, 15e, 16e, 17d, 19c; Balinsky 1956, Figs 2a,c, 3a,c); robust, short, broad plate = 1 (Figs 2.7, 2.12, 2.15) (Barnard 1934, Figs 7g,h, 9c, 10c 11d, 12c); elongated broad plate abruptly narrowed apical quarter = 2 (Fig. 2.22) (Barnard 1934, Fig. 21d).
27. Male paraprocts that contain long thin lateral supporting sclerites: lightly but completely sclerotized sclerite with heavily sclerotized lateral and medial margins = 0 (Balinsky 1956, Figs 2a,c, 3a,c); largely membranous with one or more heavily sclerotized thin sclerites = 1 (Fig. 2.18A3-K3) (Barnard 1934, Figs 14g,k,o,s, 15e, 16e, 17d, 19c); sclerite very thin and lies on lateral margin = 2 (Barnard 1934, Fig. 14g,k,o,s).
28. Medial supporting sclerite of male paraproct (= arch process): broad base, long, tapers to thin apex which is fused with or terminates near lateral sclerite = 0 (Barnard 1934, Fig. 14g,k,o,s); flat subrectangular plate, parallel to and shorter than lateral sclerite = 1 (Figs 2.12, 2.15) (Barnard 1934, Figs 7g,h, 9c, 10c 11d, 12c); narrow and flattened, tapers abruptly to acute apex = 2 (Fig. 2.18D3,E3,G3,H3,J3,K3) (Barnard 1934, Figs 16e, 17d, 19c); apically broadened = 3 (Fig. 2.18B3); superficial part apically broad and rounded; deep part thin apically acute = 4 (Fig. 2.18A3,C3,F3); thin, short, apically acute = 5 (Fig. 2.18I3); horseshoe shape = 6; acicular (needle-shaped) = 7 (Fig. 2.22); thin, narrow, short, apically rounded with tiny acute apical tip = 8.
29. Basal supporting process of male paraproct: absent = 0; present (may be vestigial or fused to arch process) = 1 (Fig. 2.18A3-H3,J3) (Barnard 1934, Figs 15e, 16e).
30. Basal supporting process of male paraproct: vestigial (a slight bump) = 0 (Fig. 2.18F3); short and apically rounded = 1 (Fig. 2.18A3,C3,E3,H3) (Barnard 1934, Fig. 16e); long and apically broadened = 2 (Fig. 2.18B3,D3) (Barnard 1934, Fig. 15e); fused to, forming thickened base of paraproct lateral sclerite = 3 (Fig. 2.18G3,J3).
31. Male paraproct glands: absent = 0; present = 1 (Figs 4.1-4.2). These are tubular structures which are called “glands” here but their histological structure and function are unknown.

There are two glands, each one opening into the membranous tissue at the base of each paraproct. These structures have not been described previously in Plecoptera (P. Zwick, personal communication). This character was weighted double in the *AP* analysis.

32. Male paraproct glands: short and thick with a single loop = 0 (Fig. 4.2); long, thin and convoluted = 1 (Fig. 4.1). This character was weighted double in the *AP* analysis.
33. Male paraproct membranous tip: tapers to acute apex (deviated laterad in *Aphanicercopsis outeniquae*) = 0; apically not acute = 1.
34. Male paraproct membranous apex folded over: yes = 0; no = 1.
35. Male paraproct length: long = 0; short = 1.
36. Accessory gland of seminal vesicle: absent = 0; present = 1 (Figs 4.1-4.2).This is a bilateral thin tubular structure, often difficult to see, which lies longitudinally and dorsally or laterally against and adhered to each half of the seminal vesicle, and appears to be a blind ending hollow tubular structure opening into and near the base of the seminal vesicle. This character was weighted double in the *AP* analysis.
37. Accessory gland of seminal vesicle: extends entire length of seminal vesicle = 0; shorter than seminal vesicle = 1 (Figs 4.1-4.2).

Adult female

38. Sternite (subgenital plate) bearing female genital pore: 7 = 0 (Figs 2.19A-K, 2.27D,E) (Barnard 1934, Figs 15f-l, 16f, 17e, 18b-d, 19d, 20d; Balinsky 1956, Figs 2d, 3d); 8 = 1 (Figs 2.13, 2.16, 2.23, 2.27A-C,F, 3.5A-V) (Barnard 1934, Figs 7j-n, 9d,e, 10d, 11e,f, 14b,c, 21e,f; Balinsky 1956, Fig. 1d,e; Balinsky 1967, Fig. 1D,E). This character was weighted double in the *AP* analysis.
39. Female subgenital plate produced caudad to the attachment to the membranous part of the sternite: no = 0; yes = 1.
40. Elongated female subgenital plates that are produced caudad to the attachment to the sternite: short = 0 (Fig. 3.5A-V) (Barnard 1934, Fig. 7j-n); broad median elongation = 1 (Figs 2.13, 2.16) (Barnard 1934, Fig. 9d,e, 10d, 11e,f, 14b,c; Balinsky 1956, Fig. 1d,e; Balinsky 1967, Fig. 1d,e); broad median incision = 2 (Fig. 2.23) (Barnard 1934, Fig. 21e,f); short, with median posterior margin fused to elongated soft ovipositor = 3 (Fig. 2.9); highly elongated ovipositor = 4.
41. Female paraprocts (subanal plates): short and not extending beyond cerci = 0 (Figs 2.19A-K, 2.23, 2.27D-F) (Barnard 1934, Figs 15g, 21e,f; Balinsky 1956, Figs 2d, 3d); elongated, extending beyond cerci = 1 (Fig. 2.27A-C) (Barnard 1934, Figs 7i,k, 9e, 11f, 14b; Balinsky 1956, Fig. 1d; Balinsky 1967, Fig. 1E).
42. Paired spermathecae with ducts opening into oviducts and not into the common oviduct or genital chamber: absent = 0; present = 1 (Fig. 4.3). They are globoid and white in colour

with each one situated at the base of the oviduct overlying posterior segment 7 and anterior segment 8, connected by a narrow duct to the oviduct just before it dilates prior to uniting with the contralateral oviduct into the common oviduct or genital chamber. Although it is not yet conclusive that these structures are not found in *A. outeniquae*, they were not found in spite of numerous dissections. Because their function is not yet certain, and the New Zealand notonemourid outgroup *Notonemoura* has a single spermatheca which opens into the genital chamber, *Notonemoura* was coded "0". This character was double weighted in the *AP* analysis.

43. Sternite 7 of female with swelling at posterior margin: absent = 0; present = 1 (Fig. 2.23) (Barnard 1934, Fig. 21e,f).

Adult general

44. Ventral abdominal nerve cord: the sixth abdominal ganglion comprises fusion of posterior ganglia = 0; the seventh abdominal ganglion comprises fusion of posterior ganglia = 1 (Fig. 4.3). This character was weighted double in the *AP* analysis.
45. Colouration of pronotum: brown = 0; cream = 1. State 1 is a synapomorphy of *Desmonemoura* species.
46. Adult setation of abdominal tergites: fine clothing hairs = 0; numerous thicker longer hairs in addition to fine clothing hairs = 1.
47. Banded wing pattern: absent = 0; present = 1 (Tillyard, 1931, Fig. 10). State 1 is a synapomorphy of *Desmonemoura* species.
48. Large and very obvious clear patch on forewings: absent = 0; present = 1. State 1 is a synapomorphy of *Aphanicerca* species.

Appendix 4.3. Data matrix of states for 48 morphological characters of 40 ingroup species of all six genera. Characters coded as '-' are inapplicable. *Notonemoura latipennis* is the outgroup.

	1 2 3 4 5 6 7 8 9 0	1 1 1 1 1 1 1 1 1 2 1 2 3 4 5 6 7 8 9 0	2 2 2 2 2 2 2 2 2 3 1 2 3 4 5 6 7 8 9 0	3 3 3 3 3 3 3 3 3 4 1 2 3 4 5 6 7 8 9 0	4 4 4 4 4 4 4 4 4 1 2 3 4 5 6 7 8
Afronemoura amatolae	0 1 0 0 0 - - 1 1 1	0 - - - 0 0 0 0 - 2	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 0
Afronemoura spinulata	0 1 0 0 0 - - 1 0 1	0 - - - 0 0 0 0 - 2	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 0
Aphanicerca austrocapensis sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca bicornis	1 0 0 0 1 0 0 0 - 1	1 1 1 0 0 0 0 0 0 - 3	0 0 1 0 1 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 1
Aphanicerca bovina	1 0 0 0 1 0 0 0 - 1	1 1 1 0 0 0 0 0 0 - 3	0 0 1 0 1 1 - 1 0 -	1 1 1 1 1 1 1 1 1 3	1 0 0 0 0 0 0 0 1
Aphanicerca breviloba sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca brevispina sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca capensis	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca cederbergensis sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca chanae	1 0 0 0 1 0 2 0 - 1	1 1 1 1 0 0 0 0 0 - 3	0 0 1 0 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 1
Aphanicerca gnua	1 0 0 0 1 0 0 0 - 1	1 1 1 1 0 0 0 0 0 - 3	0 0 1 0 1 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 1
Aphanicerca incisura sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca longiloba sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca lyrata	1 0 0 0 1 0 0 0 - 1	1 2 1 0 0 0 0 0 0 - 3	0 0 1 0 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 1
Aphanicerca mclellani sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca pickeri sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca swartbergensis sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca uncinata	1 0 0 0 1 0 0 0 - 1	1 0 1 0 0 0 0 0 0 - 3	0 0 1 0 1 1 - 1 0 -	1 1 1 1 1 1 1 1 1 1	1 0 0 0 0 0 0 0 1
Aphanicerca witsenbergensis sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicerca zwicki sp. n.	1 0 0 0 1 0 1 0 - 1	1 2 0 0 0 0 0 0 0 - 3	0 0 1 1 0 1 - 1 0 -	1 1 1 1 1 1 1 1 1 0	1 0 0 0 0 0 0 0 1
Aphanicercella barnardi	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 3 1 2	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella bifurcata	0 0 0 0 0 - - 0 - 0	0 - - - 1 0 1 1 1 0	1 0 0 0 0 0 1 5 0 -	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella bullata	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 2 1 1	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella cassida	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 2 1 3	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella clavata	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 2 1 2	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella flabellata	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 4 1 0	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella nigra	0 0 0 0 0 - - 0 - 0	0 - - - 1 0 1 1 1 0	1 0 0 0 0 0 1 2 0 -	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella pauletteae sp. n.	0 0 0 0 0 - - 0 - 0	0 - - - 0 0 1 1 1 0	1 0 0 0 0 0 1 8 1 3	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella quadrata	0 0 0 0 0 - - 0 - 0	0 - - - 1 0 1 1 1 0	1 0 0 0 0 0 1 2 1 3	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella scutata	0 0 0 0 0 - - 0 - 0	0 - - - 0 0 1 1 1 0	1 0 0 0 0 0 1 2 1 1	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella securata	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 4 1 1	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercella spatulata	0 0 0 0 0 - - 0 - 0	0 - - - 0 1 1 1 1 0	1 0 0 0 0 0 1 4 1 1	1 0 0 0 0 1 0 0 0 -	0 0 0 1 0 0 0 0 0
Aphanicercopsis denticulata	0 0 0 1 0 - - 0 - 1	1 3 - - 0 0 2 0 - 1	2 0 0 0 0 0 2 0 0 -	0 - 0 1 0 0 - 1 1 1	1 1 0 1 0 0 0 0 0
Aphanicercopsis hawaquae	0 0 0 1 0 - - 0 - 1	1 3 - - 0 0 2 0 - 1	2 0 0 0 0 0 2 0 0 -	0 - 0 1 0 0 - 1 1 1	1 1 0 1 0 0 0 0 0
Aphanicercopsis outeniquae	0 0 0 1 0 - - 0 - 1	1 3 - - 0 0 2 0 - 1	2 0 0 0 0 0 2 0 0 -	0 - 0 1 0 0 - 1 1 1	1 0 0 1 0 0 0 0 0
Aphanicercopsis tabularis	0 0 0 1 0 - - 0 - 1	1 3 - - 0 0 2 0 - 1	2 0 0 0 0 0 2 0 0 -	0 - 0 1 0 0 - 1 1 1	1 1 0 1 0 0 0 0 0
Balinskycercella gudu	0 0 1 0 0 - - 0 - 0	0 - - - 0 0 3 1 0 0	1 1 0 0 0 0 0 0 6 0 -	1 0 0 0 0 1 1 0 0 -	0 0 0 1 0 0 0 0 0
Balinskycercella tugelae	0 0 1 0 0 - - 0 - 0	0 - - - 0 0 3 1 0 0	1 1 0 0 0 0 0 0 6 0 -	1 0 0 0 0 1 1 0 0 -	0 0 0 1 0 0 0 0 0
Desmonemoura brevis	0 0 0 0 1 1 - 0 - 1	1 0 - - 0 0 0 1 2 2	0 0 0 0 0 2 - 7 0 -	1 1 0 1 0 1 1 1 1 2	0 0 1 0 1 1 1 1 0
Desmonemoura pulchellum	0 0 0 0 1 1 - 0 - 1	1 0 - - 0 0 0 1 2 2	0 0 0 0 0 2 - 7 0 -	1 1 0 1 0 1 1 1 1 2	0 0 1 0 1 1 1 1 0
Notonemoura latipennis	0 0 0 0 0 - - 0 - 2	0 - - - 0 0 4 - - -	3 0 0 0 0 - - 7 0 -	0 - 0 1 1 0 - 1 1 4	0 0 0 1 0 0 0 0 0

Appendix 4.4. Character consistency (Ci) and retention indices (Ri), and plesiomorphic characters states. Character state polarity under equal (EW) and *a priori* (*AP*) weighted unambiguous (UNAMB) and accelerated transformation (ACCTRAN) optimizations was obtained as a result of the parsimony analysis. A "?" indicates ambiguity; uninf = uninformative character. Figures in bold font highlight the increased consistency index (Ci) and retention index (Ri) from EW to *AP*.

					Plesiomorphic character states			
	Ci	Ci	Ri	Ri	EW	EW	*AP*	*AP*
Character	EW	AP	EW	AP	UNAMB	ACCTRAN	UNAMB	ACCTRAN
1	100	100	100	100	0	0	0	0
2	100	100	100	100	0	0	0	0
3	100	100	100	100	0	0	0	0
4	100	100	100	100	0	0	0	0
5	50	50	94	94	0	0	0	0
6	100	100	100	100	?	0	?	0
7	100	100	100	100	0	0	0	0
8	100	100	100	100	0	0	0	0
9	uninf	uninf	uninf	uninf	?	0	?	0
10	100	100	100	100	?	1	?	1
11	33	33	87	87	?	0	?	0
12	100	100	100	100	0	0	?	0
13	100	100	100	100	1	1	1	1
14	100	100	100	100	0	0	0	0
15	33	33	0	0	0	0	0	0
16	20	20	33	33	0	0	0	0
17	100	100	100	100	?	0	?	0
18	50	50	93	93	?	0	?	0
19	100	100	100	100	?	0	?	0
20	100	100	100	100	2	2	?	0
21	100	100	100	100	?	0	?	0
22	100	100	100	100	0	0	0	0
23	100	100	100	100	0	0	0	0
24	50	50	92	92	0	0	0	0
25	50	50	66	66	0	0	0	0
26	100	100	100	100	0	0	0	0
27	100	100	100	100	?	0	?	0
28	100	100	100	100	7	7	7	7
29	33	33	77	77	0	0	0	0
30	50	50	0	0	?	1	?	1
31	50	**100**	75	**100**	?	0	0	0
32	100	100	100	100	1	1	?	0
33	100	100	100	100	0	0	0	0
34	100	100	100	100	1	1	1	1
35	50	50	94	94	?	0	?	0
36	50	**100**	75	**100**	?	0	0	0
37	100	100	100	100	1	1	1	1
38	100	100	100	100	1	1	1	1
39	100	100	100	100	1	1	1	1
40	100	100	100	100	?	1	?	1
41	50	50	93	93	0	0	0	0
42	100	100	100	100	0	0	0	0
43	100	100	100	100	0	0	0	0
44	50	**100**	94	**100**	1	1	1	1
45	100	100	100	100	0	0	0	0
46	100	100	100	100	0	0	0	0
47	100	100	100	100	0	0	0	0
48	100	100	100	100	0	0	0	0

Appendix 4.5. Uncorrected p-distances between the 102 local notonemourid individuals of 39 species and one outgroup taxon sampled. The code to the left of the taxon name is the specimen field code. The exact localities are provided in Table 4.2. To calculate percentage difference, multiply the value by 100.

	1	2	3	4	5	6	7	8	9	10	11
1 IM1 *Notonemoura latipennis*	-										
2 CC8 *A. clavata*	0.20287	-									
3 CC9 *A. clavata*	0.19928	0.00539	-								
4 JJ3 *A. denticulata*	0.20826	0.16517	0.16697	-							
5 JJ4 *A. denticulata*	0.20826	0.16517	0.16697	0.00539	-						
6 JJ5 *A. denticulata*	0.21005	0.16517	0.16697	0.00539	0.00718	-					
7 MM4 *A. tabularis*	0.20287	0.15978	0.16338	0.07720	0.07540	0.07720	-				
8 EE6 *A. nigra*	0.22621	0.14004	0.14183	0.17056	0.16876	0.16876	0.17415	-			
9 EE7 *A. nigra*	0.22621	0.13645	0.13824	0.17056	0.16876	0.16876	0.17594	0.00898	-		
10 L4a *A. mclellani*	0.19210	0.16158	0.16338	0.17415	0.17594	0.17594	0.17594	0.17594	0.17235	-	
11 G2 *A. witsenbergensis*	0.19210	0.16158	0.16338	0.17415	0.17594	0.17594	0.17594	0.17594	0.17235	0.00000	-
12 I3 *A. longiloba*	0.19210	0.16158	0.16338	0.17415	0.17594	0.17594	0.17594	0.17594	0.17235	0.00000	0.00000
13 L3 *A. mclellani*	0.19210	0.16158	0.16338	0.17415	0.17594	0.17594	0.17594	0.17594	0.17235	0.00000	0.00000
14 G1 *A. witsenbergensis*	0.19210	0.16158	0.16338	0.17415	0.17594	0.17594	0.17594	0.17594	0.17235	0.00000	0.00000
15 P4 *A. incisura*	0.19210	0.16517	0.16697	0.17415	0.17594	0.17594	0.17594	0.17415	0.17056	0.00539	0.00539
16 P1 *A. incisura*	0.19210	0.16338	0.16517	0.17235	0.17415	0.17415	0.17415	0.17415	0.17056	0.00359	0.00359
17 P5 *A. incisura*	0.19390	0.16338	0.16517	0.17235	0.17415	0.17415	0.17415	0.17415	0.17056	0.00180	0.00180
18 I2 *A. longiloba*	0.19390	0.16158	0.16338	0.17235	0.17415	0.17415	0.17415	0.17594	0.17235	0.00180	0.00180
19 I1 *A. longiloba*	0.19569	0.16158	0.16338	0.17415	0.17594	0.17594	0.17953	0.17235	0.16876	0.00359	0.00359
20 J2 *A. swartbergensis*	0.19390	0.16338	0.16517	0.17415	0.17594	0.17594	0.17774	0.17774	0.17415	0.00718	0.00718
21 J1 *A. swartbergensis*	0.19210	0.16158	0.16338	0.17415	0.17594	0.17594	0.17774	0.17953	0.17594	0.00539	0.00539
22 E6 *A. mclellani*	0.19031	0.16517	0.16697	0.17235	0.17415	0.17415	0.17415	0.17774	0.17415	0.00359	0.00359
23 L5 *A. mclellani*	0.19031	0.16517	0.16697	0.17235	0.17415	0.17415	0.17415	0.17774	0.17415	0.00359	0.00359
24 E1 *A. mclellani*	0.19031	0.16517	0.16697	0.17235	0.17415	0.17415	0.17415	0.17774	0.17415	0.00359	0.00359
25 L2 *A. mclellani*	0.19210	0.16697	0.16876	0.17056	0.17594	0.17594	0.17594	0.17953	0.17594	0.00539	0.00539
26 P3 *A. incisura*	0.19390	0.16697	0.16876	0.17235	0.17415	0.17415	0.18133	0.17953	0.17594	0.00718	0.00718
27 B5 *A. zwicki*	0.19928	0.16517	0.16697	0.17056	0.17235	0.17235	0.17415	0.17415	0.17056	0.01436	0.01436
28 B1 *A. zwicki*	0.19749	0.16338	0.16517	0.16876	0.17056	0.17056	0.17235	0.17235	0.16876	0.01257	0.01257
29 B2 *A. zwicki*	0.19749	0.16338	0.16517	0.16876	0.17056	0.17056	0.17235	0.17235	0.16876	0.01257	0.01257
30 B4 *A. zwicki*	0.19569	0.16158	0.16338	0.16697	0.16876	0.16876	0.17235	0.17415	0.17056	0.01436	0.01436
31 DDD2 *A. swartbergensis*	0.19210	0.15978	0.16158	0.17235	0.17415	0.17415	0.17594	0.17774	0.17415	0.00898	0.00898
32 O1 *A. swartbergensis*	0.19390	0.16338	0.16517	0.17235	0.17415	0.17415	0.17953	0.17953	0.17594	0.00898	0.00898
33 O2 *A. swartbergensis*	0.19749	0.16517	0.16697	0.17774	0.17953	0.17953	0.18133	0.17953	0.17594	0.01077	0.01077
34 C2 *A. breviloba*	0.19928	0.16697	0.16876	0.17594	0.17774	0.17774	0.18133	0.18133	0.17774	0.00718	0.00718
35 C1 *A. breviloba*	0.19928	0.16697	0.16876	0.17594	0.17774	0.17774	0.18133	0.18133	0.17774	0.00718	0.00718
36 H3 *A. cederbergensis*	0.19928	0.15978	0.16158	0.16517	0.16697	0.16697	0.17594	0.16876	0.16517	0.01616	0.01616
37 H2 *A. cederbergensis*	0.19749	0.16158	0.16338	0.16338	0.16517	0.16517	0.17415	0.17056	0.16697	0.01436	0.01436
38 N2 *A. austrocapensis*	0.19749	0.16697	0.16876	0.17056	0.17235	0.17235	0.17235	0.16697	0.16158	0.02693	0.02693
39 CCC3 *A. austrocapensis*	0.19749	0.16697	0.16876	0.17056	0.17235	0.17235	0.17235	0.16697	0.16158	0.02693	0.02693
40 N3 *A. austrocapensis*	0.19749	0.16697	0.16876	0.17056	0.17235	0.17235	0.17235	0.16697	0.16158	0.02693	0.02693
41 N4 *A. austrocapensis*	0.19749	0.16697	0.16876	0.17056	0.17235	0.17235	0.17235	0.16697	0.16158	0.02693	0.02693
42 M2 *A. austrocapensis*	0.19749	0.16697	0.16876	0.17235	0.17415	0.17415	0.17415	0.16697	0.16158	0.02693	0.02693
43 CCC1 *A. austrocapensis*	0.19569	0.16517	0.16697	0.16876	0.17056	0.17056	0.17056	0.16517	0.15978	0.02873	0.02873
44 D4 *A. brevispina*	0.19210	0.15978	0.16158	0.17056	0.17235	0.16876	0.17415	0.17953	0.17594	0.03052	0.03052
45 D3 *A. brevispina*	0.19569	0.16338	0.16517	0.17235	0.17415	0.17056	0.17594	0.17953	0.17594	0.03052	0.03052
46 F4 *A. pickeri*	0.19569	0.15799	0.15978	0.17415	0.17415	0.17594	0.17415	0.17594	0.17594	0.02513	0.02513
47 F2 *A. pickeri*	0.19928	0.16158	0.16338	0.17774	0.17774	0.17953	0.17774	0.17953	0.17953	0.02873	0.02873
48 X3 *A. uncinata*	0.20287	0.16158	0.16338	0.17774	0.17953	0.17953	0.17774	0.17415	0.17415	0.02693	0.02693
49 X4 *A. uncinata*	0.19928	0.16158	0.16338	0.17953	0.18133	0.18133	0.17953	0.17415	0.17415	0.02693	0.02693
50 T2 *A. bovina*	0.20467	0.16517	0.16697	0.17235	0.17056	0.17415	0.17415	0.15440	0.15260	0.06643	0.06643
51 T1 *A. bovina*	0.20467	0.15978	0.16158	0.16876	0.16697	0.17056	0.17056	0.15081	0.14901	0.06643	0.06643
52 A2 *A. capensis*	0.20467	0.16876	0.16697	0.16876	0.17056	0.17056	0.19210	0.16338	0.16158	0.06463	0.06463
53 A1 *A. capensis*	0.20467	0.16876	0.16697	0.16876	0.17056	0.17056	0.19210	0.16338	0.16158	0.06463	0.06463
54 U3 *A. chanae*	0.20108	0.15081	0.15260	0.16158	0.15978	0.16338	0.16158	0.16697	0.16338	0.06463	0.06463
55 U4 *A. chanae*	0.20108	0.14722	0.14901	0.16158	0.15978	0.16338	0.16158	0.16158	0.15799	0.06463	0.06463
56 S3 *A. bicornis*	0.19210	0.14542	0.14722	0.16158	0.16338	0.16338	0.16338	0.15799	0.15440	0.08977	0.08977
57 S5 *A. bicornis*	0.19210	0.14542	0.14722	0.16158	0.16338	0.16338	0.16338	0.15799	0.15440	0.08977	0.08977
58 S4 *A. bicornis*	0.19569	0.14901	0.15081	0.16158	0.16338	0.16338	0.16697	0.15799	0.15440	0.09336	0.09336
59 W2 *A. lyrata*	0.19569	0.14363	0.14542	0.16338	0.16517	0.16517	0.16876	0.15081	0.14722	0.08618	0.08618
60 W3 *A. lyrata*	0.19569	0.14363	0.14542	0.16338	0.16517	0.16517	0.16876	0.15081	0.14722	0.08618	0.08618
61 II3 *A. spatulata*	0.21364	0.05386	0.05566	0.18133	0.17953	0.18133	0.17594	0.13285	0.12926	0.16158	0.16158
62 HH4 *A. securata*	0.21544	0.05566	0.05745	0.18312	0.18133	0.18312	0.17774	0.13465	0.13106	0.16158	0.16158
63 Y1 *A. barnardi*	0.21185	0.05206	0.05386	0.18133	0.17953	0.18133	0.17594	0.13106	0.12747	0.15978	0.15978
64 Y4 *A. barnardi*	0.21364	0.05386	0.05566	0.18133	0.17953	0.18133	0.17594	0.13285	0.12926	0.16158	0.16158
65 II5 *A. spatulata*	0.21005	0.05566	0.05745	0.18492	0.18312	0.18492	0.17953	0.13106	0.12747	0.16338	0.16338

Appendix 4.5. Continued. Uncorrected p-distances.

		1	2	3	4	5	6	7	8	9	10	11
66	HH1 *A. securata*	0.21544	0.05745	0.05925	0.18671	0.18492	0.18671	0.17774	0.12747	0.12388	0.15978	0.15978
67	AA2 *A. bullata*	0.21005	0.05386	0.05566	0.17953	0.17774	0.17953	0.17953	0.13106	0.12747	0.16338	0.16338
68	CC2 *A. clavata*	0.21185	0.05386	0.05566	0.18133	0.17953	0.18133	0.17594	0.13106	0.12747	0.16338	0.16338
69	CC7 *A. clavata*	0.21185	0.05027	0.05206	0.18133	0.17953	0.18133	0.17594	0.13106	0.12747	0.15978	0.15978
70	FF2 *A. quadrata*	0.21005	0.05206	0.05386	0.18133	0.17953	0.18133	0.17594	0.13645	0.13285	0.16338	0.16338
71	AA5 *A. bullata*	0.20826	0.05745	0.05925	0.18312	0.18133	0.18312	0.17774	0.13645	0.13285	0.16158	0.16158
72	GG1 *A. scutata*	0.20826	0.05027	0.04847	0.18312	0.18312	0.18312	0.17235	0.13106	0.12388	0.15978	0.15978
73	GG2 *A. scutata*	0.21005	0.05745	0.05566	0.19031	0.19031	0.19031	0.17774	0.13465	0.12926	0.16338	0.16338
74	AA6 *A. bullata*	0.20826	0.06104	0.06284	0.18312	0.18133	0.18312	0.17415	0.13465	0.13285	0.17594	0.17594
75	DD2 *A. flabellata*	0.21724	0.07540	0.07540	0.17953	0.17953	0.17594	0.17235	0.13824	0.13285	0.16697	0.16697
76	DD1 *A. flabellata*	0.21724	0.07181	0.07181	0.18133	0.18133	0.17774	0.17415	0.13645	0.13106	0.16697	0.16697
77	AA7 *A. bullata*	0.21185	0.08079	0.08259	0.17774	0.17774	0.17774	0.16876	0.14901	0.14542	0.15619	0.15619
78	BB1 *A. cassida*	0.21903	0.11311	0.11490	0.17594	0.17594	0.17594	0.18133	0.15440	0.15260	0.15978	0.15978
79	JC1 *A. cassida*	0.21724	0.11670	0.11490	0.17415	0.17415	0.17415	0.17953	0.15440	0.15260	0.15799	0.15799
80	JD2 *A. cassida*	0.21005	0.11670	0.11490	0.17056	0.17056	0.17056	0.17415	0.15440	0.15260	0.14901	0.14901
81	JA5 *A. cassida*	0.21724	0.12388	0.12388	0.17953	0.17953	0.17953	0.18312	0.14004	0.13824	0.15440	0.15440
82	AB1 *A. pauletteae*	0.21903	0.10592	0.10772	0.16876	0.17235	0.17235	0.16517	0.13285	0.12926	0.16697	0.16697
83	AB2 *A. pauletteae*	0.21724	0.10772	0.10952	0.17056	0.17415	0.17415	0.16697	0.13106	0.12747	0.16876	0.16876
84	Z1 *A. bifurcata*	0.20108	0.11849	0.12029	0.16697	0.16697	0.16697	0.16338	0.14363	0.14722	0.17774	0.17774
85	Z3 *A. bifurcata*	0.22442	0.11849	0.12029	0.17953	0.17953	0.17953	0.17774	0.14901	0.14901	0.18312	0.18312
86	JJ2 *A. denticulata*	0.20646	0.16338	0.16517	0.00180	0.00359	0.00359	0.07540	0.16876	0.16876	0.17235	0.17235
87	MM1 *A. tabularis*	0.20467	0.15799	0.16158	0.07361	0.07540	0.07361	0.00359	0.17415	0.17594	0.17235	0.17235
88	KK2 *A. hawaquae*	0.19390	0.15081	0.15081	0.12388	0.12567	0.12388	0.10592	0.16697	0.16517	0.14542	0.14542
89	KK1 *A. hawaquae*	0.20287	0.14722	0.14722	0.13824	0.13645	0.13465	0.12567	0.15978	0.15619	0.17235	0.17235
90	LL1 *A. outeniquae*	0.18133	0.16517	0.16697	0.13465	0.13824	0.13824	0.11670	0.16876	0.16876	0.16158	0.16158
91	LL2 *A. outeniquae*	0.18312	0.16517	0.16697	0.13824	0.13824	0.13824	0.11670	0.16517	0.16517	0.15799	0.15799
92	Q2 *A. amatolae*	0.18312	0.16876	0.17235	0.16697	0.16876	0.16876	0.16158	0.17235	0.17056	0.13285	0.13285
93	Q3 *A. amatolae*	0.18492	0.17056	0.17415	0.16876	0.17056	0.17056	0.16338	0.17056	0.16876	0.13465	0.13465
94	R2 *A. spinulata*	0.19569	0.15978	0.16158	0.17953	0.17774	0.18133	0.17594	0.17953	0.17953	0.13106	0.13106
95	R3 *A. spinulata*	0.19569	0.15978	0.16158	0.17953	0.17774	0.18133	0.17594	0.17953	0.17953	0.13106	0.13106
96	QQ1 *D. pulchellum*	0.21903	0.16517	0.16697	0.18133	0.17953	0.18133	0.16517	0.16517	0.16158	0.15440	0.15440
97	QQ3 *D. pulchellum*	0.21903	0.16517	0.16697	0.18133	0.17953	0.18133	0.16517	0.16517	0.16158	0.15440	0.15440
98	PP1 *D. brevis*	0.21724	0.16697	0.16876	0.17953	0.17774	0.17953	0.16338	0.16697	0.16338	0.15260	0.15260
99	PP3 *D. brevis*	0.21544	0.16876	0.17056	0.17953	0.17774	0.17953	0.16517	0.16517	0.16517	0.15081	0.15081
100	NN2 *B. gudu*	0.20826	0.17235	0.17774	0.17594	0.18133	0.18133	0.17415	0.17415	0.17056	0.17953	0.17953
101	NN1 *B. gudu*	0.20646	0.16697	0.17235	0.17953	0.18492	0.18492	0.17056	0.17235	0.16876	0.17415	0.17415
102	OO3 *B. tugelae*	0.20467	0.16158	0.16697	0.18312	0.18851	0.18851	0.17235	0.17056	0.16697	0.17415	0.17415
103	OO2 *B. tugelae*	0.20646	0.16158	0.16697	0.18312	0.18851	0.18851	0.17235	0.17056	0.16697	0.17594	0.17594

Appendix 4.5. Continued. Uncorrected p-distances.

		12	13	14	15	16	17	18	19	20	21	22
12	I3 *A. longiloba*	-										
13	L3 *A. mclellani*	0.00000	-									
14	G1 *A. witsenbergensis*	0.00000	0.00000	-								
15	P4 *A. incisura*	0.00539	0.00539	0.00539	-							
16	P1 *A. incisura*	0.00359	0.00359	0.00359	0.00180	-						
17	P5 *A. incisura*	0.00180	0.00180	0.00180	0.00359	0.00180	-					
18	I2 *A. longiloba*	0.00180	0.00180	0.00180	0.00718	0.00539	0.00359	-				
19	I1 *A. longiloba*	0.00359	0.00359	0.00359	0.00898	0.00718	0.00539	0.00539	-			
20	J2 *A. swartbergensis*	0.00718	0.00718	0.00718	0.01257	0.01077	0.00898	0.00898	0.01077	-		
21	J1 *A. swartbergensis*	0.00539	0.00539	0.00539	0.01077	0.00898	0.00718	0.00718	0.00898	0.00180	-	
22	E6 *A. mclellani*	0.00359	0.00359	0.00359	0.00898	0.00718	0.00539	0.00539	0.00718	0.01077	0.00898	-
23	L5 *A. mclellani*	0.00359	0.00359	0.00359	0.00898	0.00718	0.00539	0.00539	0.00718	0.01077	0.00898	0.00000
24	E1 *A. mclellani*	0.00359	0.00359	0.00359	0.00898	0.00718	0.00539	0.00539	0.00718	0.01077	0.00898	0.00000
25	L2 *A. mclellani*	0.00539	0.00539	0.00539	0.01077	0.00898	0.00718	0.00718	0.00898	0.01257	0.01077	0.00180
26	P3 *A. incisura*	0.00718	0.00718	0.00718	0.01257	0.01077	0.00898	0.00898	0.01077	0.01436	0.01257	0.01077
27	B5 *A. zwicki*	0.01436	0.01436	0.01436	0.01795	0.01616	0.01616	0.01616	0.01795	0.02154	0.01975	0.01436
28	B1 *A. zwicki*	0.01257	0.01257	0.01257	0.01616	0.01436	0.01436	0.01436	0.01616	0.01975	0.01795	0.01257
29	B2 *A. zwicki*	0.01257	0.01257	0.01257	0.01616	0.01436	0.01436	0.01436	0.01616	0.01975	0.01795	0.01257
30	B4 *A. zwicki*	0.01436	0.01436	0.01436	0.01795	0.01616	0.01616	0.01616	0.01795	0.02154	0.01975	0.01436
31	DDD2 *A. swartbergensis*	0.00898	0.00898	0.00898	0.01436	0.01257	0.01077	0.00898	0.01257	0.00539	0.00359	0.01257
32	O1 *A. swartbergensis*	0.00898	0.00898	0.00898	0.01436	0.01257	0.01077	0.00898	0.01257	0.00539	0.00359	0.01257
33	O2 *A. swartbergensis*	0.01077	0.01077	0.01077	0.01616	0.01436	0.01257	0.01077	0.01436	0.00718	0.00539	0.01436
34	C2 *A. breviloba*	0.00718	0.00718	0.00718	0.01257	0.01077	0.00898	0.00718	0.01077	0.01436	0.01257	0.01077
35	C1 *A. breviloba*	0.00718	0.00718	0.00718	0.01257	0.01077	0.00898	0.00718	0.01077	0.01436	0.01257	0.01077
36	H3 *A. cederbergensis*	0.01616	0.01616	0.01616	0.01975	0.01795	0.01795	0.01795	0.01616	0.02334	0.02154	0.01975
37	H2 *A. cederbergensis*	0.01436	0.01436	0.01436	0.01795	0.01616	0.01616	0.01616	0.01436	0.02154	0.01975	0.01795

Appendix 4.5. Continued. Uncorrected p-distances.

		12	13	14	15	16	17	18	19	20	21	22
38	N2 *A. austrocapensis*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
39	CCC3 *A. austrocapensis*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
40	N3 *A. austrocapensis*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
41	N4 *A. austrocapensis*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
42	M2 *A. austrocapensis*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
43	CCC1 *A. austrocapensis*	0.02873	0.02873	0.02873	0.03232	0.03052	0.03052	0.03052	0.03232	0.03591	0.03411	0.02873
44	D4 *A. brevispina*	0.03052	0.03052	0.03052	0.03411	0.03232	0.03232	0.03232	0.03411	0.03770	0.03591	0.03052
45	D3 *A. brevispina*	0.03052	0.03052	0.03052	0.03411	0.03232	0.03232	0.03232	0.03411	0.03770	0.03591	0.03052
46	F4 *A. pickeri*	0.02513	0.02513	0.02513	0.02873	0.02693	0.02693	0.02693	0.02873	0.03232	0.03052	0.02873
47	F2 *A. pickeri*	0.02873	0.02873	0.02873	0.03232	0.03052	0.03052	0.03052	0.03232	0.03591	0.03411	0.03232
48	X3 *A. uncinata*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
49	X4 *A. uncinata*	0.02693	0.02693	0.02693	0.03052	0.02873	0.02873	0.02873	0.03052	0.03411	0.03232	0.02693
50	T2 *A. bovina*	0.06643	0.06643	0.06643	0.06463	0.06463	0.06463	0.06822	0.06643	0.07361	0.07181	0.07002
51	T1 *A. bovina*	0.06643	0.06643	0.06643	0.06463	0.06463	0.06463	0.06822	0.06643	0.07361	0.07181	0.07002
52	A2 *A. capensis*	0.06463	0.06463	0.06463	0.06822	0.06643	0.06643	0.06643	0.06822	0.06822	0.06643	0.06463
53	A1 *A. capensis*	0.06463	0.06463	0.06463	0.06822	0.06643	0.06643	0.06643	0.06822	0.06822	0.06643	0.06463
54	U3 *A. chanae*	0.06463	0.06463	0.06463	0.06463	0.06284	0.06284	0.06284	0.06822	0.06822	0.06643	0.06822
55	U4 *A. chanae*	0.06463	0.06463	0.06463	0.06463	0.06284	0.06284	0.06284	0.06822	0.06822	0.06643	0.06822
56	S3 *A. bicornis*	0.08977	0.08977	0.08977	0.08797	0.08797	0.08797	0.08797	0.09336	0.09695	0.09515	0.09336
57	S5 *A. bicornis*	0.08977	0.08977	0.08977	0.08797	0.08797	0.08797	0.08797	0.09336	0.09695	0.09515	0.09336
58	S4 *A. bicornis*	0.09336	0.09336	0.09336	0.09156	0.09156	0.09156	0.09156	0.09336	0.10054	0.09874	0.09695
59	W2 *A. lyrata*	0.08618	0.08618	0.08618	0.08438	0.08438	0.08438	0.08618	0.08977	0.09336	0.09156	0.08977
60	W3 *A. lyrata*	0.08618	0.08618	0.08618	0.08438	0.08438	0.08438	0.08618	0.08977	0.09336	0.09156	0.08977
61	II3 *A. spatulata*	0.16158	0.16158	0.16158	0.16338	0.16338	0.16338	0.16158	0.16158	0.16338	0.16517	0.16517
62	HH4 *A. securata*	0.16158	0.16158	0.16158	0.16338	0.16338	0.16338	0.16158	0.16158	0.16338	0.16517	0.16517
63	Y1 *A. barnardi*	0.15978	0.15978	0.15978	0.16158	0.16158	0.16158	0.15978	0.15978	0.16158	0.16338	0.16338
64	Y4 *A. barnardi*	0.16158	0.16158	0.16158	0.16338	0.16338	0.16338	0.16158	0.16158	0.16338	0.16517	0.16517
65	II5 *A. spatulata*	0.16338	0.16338	0.16338	0.16517	0.16517	0.16517	0.16338	0.16338	0.16517	0.16697	0.16697
66	HH1 *A. securata*	0.15978	0.15978	0.15978	0.16158	0.16158	0.16158	0.15978	0.15978	0.16158	0.16338	0.16338
67	AA2 *A. bullata*	0.16338	0.16338	0.16338	0.16517	0.16517	0.16517	0.16338	0.15978	0.16517	0.16697	0.16697
68	CC2 *A. clavata*	0.16338	0.16338	0.16338	0.16517	0.16517	0.16517	0.16338	0.16338	0.16517	0.16697	0.16697
69	CC7 *A. clavata*	0.15978	0.15978	0.15978	0.16158	0.16158	0.16158	0.15978	0.15978	0.16158	0.16338	0.16338
70	FF2 *A. quadrata*	0.16338	0.16338	0.16338	0.16517	0.16517	0.16517	0.16338	0.16338	0.16517	0.16697	0.16697
71	AA5 *A. bullata*	0.16158	0.16158	0.16158	0.16338	0.16338	0.16338	0.16158	0.16158	0.16338	0.16517	0.16517
72	GG1 *A. scutata*	0.15978	0.15978	0.15978	0.16158	0.16158	0.16158	0.15978	0.15978	0.16517	0.16338	0.16338
73	GG2 *A. scutata*	0.16338	0.16338	0.16338	0.16517	0.16517	0.16517	0.16338	0.16338	0.16876	0.16697	0.16697
74	AA6 *A. bullata*	0.17594	0.17594	0.17594	0.17415	0.17415	0.17415	0.17594	0.17594	0.17594	0.17774	0.17594
75	DD2 *A. flabellata*	0.16697	0.16697	0.16697	0.16517	0.16517	0.16517	0.16517	0.16697	0.17056	0.16876	0.17056
76	DD1 *A. flabellata*	0.16697	0.16697	0.16697	0.16517	0.16517	0.16517	0.16517	0.16697	0.17056	0.16876	0.17056
77	AA7 *A. bullata*	0.15619	0.15619	0.15619	0.15799	0.15799	0.15799	0.15619	0.15619	0.16158	0.15978	0.15978
78	BB1 *A. cassida*	0.15978	0.15978	0.15978	0.15799	0.15799	0.15799	0.15978	0.15978	0.16517	0.16338	0.16338
79	JC1 *A. cassida*	0.15799	0.15799	0.15799	0.15619	0.15619	0.15619	0.15799	0.15799	0.16338	0.16158	0.16158
80	JD2 *A. cassida*	0.14901	0.14901	0.14901	0.14722	0.14722	0.14722	0.14901	0.14901	0.15440	0.15260	0.15260
81	JA5 *A. cassida*	0.15440	0.15440	0.15440	0.15260	0.15260	0.15260	0.15440	0.15081	0.15978	0.15799	0.15799
82	AB1 *A. pauletteae*	0.16697	0.16697	0.16697	0.16697	0.16517	0.16517	0.16517	0.16697	0.16876	0.16697	0.17056
83	AB2 *A. pauletteae*	0.16876	0.16876	0.16876	0.16697	0.16697	0.16697	0.16697	0.16876	0.17056	0.16876	0.17235
84	Z1 *A. bifurcata*	0.17774	0.17774	0.17774	0.17594	0.17594	0.17594	0.17774	0.17415	0.17774	0.17953	0.18133
85	Z3 *A. bifurcata*	0.18312	0.18312	0.18312	0.18133	0.18133	0.18133	0.18312	0.18312	0.18312	0.18492	0.18671
86	JJ2 *A. denticulata*	0.17235	0.17235	0.17235	0.17235	0.17056	0.17056	0.17056	0.17235	0.17235	0.17235	0.17056
87	MM1 *A. tabularis*	0.17235	0.17235	0.17235	0.17235	0.17056	0.17056	0.17056	0.17594	0.17415	0.17415	0.17056
88	KK2 *A. hawaquae*	0.14542	0.14542	0.14542	0.14901	0.14722	0.14722	0.14363	0.14542	0.14722	0.14722	0.14542
89	KK1 *A. hawaquae*	0.17235	0.17235	0.17235	0.17594	0.17415	0.17415	0.17235	0.17594	0.17594	0.17415	0.17235
90	LL1 *A. outeniquae*	0.16158	0.16158	0.16158	0.16338	0.16158	0.15978	0.15978	0.16517	0.16158	0.16338	0.15978
91	LL2 *A. outeniquae*	0.15799	0.15799	0.15799	0.15978	0.15799	0.15619	0.15619	0.16158	0.15799	0.15978	0.15619
92	Q2 *A. amatolae*	0.13285	0.13285	0.13285	0.13285	0.13285	0.13106	0.13465	0.13285	0.13645	0.13465	0.13645
93	Q3 *A. amatolae*	0.13465	0.13465	0.13465	0.13465	0.13465	0.13285	0.13645	0.13465	0.13824	0.13645	0.13824
94	R2 *A. spinulata*	0.13106	0.13106	0.13106	0.13285	0.13106	0.12926	0.12926	0.12747	0.13285	0.13106	0.13106
95	R3 *A. spinulata*	0.13106	0.13106	0.13106	0.13285	0.13106	0.12926	0.12926	0.12747	0.13285	0.13106	0.13106
96	QQ1 *D. pulchellum*	0.15440	0.15440	0.15440	0.15799	0.15799	0.15619	0.15440	0.15799	0.15799	0.15978	0.15619
97	QQ3 *D. pulchellum*	0.15440	0.15440	0.15440	0.15799	0.15799	0.15619	0.15440	0.15799	0.15799	0.15978	0.15619
98	PP1 *D. brevis*	0.15260	0.15260	0.15260	0.15619	0.15619	0.15440	0.15260	0.15619	0.15260	0.15440	0.15440
99	PP3 *D. brevis*	0.15081	0.15081	0.15081	0.15440	0.15440	0.15260	0.15081	0.15440	0.15440	0.15619	0.15260
100	NN2 *B. gudu*	0.17953	0.17953	0.17953	0.17953	0.17953	0.17774	0.18133	0.17953	0.17594	0.17774	0.18312
101	NN1 *B. gudu*	0.17415	0.17415	0.17415	0.17415	0.17415	0.17235	0.17594	0.17415	0.17056	0.17235	0.17774
102	OO3 *B. tugelae*	0.17415	0.17415	0.17415	0.17415	0.17415	0.17235	0.17594	0.17415	0.17056	0.17235	0.17774
103	OO2 *B. tugelae*	0.17594	0.17594	0.17594	0.17594	0.17594	0.17415	0.17594	0.17594	0.17235	0.17415	0.17953

Appendix 4.5. Continued. Uncorrected p-distances.

		23	24	25	26	27	28	29	30	31	32	33
23	L5 *A. mclellani*	-										
24	E1 *A. mclellani*	0.00000	-									
25	L2 *A. mclellani*	0.00180	0.00180	-								
26	P3 *A. incisura*	0.01077	0.01077	0.01257	-							
27	B5 *A. zwicki*	0.01436	0.01436	0.01616	0.02154	-						
28	B1 *A. zwicki*	0.01257	0.01257	0.01436	0.01975	0.00180	-					
29	B2 *A. zwicki*	0.01257	0.01257	0.01436	0.01975	0.00180	0.00000	-				
30	B4 *A. zwicki*	0.01436	0.01436	0.01616	0.02154	0.00359	0.00180	0.00180	-			
31	DDD2 *A. swartbergensis*	0.01257	0.01257	0.01436	0.01616	0.02334	0.02154	0.02154	0.02334	-		
32	O1 *A. swartbergensis*	0.01257	0.01257	0.01436	0.01257	0.02334	0.02154	0.02154	0.02334	0.00359	-	
33	O2 *A. swartbergensis*	0.01436	0.01436	0.01616	0.01795	0.02513	0.02334	0.02334	0.02513	0.00539	0.00539	-
34	C2 *A. breviloba*	0.01077	0.01077	0.01257	0.01077	0.02154	0.01975	0.01975	0.02154	0.01257	0.01257	0.01436
35	C1 *A. breviloba*	0.01077	0.01077	0.01257	0.01077	0.02154	0.01975	0.01975	0.02154	0.01257	0.01257	0.01436
36	H3 *A. cederbergensis*	0.01975	0.01975	0.02154	0.02334	0.01616	0.01436	0.01436	0.01616	0.02154	0.02513	0.02693
37	H2 *A. cederbergensis*	0.01795	0.01795	0.01975	0.02154	0.01436	0.01257	0.01257	0.01436	0.01975	0.02334	0.02513
38	N2 *A. austrocapensis*	0.02693	0.02693	0.02873	0.03411	0.01975	0.01795	0.01795	0.01616	0.03591	0.03591	0.03770
39	CCC3 *A. austrocapensis*	0.02693	0.02693	0.02873	0.03411	0.01975	0.01795	0.01795	0.01616	0.03591	0.03591	0.03770
40	N3 *A. austrocapensis*	0.02693	0.02693	0.02873	0.03411	0.01975	0.01795	0.01795	0.01616	0.03591	0.03591	0.03770
41	N4 *A. austrocapensis*	0.02693	0.02693	0.02873	0.03411	0.01975	0.01795	0.01795	0.01616	0.03591	0.03591	0.03770
42	M2 *A. austrocapensis*	0.02693	0.02693	0.02873	0.03411	0.01975	0.01795	0.01795	0.01616	0.03591	0.03591	0.03770
43	CCC1 *A. austrocapensis*	0.02873	0.02873	0.03052	0.03591	0.02154	0.01975	0.01975	0.01795	0.03770	0.03770	0.03950
44	D4 *A. brevispina*	0.03052	0.03052	0.03232	0.03770	0.02693	0.02513	0.02513	0.02693	0.03591	0.03950	0.04129
45	D3 *A. brevispina*	0.03052	0.03052	0.03232	0.03770	0.02693	0.02513	0.02513	0.02693	0.03591	0.03950	0.04129
46	F4 *A. pickeri*	0.02873	0.02873	0.03052	0.03232	0.02513	0.02334	0.02334	0.02513	0.03052	0.03411	0.03591
47	F2 *A. pickeri*	0.03232	0.03232	0.03411	0.03232	0.02873	0.02693	0.02693	0.02873	0.03411	0.03770	0.03950
48	X3 *A. uncinata*	0.02693	0.02693	0.02873	0.03232	0.02334	0.02154	0.02154	0.02334	0.03591	0.03591	0.03770
49	X4 *A. uncinata*	0.02693	0.02693	0.02873	0.03232	0.02334	0.02154	0.02154	0.02334	0.03591	0.03591	0.03770
50	T2 *A. bovina*	0.07002	0.07002	0.07181	0.07361	0.06643	0.06822	0.06822	0.07002	0.07540	0.07540	0.07720
51	T1 *A. bovina*	0.07002	0.07002	0.07181	0.07361	0.06643	0.06822	0.06822	0.07002	0.07540	0.07540	0.07720
52	A2 *A. capensis*	0.06463	0.06463	0.06643	0.06822	0.06463	0.06284	0.06284	0.06463	0.07002	0.06643	0.06822
53	A1 *A. capensis*	0.06463	0.06463	0.06643	0.06822	0.06463	0.06284	0.06284	0.06463	0.07002	0.06643	0.06822
54	U3 *A. chanae*	0.06822	0.06822	0.07002	0.07181	0.06822	0.06643	0.06643	0.06643	0.06463	0.06822	0.07002
55	U4 *A. chanae*	0.06822	0.06822	0.07002	0.07181	0.06822	0.06643	0.06643	0.06643	0.06463	0.06822	0.07002
56	S3 *A. bicornis*	0.09336	0.09336	0.09515	0.09695	0.09336	0.09156	0.09156	0.09336	0.09336	0.09695	0.09874
57	S5 *A. bicornis*	0.09336	0.09336	0.09515	0.09695	0.09336	0.09156	0.09156	0.09336	0.09336	0.09695	0.09874
58	S4 *A. bicornis*	0.09695	0.09695	0.09874	0.10054	0.09695	0.09515	0.09515	0.09695	0.09695	0.10054	0.10233
59	W2 *A. lyrata*	0.08977	0.08977	0.09156	0.09336	0.08977	0.08797	0.08797	0.08977	0.08977	0.09336	0.09156
60	W3 *A. lyrata*	0.08977	0.08977	0.09156	0.09336	0.08977	0.08797	0.08797	0.08977	0.08977	0.09336	0.09156
61	II3 *A. spatulata*	0.16517	0.16517	0.16697	0.16517	0.16697	0.16517	0.16517	0.16338	0.16338	0.16517	0.16876
62	HH4 *A. securata*	0.16517	0.16517	0.16697	0.16517	0.16697	0.16517	0.16517	0.16338	0.16338	0.16517	0.16876
63	Y1 *A. barnardi*	0.16338	0.16338	0.16517	0.16338	0.16697	0.16517	0.16517	0.16338	0.16158	0.16338	0.16697
64	Y4 *A. barnardi*	0.16517	0.16517	0.16697	0.16517	0.16697	0.16517	0.16517	0.16338	0.16338	0.16517	0.16876
65	II5 *A. spatulata*	0.16697	0.16697	0.16876	0.16697	0.16876	0.16697	0.16697	0.16517	0.16517	0.16697	0.17056
66	HH1 *A. securata*	0.16338	0.16338	0.16517	0.16338	0.16697	0.16517	0.16517	0.16338	0.16158	0.16338	0.16697
67	AA2 *A. bullata*	0.16697	0.16697	0.16876	0.16697	0.16876	0.16697	0.16697	0.16517	0.16517	0.16697	0.17056
68	CC2 *A. clavata*	0.16697	0.16697	0.16876	0.16697	0.16876	0.16697	0.16697	0.16517	0.16517	0.16697	0.17056
69	CC7 *A. clavata*	0.16338	0.16338	0.16517	0.16338	0.16517	0.16338	0.16338	0.16158	0.16158	0.16338	0.16697
70	FF2 *A. quadrata*	0.16697	0.16697	0.16876	0.16697	0.16876	0.16697	0.16697	0.16517	0.16517	0.16697	0.17056
71	AA5 *A. bullata*	0.16517	0.16517	0.16697	0.16517	0.16697	0.16517	0.16517	0.16338	0.16338	0.16517	0.16876
72	GG1 *A. scutata*	0.16338	0.16338	0.16517	0.16338	0.16517	0.16338	0.16338	0.16517	0.16158	0.16338	0.16697
73	GG2 *A. scutata*	0.16697	0.16697	0.16876	0.16697	0.16876	0.16697	0.16697	0.16876	0.16517	0.16697	0.17056
74	AA6 *A. bullata*	0.17594	0.17594	0.17774	0.17953	0.17774	0.17594	0.17594	0.17415	0.17594	0.17774	0.18133
75	DD2 *A. flabellata*	0.17056	0.17056	0.17235	0.17056	0.17235	0.17056	0.17056	0.16876	0.17056	0.16876	0.17235
76	DD1 *A. flabellata*	0.17056	0.17056	0.17235	0.17056	0.17235	0.17056	0.17056	0.16876	0.17056	0.16876	0.17235
77	AA7 *A. bullata*	0.15978	0.15978	0.16158	0.15978	0.15978	0.15799	0.15799	0.15619	0.15799	0.15978	0.15978
78	BB1 *A. cassida*	0.16338	0.16338	0.16517	0.16517	0.16697	0.16517	0.16517	0.16338	0.16517	0.16338	0.16338
79	JC1 *A. cassida*	0.16158	0.16158	0.16338	0.16338	0.16517	0.16338	0.16338	0.16158	0.16338	0.16158	0.16158
80	JD2 *A. cassida*	0.15260	0.15260	0.15440	0.15440	0.15619	0.15440	0.15440	0.15260	0.15440	0.15260	0.15260
81	JA5 *A. cassida*	0.15799	0.15799	0.15978	0.15978	0.16158	0.15978	0.15978	0.15799	0.15978	0.15799	0.15799
82	AB1 *A. pauletteae*	0.17056	0.17056	0.16876	0.17056	0.16876	0.16697	0.16697	0.16697	0.16876	0.16697	0.17056
83	AB2 *A. pauletteae*	0.17235	0.17235	0.17056	0.17235	0.17056	0.16876	0.16876	0.16876	0.17056	0.16876	0.17235
84	Z1 *A. bifurcata*	0.18133	0.18133	0.18312	0.18133	0.18671	0.18492	0.18492	0.18671	0.17774	0.17953	0.17953
85	Z3 *A. bifurcata*	0.18671	0.18671	0.18851	0.18671	0.19031	0.18851	0.18851	0.19031	0.18671	0.18492	0.18133
86	JJ2 *A. denticulata*	0.17056	0.17056	0.17235	0.17056	0.16876	0.16697	0.16697	0.16517	0.17056	0.17056	0.17594
87	MM1 *A. tabularis*	0.17056	0.17056	0.17235	0.17774	0.17056	0.16876	0.16876	0.16876	0.17235	0.17594	0.17774
88	KK2 *A. hawaquae*	0.14542	0.14542	0.14722	0.15081	0.14722	0.14542	0.14542	0.14722	0.14901	0.14901	0.15081
89	KK1 *A. hawaquae*	0.17235	0.17235	0.17415	0.17415	0.17594	0.17415	0.17415	0.17594	0.17235	0.17235	0.17415

Appendix 4.5. Continued. Uncorrected p-distances.

		23	24	25	26	27	28	29	30	31	32	33
90	LL1 *A. outeniquae*	0.15978	0.15978	0.15799	0.16338	0.16697	0.16517	0.16517	0.16697	0.16517	0.16338	0.16697
91	LL2 *A. outeniquae*	0.15619	0.15619	0.15799	0.15978	0.16338	0.16158	0.16158	0.16338	0.16158	0.15978	0.16338
92	Q2 *A. amatolae*	0.13645	0.13645	0.13824	0.13465	0.13645	0.13465	0.13465	0.13285	0.13824	0.13645	0.14004
93	Q3 *A. amatolae*	0.13824	0.13824	0.14004	0.13645	0.13824	0.13645	0.13645	0.13465	0.14004	0.13824	0.14183
94	R2 *A. spinulata*	0.13106	0.13106	0.13285	0.13824	0.13645	0.13465	0.13465	0.13285	0.13285	0.13285	0.13465
95	R3 *A. spinulata*	0.13106	0.13106	0.13285	0.13824	0.13645	0.13465	0.13465	0.13285	0.13285	0.13285	0.13465
96	QQ1 *D. pulchellum*	0.15619	0.15619	0.15799	0.15440	0.15619	0.15799	0.15799	0.15978	0.16158	0.15978	0.15978
97	QQ3 *D. pulchellum*	0.15619	0.15619	0.15799	0.15440	0.15619	0.15799	0.15799	0.15978	0.16158	0.15978	0.15978
98	PP1 *D. brevis*	0.15440	0.15440	0.15619	0.15260	0.15440	0.15619	0.15619	0.15799	0.15619	0.15440	0.15440
99	PP3 *D. brevis*	0.15260	0.15260	0.15440	0.15081	0.15260	0.15440	0.15440	0.15619	0.15799	0.15619	0.15619
100	NN2 *B. gudu*	0.18312	0.18312	0.18133	0.18492	0.18671	0.18851	0.18851	0.19031	0.18133	0.18133	0.18312
101	NN1 *B. gudu*	0.17774	0.17774	0.17594	0.17953	0.18133	0.18312	0.18312	0.18492	0.17594	0.17594	0.17774
102	OO3 *B. tugelae*	0.17774	0.17774	0.17594	0.17594	0.18133	0.18312	0.18312	0.18492	0.17594	0.17235	0.17774
103	OO2 *B. tugelae*	0.17953	0.17953	0.17774	0.17774	0.18312	0.18492	0.18492	0.18671	0.17415	0.17056	0.17594

Appendix 4.5. Continued. Uncorrected p-distances.

		34	35	36	37	38	39	40	41	42	43	44
34	C2 *A. breviloba*	-										
35	C1 *A. breviloba*	0.00000	-									
36	H3 *A. cederbergensis*	0.01975	0.01975	-								
37	H2 *A. cederbergensis*	0.02154	0.02154	0.00539	-							
38	N2 *A. austrocapensis*	0.03411	0.03411	0.02873	0.02693	-						
39	CCC3 *A. austrocapensis*	0.03411	0.03411	0.02873	0.02693	0.00000	-					
40	N3 *A. austrocapensis*	0.03411	0.03411	0.02873	0.02693	0.00000	0.00000	-				
41	N4 *A. austrocapensis*	0.03411	0.03411	0.02873	0.02693	0.00000	0.00000	0.00000	-			
42	M2 *A. austrocapensis*	0.03411	0.03411	0.02873	0.02693	0.00359	0.00359	0.00359	0.00359	-		
43	CCC1 *A. austrocapensis*	0.03591	0.03591	0.03052	0.02873	0.00180	0.00180	0.00180	0.00180	0.00539	-	
44	D4 *A. brevispina*	0.03770	0.03770	0.02873	0.02693	0.02873	0.02873	0.02873	0.02873	0.02873	0.03052	-
45	D3 *A. brevispina*	0.03770	0.03770	0.02873	0.02693	0.02873	0.02873	0.02873	0.02873	0.02873	0.03052	0.00359
46	F4 *A. pickeri*	0.03232	0.03232	0.02334	0.02154	0.02693	0.02693	0.02693	0.02693	0.02693	0.02873	0.01257
47	F2 *A. pickeri*	0.03232	0.03232	0.02693	0.02513	0.03052	0.03052	0.03052	0.03052	0.03052	0.03232	0.01257
48	X3 *A. uncinata*	0.03411	0.03411	0.02873	0.02693	0.02513	0.02513	0.02513	0.02513	0.02513	0.02693	0.01795
49	X4 *A. uncinata*	0.03411	0.03411	0.02873	0.02693	0.02513	0.02513	0.02513	0.02513	0.02513	0.02693	0.01795
50	T2 *A. bovina*	0.07361	0.07361	0.06463	0.06284	0.06284	0.06284	0.06284	0.06284	0.06284	0.06463	0.07899
51	T1 *A. bovina*	0.07361	0.07361	0.06463	0.06284	0.06284	0.06284	0.06284	0.06284	0.06284	0.06463	0.07899
52	A2 *A. capensis*	0.06643	0.06643	0.06284	0.06463	0.06643	0.06643	0.06643	0.06643	0.06643	0.06822	0.07361
53	A1 *A. capensis*	0.06643	0.06643	0.06284	0.06463	0.06643	0.06643	0.06643	0.06643	0.06643	0.06822	0.07361
54	U3 *A. chanae*	0.06643	0.06643	0.05925	0.06104	0.06104	0.06104	0.06104	0.06104	0.06104	0.06284	0.07002
55	U4 *A. chanae*	0.06643	0.06643	0.05925	0.06104	0.06104	0.06104	0.06104	0.06104	0.06104	0.06284	0.07002
56	S3 *A. bicornis*	0.08797	0.08797	0.08977	0.09156	0.09336	0.09336	0.09336	0.09336	0.09336	0.09156	0.09695
57	S5 *A. bicornis*	0.08797	0.08797	0.08977	0.09156	0.09336	0.09336	0.09336	0.09336	0.09336	0.09156	0.09695
58	S4 *A. bicornis*	0.09156	0.09156	0.08977	0.09156	0.09695	0.09695	0.09695	0.09695	0.09695	0.09515	0.10054
59	W2 *A. lyrata*	0.08797	0.08797	0.08618	0.08797	0.08977	0.08977	0.08977	0.08977	0.08977	0.08797	0.09336
60	W3 *A. lyrata*	0.08797	0.08797	0.08618	0.08797	0.08977	0.08977	0.08977	0.08977	0.08977	0.08797	0.09336
61	II3 *A. spatulata*	0.16338	0.16338	0.15799	0.16338	0.16517	0.16517	0.16517	0.16517	0.16517	0.16338	0.16158
62	HH4 *A. securata*	0.16338	0.16338	0.15799	0.16338	0.16517	0.16517	0.16517	0.16517	0.16517	0.16338	0.16158
63	Y1 *A. barnardi*	0.16158	0.16158	0.15799	0.16338	0.16517	0.16517	0.16517	0.16517	0.16517	0.16338	0.16158
64	Y4 *A. barnardi*	0.16338	0.16338	0.15799	0.16338	0.16517	0.16517	0.16517	0.16517	0.16517	0.16338	0.16158
65	II5 *A. spatulata*	0.16517	0.16517	0.15978	0.16517	0.16338	0.16338	0.16338	0.16338	0.16338	0.16158	0.16338
66	HH1 *A. securata*	0.16158	0.16158	0.15799	0.16338	0.16158	0.16158	0.16158	0.16158	0.16158	0.15978	0.16517
67	AA2 *A. bullata*	0.16517	0.16517	0.15619	0.16158	0.16338	0.16338	0.16338	0.16338	0.16338	0.16158	0.16338
68	CC2 *A. clavata*	0.16517	0.16517	0.15978	0.16517	0.16338	0.16338	0.16338	0.16338	0.16338	0.16158	0.16338
69	CC7 *A. clavata*	0.16158	0.16158	0.15619	0.16158	0.16338	0.16338	0.16338	0.16338	0.16338	0.16158	0.15978
70	FF2 *A. quadrata*	0.16517	0.16517	0.15978	0.16517	0.16338	0.16338	0.16338	0.16338	0.16338	0.16158	0.16338
71	AA5 *A. bullata*	0.16338	0.16338	0.15799	0.16338	0.16158	0.16158	0.16158	0.16158	0.16158	0.15978	0.16517
72	GG1 *A. scutata*	0.16517	0.16517	0.15978	0.16158	0.16338	0.16338	0.16338	0.16338	0.16338	0.16158	0.15619
73	GG2 *A. scutata*	0.16876	0.16876	0.16338	0.16517	0.16697	0.16697	0.16697	0.16697	0.16697	0.16517	0.15978
74	AA6 *A. bullata*	0.17774	0.17774	0.17235	0.17774	0.17056	0.17056	0.17056	0.17056	0.17056	0.16876	0.16876
75	DD2 *A. flabellata*	0.17235	0.17235	0.17056	0.16876	0.16697	0.16697	0.16697	0.16697	0.16697	0.16517	0.16517
76	DD1 *A. flabellata*	0.17235	0.17235	0.16697	0.16876	0.16697	0.16697	0.16697	0.16697	0.16697	0.16517	0.16517
77	AA7 *A. bullata*	0.15799	0.15799	0.15081	0.15619	0.15799	0.15799	0.15799	0.15799	0.15799	0.15619	0.15799
78	BB1 *A. cassida*	0.16158	0.16158	0.16158	0.16338	0.16517	0.16517	0.16517	0.16517	0.16517	0.16697	0.15978
79	JC1 *A. cassida*	0.15978	0.15978	0.15978	0.16158	0.16338	0.16338	0.16338	0.16338	0.16338	0.16517	0.15799
80	JD2 *A. cassida*	0.15440	0.15440	0.15440	0.15260	0.15440	0.15440	0.15440	0.15440	0.15440	0.15619	0.14901
81	JA5 *A. cassida*	0.15978	0.15978	0.15619	0.15440	0.15978	0.15978	0.15978	0.15978	0.15978	0.16158	0.15799

Appendix 4.5. Continued. Uncorrected p-distances.

		34	35	36	37	38	39	40	41	42	43	44
82	AB1 *A. pauletteae*	0.17235	0.17235	0.16697	0.16517	0.16517	0.16517	0.16517	0.16517	0.16517	0.16338	0.16517
83	AB2 *A. pauletteae*	0.17415	0.17415	0.16876	0.16697	0.16697	0.16697	0.16697	0.16697	0.16697	0.16517	0.16697
84	Z1 *A. bifurcata*	0.17953	0.17953	0.17594	0.17774	0.17953	0.17953	0.17953	0.17953	0.18133	0.17774	0.16876
85	Z3 *A. bifurcata*	0.18492	0.18492	0.18312	0.18492	0.18492	0.18492	0.18492	0.18492	0.18492	0.18312	0.17594
86	JJ2 *A. denticulata*	0.17415	0.17415	0.16338	0.16158	0.16876	0.16876	0.16876	0.16876	0.17056	0.16697	0.16876
87	MM1 *A. tabularis*	0.17774	0.17774	0.17235	0.17056	0.16876	0.16876	0.16876	0.16876	0.17056	0.16697	0.17056
88	KK2 *A. hawaquae*	0.15081	0.15081	0.14722	0.14542	0.14542	0.14542	0.14542	0.14542	0.14722	0.14363	0.14722
89	KK1 *A. hawaquae*	0.17774	0.17774	0.17235	0.17056	0.16697	0.16697	0.16697	0.16697	0.16876	0.16517	0.16876
90	LL1 *A. outeniquae*	0.16697	0.16697	0.16697	0.16876	0.16517	0.16517	0.16517	0.16517	0.16697	0.16338	0.16697
91	LL2 *A. outeniquae*	0.16338	0.16338	0.16338	0.16517	0.16158	0.16158	0.16158	0.16158	0.16338	0.15978	0.16338
92	Q2 *A. amatolae*	0.13645	0.13645	0.13645	0.13824	0.13465	0.13465	0.13465	0.13465	0.13465	0.13285	0.14004
93	Q3 *A. amatolae*	0.13824	0.13824	0.13824	0.14004	0.13645	0.13645	0.13645	0.13645	0.13645	0.13465	0.14183
94	R2 *A. spinulata*	0.13645	0.13645	0.13285	0.13465	0.13106	0.13106	0.13106	0.13106	0.13106	0.12926	0.12926
95	R3 *A. spinulata*	0.13645	0.13645	0.13285	0.13465	0.13106	0.13106	0.13106	0.13106	0.13106	0.12926	0.12926
96	QQ1 *D. pulchellum*	0.15978	0.15978	0.15978	0.16158	0.15440	0.15440	0.15440	0.15440	0.15440	0.15260	0.15260
97	QQ3 *D. pulchellum*	0.15978	0.15978	0.15978	0.16158	0.15440	0.15440	0.15440	0.15440	0.15440	0.15260	0.15260
98	PP1 *D. brevis*	0.15799	0.15799	0.16158	0.15978	0.15260	0.15260	0.15260	0.15260	0.15260	0.15081	0.15081
99	PP3 *D. brevis*	0.15619	0.15619	0.15978	0.15799	0.15440	0.15440	0.15440	0.15440	0.15440	0.15260	0.14901
100	NN2 *B. gudu*	0.18492	0.18492	0.18133	0.18492	0.19031	0.19031	0.19031	0.19031	0.19031	0.18851	0.18851
101	NN1 *B. gudu*	0.17953	0.17953	0.17594	0.17953	0.18492	0.18492	0.18492	0.18492	0.18492	0.18312	0.18671
102	OO3 *B. tugelae*	0.17953	0.17953	0.17953	0.18312	0.18492	0.18492	0.18492	0.18492	0.18492	0.18492	0.18312
103	OO2 *B. tugelae*	0.17774	0.17774	0.18133	0.18492	0.18671	0.18671	0.18671	0.18671	0.18671	0.18671	0.18492

Appendix 4.5. Continued. Uncorrected p-distances.

		45	46	47	48	49	50	51	52	53	54	55
45	D3 *A. brevispina*	-										
46	F4 *A. pickeri*	0.01257	-									
47	F2 *A. pickeri*	0.01257	0.00359	-								
48	X3 *A. uncinata*	0.01795	0.01616	0.01616	-							
49	X4 *A. uncinata*	0.01795	0.01616	0.01616	0.00359	-						
50	T2 *A. bovina*	0.07540	0.07361	0.07720	0.07540	0.07540	-					
51	T1 *A. bovina*	0.07540	0.07361	0.07720	0.07540	0.07540	0.00718	-				
52	A2 *A. capensis*	0.07361	0.07181	0.07540	0.06643	0.06643	0.07540	0.07181	-			
53	A1 *A. capensis*	0.07361	0.07181	0.07540	0.06643	0.06643	0.07540	0.07181	0.00000	-		
54	U3 *A. chanae*	0.07002	0.06463	0.06822	0.06643	0.06643	0.07540	0.07540	0.07361	0.07361	-	
55	U4 *A. chanae*	0.07002	0.06463	0.06822	0.07002	0.07002	0.07181	0.07181	0.07720	0.07720	0.00718	-
56	S3 *A. bicornis*	0.09336	0.09156	0.09515	0.09515	0.09695	0.09515	0.09515	0.09695	0.09695	0.07899	0.07720
57	S5 *A. bicornis*	0.09336	0.09156	0.09515	0.09515	0.09695	0.09515	0.09515	0.09695	0.09695	0.07899	0.07720
58	S4 *A. bicornis*	0.09695	0.09515	0.09874	0.09874	0.10054	0.09515	0.09515	0.10054	0.10054	0.08259	0.08079
59	W2 *A. lyrata*	0.08977	0.08797	0.09156	0.09156	0.09336	0.09515	0.09515	0.09336	0.09336	0.08079	0.07899
60	W3 *A. lyrata*	0.08977	0.08797	0.09156	0.09156	0.09336	0.09515	0.09515	0.09336	0.09336	0.08079	0.07899
61	II3 *A. spatulata*	0.16517	0.15619	0.15978	0.16338	0.16338	0.15978	0.15799	0.16876	0.16876	0.15440	0.15081
62	HH4 *A. securata*	0.16517	0.15619	0.15978	0.16338	0.16338	0.15978	0.15799	0.16876	0.16876	0.15440	0.15081
63	Y1 *A. barnardi*	0.16517	0.15619	0.15978	0.16338	0.16338	0.15799	0.15619	0.16697	0.16697	0.15260	0.14901
64	Y4 *A. barnardi*	0.16517	0.15619	0.15978	0.16338	0.16338	0.15978	0.15799	0.16876	0.16876	0.15440	0.15081
65	II5 *A. spatulata*	0.16697	0.15799	0.16158	0.16517	0.16517	0.15978	0.15799	0.17235	0.17235	0.15440	0.15081
66	HH1 *A. securata*	0.16517	0.15619	0.15978	0.16338	0.16338	0.15619	0.15440	0.16876	0.16876	0.15081	0.14722
67	AA2 *A. bullata*	0.16697	0.15799	0.16158	0.16517	0.16517	0.15440	0.15260	0.17056	0.17056	0.15260	0.14901
68	CC2 *A. clavata*	0.16697	0.15799	0.16158	0.16158	0.16517	0.15978	0.15799	0.17056	0.17056	0.15260	0.14901
69	CC7 *A. clavata*	0.16338	0.15440	0.15799	0.16158	0.16158	0.15978	0.15799	0.16697	0.16697	0.15260	0.14901
70	FF2 *A. quadrata*	0.16697	0.15799	0.16158	0.16517	0.16517	0.16158	0.15978	0.17056	0.17056	0.15260	0.14901
71	AA5 *A. bullata*	0.16876	0.15978	0.16338	0.16338	0.16338	0.16338	0.16158	0.17235	0.17235	0.15440	0.15081
72	GG1 *A. scutata*	0.15978	0.15081	0.15440	0.15799	0.15799	0.15799	0.15260	0.16876	0.16876	0.15619	0.15260
73	GG2 *A. scutata*	0.15978	0.15440	0.15440	0.15799	0.15799	0.16338	0.15799	0.17953	0.17953	0.16338	0.15978
74	AA6 *A. bullata*	0.17235	0.16697	0.17056	0.17594	0.17594	0.16517	0.16338	0.17594	0.17594	0.15978	0.15619
75	DD2 *A. flabellata*	0.16697	0.16517	0.16876	0.16876	0.16876	0.16876	0.16338	0.17594	0.17594	0.15799	0.15440
76	DD1 *A. flabellata*	0.16697	0.16517	0.16876	0.16876	0.16876	0.16517	0.15978	0.17594	0.17594	0.15799	0.15440
77	AA7 *A. bullata*	0.15799	0.15260	0.15619	0.15978	0.15978	0.16158	0.15978	0.16697	0.16697	0.15260	0.15081
78	BB1 *A. cassida*	0.16158	0.16338	0.16338	0.16338	0.16158	0.15799	0.15619	0.15440	0.15440	0.15440	0.15260
79	JC1 *A. cassida*	0.15978	0.16158	0.16158	0.16158	0.15978	0.15619	0.15440	0.15260	0.15260	0.15260	0.15081
80	JD2 *A. cassida*	0.15081	0.15260	0.15260	0.15440	0.15260	0.14722	0.14722	0.15440	0.15440	0.14901	0.14542
81	JA5 *A. cassida*	0.15978	0.16158	0.16158	0.15799	0.15619	0.15440	0.15260	0.15978	0.15978	0.15440	0.15260
82	AB1 *A. pauletteae*	0.16517	0.15978	0.16338	0.16158	0.16158	0.15978	0.15978	0.16158	0.16158	0.14542	0.14004
83	AB2 *A. pauletteae*	0.16697	0.16158	0.16517	0.16338	0.16338	0.15799	0.15799	0.16338	0.16338	0.14722	0.14183
84	Z1 *A. bifurcata*	0.17056	0.17056	0.17415	0.17594	0.17594	0.16697	0.17056	0.18851	0.18851	0.16338	0.16158
85	Z3 *A. bifurcata*	0.17774	0.17774	0.18133	0.17953	0.17953	0.16876	0.17235	0.18133	0.18133	0.17415	0.17235

Appendix 4.5. Continued. Uncorrected p-distances.

		45	46	47	48	49	50	51	52	53	54	55
86	JJ2 *A. denticulata*	0.17056	0.17235	0.17594	0.17594	0.17774	0.17056	0.16697	0.16697	0.16697	0.15978	0.15978
87	MM1 *A. tabularis*	0.17235	0.17056	0.17415	0.17415	0.17594	0.17415	0.17056	0.18851	0.18851	0.16158	0.16158
88	KK2 *A. hawaquae*	0.15081	0.14901	0.15260	0.15260	0.15260	0.14542	0.14183	0.16158	0.16158	0.14722	0.14542
89	KK1 *A. hawaquae*	0.17235	0.16876	0.17235	0.17594	0.17774	0.17235	0.16876	0.16517	0.16517	0.15799	0.15081
90	LL1 *A. outeniquae*	0.17056	0.16697	0.17056	0.17056	0.17056	0.16338	0.16697	0.17594	0.17594	0.16876	0.16158
91	LL2 *A. outeniquae*	0.16697	0.16338	0.16697	0.16697	0.16697	0.15978	0.16338	0.17235	0.17235	0.16517	0.15799
92	Q2 *A. amatolae*	0.13824	0.13824	0.14183	0.14004	0.14004	0.13645	0.13285	0.14363	0.14363	0.13285	0.12926
93	Q3 *A. amatolae*	0.14004	0.14004	0.14363	0.14183	0.14183	0.13824	0.13465	0.14183	0.14183	0.13465	0.13106
94	R2 *A. spinulata*	0.12747	0.12747	0.13106	0.12926	0.12926	0.13106	0.13465	0.14363	0.14363	0.12567	0.12388
95	R3 *A. spinulata*	0.12747	0.12747	0.13106	0.12926	0.12926	0.13106	0.13465	0.14363	0.14363	0.12567	0.12388
96	QQ1 *D. pulchellum*	0.15440	0.15799	0.15978	0.15440	0.15440	0.16517	0.16158	0.16338	0.16338	0.17056	0.17056
97	QQ3 *D. pulchellum*	0.15440	0.15799	0.15978	0.15440	0.15440	0.16517	0.16158	0.16338	0.16338	0.17056	0.17056
98	PP1 *D. brevis*	0.15260	0.15619	0.15799	0.15260	0.15260	0.16697	0.16338	0.16876	0.16876	0.16876	0.16876
99	PP3 *D. brevis*	0.15081	0.15440	0.15619	0.15081	0.15081	0.16338	0.15978	0.16876	0.16876	0.16876	0.16876
100	NN2 *B. gudu*	0.19210	0.19031	0.19390	0.18492	0.18492	0.18671	0.18492	0.19749	0.19749	0.19569	0.19390
101	NN1 *B. gudu*	0.19031	0.18492	0.18851	0.18133	0.17953	0.18133	0.17953	0.19569	0.19569	0.19031	0.18851
102	OO3 *B. tugelae*	0.18671	0.18133	0.18492	0.18133	0.17953	0.18312	0.17953	0.18851	0.18851	0.18671	0.18492
103	OO2 *B. tugelae*	0.18851	0.18312	0.18671	0.18312	0.18133	0.18492	0.18133	0.19031	0.19031	0.18671	0.18492

Appendix 4.5. Continued. Uncorrected p-distances.

		56	57	58	59	60	61	62	63	64	65	66
56	S3 *A. bicornis*	-										
57	S5 *A. bicornis*	0.00000	-									
58	S4 *A. bicornis*	0.00359	0.00359	-								
59	W2 *A. lyrata*	0.01257	0.01257	0.01257	-							
60	W3 *A. lyrata*	0.01257	0.01257	0.01257	0.00000	-						
61	II3 *A. spatulata*	0.14542	0.14542	0.14901	0.14363	0.14363	-					
62	HH4 *A. securata*	0.14542	0.14542	0.14901	0.14363	0.14363	0.00180	-				
63	Y1 *A. barnardi*	0.14363	0.14363	0.14722	0.14183	0.14183	0.00180	0.00359	-			
64	Y4 *A. barnardi*	0.14542	0.14542	0.14901	0.14363	0.14363	0.00000	0.00180	0.00180	-		
65	II5 *A. spatulata*	0.14363	0.14363	0.14722	0.14183	0.14183	0.00539	0.00718	0.00718	0.00539	-	
66	HH1 *A. securata*	0.14004	0.14004	0.14363	0.13824	0.13824	0.00718	0.00898	0.00898	0.00718	0.00539	-
67	AA2 *A. bullata*	0.14183	0.14183	0.14183	0.14004	0.14004	0.01077	0.01257	0.01257	0.01077	0.00898	0.01077
68	CC2 *A. clavata*	0.14004	0.14004	0.14363	0.13824	0.13824	0.00898	0.01077	0.01077	0.00898	0.00718	0.00898
69	CC7 *A. clavata*	0.14183	0.14183	0.14542	0.14004	0.14004	0.00539	0.00718	0.00718	0.00539	0.00718	0.00898
70	FF2 *A. quadrata*	0.14183	0.14183	0.14542	0.14004	0.14004	0.01257	0.01436	0.01436	0.01257	0.01077	0.01257
71	AA5 *A. bullata*	0.14363	0.14363	0.14722	0.14183	0.14183	0.01795	0.01975	0.01975	0.01795	0.01616	0.01795
72	GG1 *A. scutata*	0.14363	0.14363	0.14722	0.14183	0.14183	0.02513	0.02693	0.02693	0.02513	0.02334	0.02513
73	GG2 *A. scutata*	0.14901	0.14901	0.15260	0.14722	0.14722	0.03950	0.04129	0.04129	0.03950	0.03770	0.03591
74	AA6 *A. bullata*	0.14722	0.14722	0.15081	0.14542	0.14542	0.02693	0.02873	0.02873	0.02693	0.02513	0.02513
75	DD2 *A. flabellata*	0.15440	0.15440	0.15799	0.15978	0.15978	0.06284	0.06463	0.06463	0.06284	0.06463	0.06463
76	DD1 *A. flabellata*	0.15440	0.15440	0.15799	0.15978	0.15978	0.05925	0.06104	0.06104	0.05925	0.06104	0.06104
77	AA7 *A. bullata*	0.15081	0.15081	0.15440	0.14901	0.14901	0.05566	0.05745	0.05566	0.05566	0.05925	0.05745
78	BB1 *A. cassida*	0.14542	0.14542	0.14901	0.14363	0.14363	0.10592	0.10772	0.10413	0.10592	0.10952	0.10952
79	JC1 *A. cassida*	0.14542	0.14542	0.14901	0.14363	0.14363	0.10592	0.10772	0.10413	0.10592	0.10952	0.10952
80	JD2 *A. cassida*	0.14004	0.14004	0.14363	0.13824	0.13824	0.10952	0.11131	0.10772	0.10952	0.11311	0.11311
81	JA5 *A. cassida*	0.15440	0.15440	0.15440	0.15081	0.15081	0.11670	0.11849	0.11490	0.11670	0.12029	0.12029
82	AB1 *A. pauletteae*	0.13824	0.13824	0.14183	0.14363	0.14363	0.10054	0.10233	0.10233	0.10054	0.09874	0.09515
83	AB2 *A. pauletteae*	0.13645	0.13645	0.14004	0.14183	0.14183	0.09874	0.10054	0.10054	0.09874	0.09695	0.09336
84	Z1 *A. bifurcata*	0.15978	0.15978	0.15978	0.15799	0.15799	0.10054	0.10233	0.10233	0.10054	0.10233	0.10413
85	Z3 *A. bifurcata*	0.16697	0.16697	0.16697	0.15799	0.15799	0.10413	0.10592	0.10592	0.10413	0.10952	0.10772
86	JJ2 *A. denticulata*	0.15978	0.15978	0.15978	0.16158	0.16158	0.17953	0.18133	0.17953	0.17953	0.18312	0.18492
87	MM1 *A. tabularis*	0.15978	0.15978	0.16338	0.16517	0.16517	0.17415	0.17594	0.17415	0.17415	0.17774	0.17594
88	KK2 *A. hawaquae*	0.15978	0.15978	0.15978	0.16158	0.16158	0.15440	0.15619	0.15260	0.15440	0.15619	0.15619
89	KK1 *A. hawaquae*	0.15440	0.15440	0.15799	0.14901	0.14901	0.14722	0.14901	0.14542	0.14722	0.14901	0.14901
90	LL1 *A. outeniquae*	0.16158	0.16158	0.16517	0.16338	0.16338	0.16158	0.16158	0.15978	0.16158	0.16338	0.16876
91	LL2 *A. outeniquae*	0.15978	0.15978	0.16338	0.16158	0.16158	0.15799	0.15799	0.15619	0.15799	0.15978	0.16517
92	Q2 *A. amatolae*	0.12747	0.12747	0.13106	0.13106	0.13106	0.16517	0.16517	0.16338	0.16517	0.16338	0.16338
93	Q3 *A. amatolae*	0.12926	0.12926	0.13285	0.13285	0.13285	0.16697	0.16697	0.16517	0.16697	0.16517	0.16517
94	R2 *A. spinulata*	0.13285	0.13285	0.13285	0.13106	0.13106	0.17415	0.17415	0.17235	0.17415	0.17594	0.17235
95	R3 *A. spinulata*	0.13285	0.13285	0.13285	0.13106	0.13106	0.17415	0.17415	0.17235	0.17415	0.17594	0.17235
96	QQ1 *D. pulchellum*	0.15440	0.15440	0.15799	0.15619	0.15619	0.16158	0.16338	0.16338	0.16158	0.16517	0.16338
97	QQ3 *D. pulchellum*	0.15440	0.15440	0.15799	0.15619	0.15619	0.16158	0.16338	0.16338	0.16158	0.16517	0.16338
98	PP1 *D. brevis*	0.15260	0.15260	0.15619	0.15440	0.15440	0.16338	0.16517	0.16517	0.16338	0.16697	0.16517
99	PP3 *D. brevis*	0.15619	0.15619	0.15978	0.15799	0.15799	0.16158	0.16338	0.16338	0.16158	0.16517	0.16338

Appendix 5 continued. Uncorrected p-distances.

		56	57	58	59	60	61	62	63	64	65	66
100	NN2 *B. gudu*	0.17594	0.17594	0.17594	0.17594	0.17594	0.17774	0.17953	0.17953	0.17774	0.17774	0.17953
101	NN1 *B. gudu*	0.17235	0.17235	0.17235	0.17235	0.17235	0.17235	0.17415	0.17415	0.17235	0.17235	0.17415
102	OO3 *B. tugelae*	0.17415	0.17415	0.17774	0.17415	0.17415	0.16338	0.16517	0.16517	0.16338	0.16517	0.16697
103	OO2 *B. tugelae*	0.17415	0.17415	0.17774	0.17415	0.17415	0.16338	0.16517	0.16517	0.16338	0.16517	0.16697

Appendix 4.5. Continued. Uncorrected p-distances.

		67	68	69	70	71	72	73	74	75	76	77
67	AA2 *A. bullata*	-										
68	CC2 *A. clavata*	0.01257	-									
69	CC7 *A. clavata*	0.01257	0.00359	-								
70	FF2 *A. quadrata*	0.00898	0.01257	0.01257	-							
71	AA5 *A. bullata*	0.01436	0.01795	0.01795	0.00898	-						
72	GG1 *A. scutata*	0.02513	0.02693	0.02693	0.02693	0.03232	-					
73	GG2 *A. scutata*	0.03232	0.04129	0.04129	0.03770	0.03950	0.01975	-				
74	AA6 *A. bullata*	0.02334	0.02513	0.02513	0.02334	0.02873	0.03591	0.04668	-			
75	DD2 *A. flabellata*	0.06643	0.06463	0.06463	0.06643	0.07181	0.06643	0.07540	0.06822	-		
76	DD1 *A. flabellata*	0.06284	0.06104	0.06104	0.06284	0.06822	0.06284	0.07181	0.06463	0.00359	-	
77	AA7 *A. bullata*	0.05745	0.05925	0.05745	0.05566	0.06104	0.06643	0.07361	0.06284	0.07899	0.07540	-
78	BB1 *A. cassida*	0.10592	0.10592	0.10413	0.10592	0.11131	0.11311	0.10952	0.11131	0.10772	0.10772	0.08977
79	JC1 *A. cassida*	0.10592	0.10592	0.10413	0.10592	0.11131	0.11311	0.10952	0.11131	0.10592	0.10592	0.08977
80	JD2 *A. cassida*	0.10952	0.10952	0.10772	0.10952	0.11490	0.11311	0.10952	0.11490	0.10592	0.10592	0.09695
81	JA5 *A. cassida*	0.11311	0.11670	0.11490	0.11670	0.11849	0.12208	0.11849	0.12208	0.11849	0.11490	0.10054
82	AB1 *A. pauletteae*	0.09695	0.09695	0.09695	0.09695	0.10233	0.09874	0.09695	0.10054	0.09874	0.09515	0.10413
83	AB2 *A. pauletteae*	0.09515	0.09515	0.09515	0.09515	0.10054	0.09695	0.09515	0.09874	0.09695	0.09336	0.10233
84	Z1 *A. bifurcata*	0.09874	0.09874	0.09874	0.10054	0.09874	0.10413	0.10952	0.09695	0.11490	0.11490	0.11490
85	Z3 *A. bifurcata*	0.10952	0.10592	0.10233	0.10772	0.10592	0.11131	0.11670	0.10413	0.12747	0.12388	0.11670
86	JJ2 *A. denticulata*	0.17774	0.17953	0.17953	0.17953	0.18133	0.18133	0.18851	0.18133	0.17774	0.17953	0.17594
87	MM1 *A. tabularis*	0.17774	0.17415	0.17415	0.17415	0.17594	0.16876	0.17415	0.17235	0.17056	0.17235	0.16697
88	KK2 *A. hawaquae*	0.15260	0.15440	0.15440	0.15619	0.15440	0.14722	0.14901	0.16158	0.14901	0.14901	0.15799
89	KK1 *A. hawaquae*	0.15260	0.14542	0.14722	0.15440	0.15619	0.14004	0.15081	0.15978	0.13645	0.14004	0.15619
90	LL1 *A. outeniquae*	0.16697	0.16517	0.16158	0.16338	0.16517	0.16876	0.17415	0.16876	0.16876	0.16517	0.17594
91	LL2 *A. outeniquae*	0.16338	0.16158	0.15799	0.15978	0.16158	0.16517	0.17056	0.16517	0.16517	0.16158	0.17235
92	Q2 *A. amatolae*	0.16338	0.16876	0.16876	0.16338	0.16517	0.16338	0.16158	0.16517	0.16697	0.17056	0.16876
93	Q3 *A. amatolae*	0.16517	0.17056	0.17056	0.16517	0.16697	0.16517	0.16338	0.16697	0.16876	0.17235	0.17056
94	R2 *A. spinulata*	0.16876	0.17594	0.17594	0.17415	0.17594	0.17235	0.16517	0.16338	0.16697	0.16338	0.17056
95	R3 *A. spinulata*	0.16876	0.17594	0.17594	0.17415	0.17594	0.17235	0.16517	0.16338	0.16697	0.16338	0.17056
96	QQ1 *D. pulchellum*	0.16158	0.16338	0.16158	0.15978	0.15081	0.15440	0.15260	0.16697	0.16517	0.16158	0.15619
97	QQ3 *D. pulchellum*	0.16158	0.16338	0.16158	0.15978	0.15081	0.15440	0.15260	0.16697	0.16517	0.16158	0.15619
98	PP1 *D. brevis*	0.16338	0.16517	0.16338	0.16158	0.15260	0.15619	0.15440	0.16876	0.16338	0.16338	0.15799
99	PP3 *D. brevis*	0.16158	0.16338	0.16158	0.15978	0.15081	0.15440	0.15260	0.16517	0.16158	0.16158	0.15440
100	NN2 *B. gudu*	0.17415	0.17415	0.17415	0.17235	0.17235	0.17235	0.17774	0.18133	0.17953	0.17594	0.18133
101	NN1 *B. gudu*	0.16876	0.17056	0.16876	0.16697	0.16697	0.16697	0.17235	0.17594	0.17415	0.17056	0.17594
102	OO3 *B. tugelae*	0.16517	0.16338	0.16158	0.16338	0.16338	0.15978	0.16517	0.16517	0.16338	0.15978	0.16876
103	OO2 *B. tugelae*	0.16517	0.16338	0.16158	0.16338	0.16338	0.15978	0.16517	0.16517	0.16338	0.15978	0.16876

Appendix 4.5. Continued. Uncorrected p-distances.

		78	79	80	81	82	83	84	85	86	87	88
78	BB1 *A. cassida*	-										
79	JC1 *A. cassida*	0.00359	-									
80	JD2 *A. cassida*	0.01436	0.01077	-								
81	JA5 *A. cassida*	0.03232	0.02873	0.03232	-							
82	AB1 *A. pauletteae*	0.10952	0.11131	0.10772	0.11670	-						
83	AB2 *A. pauletteae*	0.10772	0.10952	0.10592	0.11490	0.00180	-					
84	Z1 *A. bifurcata*	0.12567	0.12567	0.12388	0.13645	0.13465	0.13285	-				
85	Z3 *A. bifurcata*	0.12208	0.12208	0.12029	0.13106	0.12747	0.12567	0.04309	-			
86	JJ2 *A. denticulata*	0.17415	0.17235	0.16876	0.17774	0.17056	0.17235	0.16517	0.17774	-		
87	MM1 *A. tabularis*	0.17953	0.17774	0.17235	0.18133	0.16338	0.16517	0.16158	0.17594	0.07181	-	
88	KK2 *A. hawaquae*	0.16876	0.16517	0.15619	0.16338	0.15619	0.15799	0.15260	0.16158	0.12208	0.10233	-
89	KK1 *A. hawaquae*	0.15260	0.14901	0.14722	0.15260	0.15081	0.15260	0.15260	0.15260	0.13645	0.12208	0.09695
90	LL1 *A. outeniquae*	0.17415	0.17235	0.16158	0.17235	0.15978	0.16158	0.16517	0.15619	0.13645	0.11490	0.11490
91	LL2 *A. outeniquae*	0.17235	0.17056	0.15978	0.17235	0.16338	0.16517	0.16158	0.15260	0.13645	0.11490	0.11490
92	Q2 *A. amatolae*	0.17235	0.17415	0.16876	0.18671	0.16517	0.16338	0.16697	0.17594	0.16517	0.15799	0.15260
93	Q3 *A. amatolae*	0.17056	0.17235	0.17056	0.18492	0.16697	0.16517	0.16876	0.17774	0.16697	0.15978	0.15440
94	R2 *A. spinulata*	0.15978	0.15619	0.15081	0.15978	0.14363	0.14542	0.16158	0.17594	0.17774	0.17594	0.16158
95	R3 *A. spinulata*	0.15978	0.15619	0.15081	0.15978	0.14363	0.14542	0.16158	0.17594	0.17774	0.17594	0.16158

Appendix 4.5. Continued. Uncorrected p-distances.

		78	79	80	81	82	83	84	85	86	87	88
96	QQ1 *D. pulchellum*	0.15440	0.15440	0.15260	0.15440	0.14722	0.14542	0.14722	0.14542	0.17953	0.16517	0.17415
97	QQ3 *D. pulchellum*	0.15440	0.15440	0.15260	0.15440	0.14722	0.14542	0.14722	0.14542	0.17953	0.16517	0.17415
98	PP1 *D. brevis*	0.15619	0.15619	0.15440	0.15619	0.14901	0.14722	0.14542	0.14722	0.17774	0.16338	0.17235
99	PP3 *D. brevis*	0.16158	0.16158	0.15978	0.16158	0.15081	0.14901	0.14183	0.14363	0.17774	0.16517	0.17415
100	NN2 *B. gudu*	0.19390	0.19390	0.19031	0.19749	0.17235	0.17056	0.17056	0.17415	0.17774	0.17056	0.18671
101	NN1 *B. gudu*	0.19210	0.19210	0.18851	0.19569	0.16697	0.16517	0.16876	0.17235	0.18133	0.16697	0.18492
102	OO3 *B. tugelae*	0.18492	0.18492	0.18312	0.19210	0.15978	0.15799	0.16697	0.16697	0.18492	0.16876	0.18671
103	OO2 *B. tugelae*	0.18492	0.18492	0.18312	0.19210	0.15978	0.15799	0.16697	0.16697	0.18492	0.16876	0.18671

Appendix 4.5. Continued. Uncorrected p-distances.

		89	90	91	92	93	94	95	96	97	98	99
89	KK1 *A. hawaquae*	-										
90	LL1 *A. outeniquae*	0.13465	-									
91	LL2 *A. outeniquae*	0.13465	0.00718	-								
92	Q2 *A. amatolae*	0.16876	0.15081	0.14542	-							
93	Q3 *A. amatolae*	0.16697	0.15260	0.14722	0.00180	-						
94	R2 *A. spinulata*	0.18492	0.16338	0.16158	0.11131	0.11311	-					
95	R3 *A. spinulata*	0.18492	0.16338	0.16158	0.11131	0.11311	0.00000	-				
96	QQ1 *D. pulchellum*	0.17594	0.17235	0.17235	0.16158	0.15978	0.15260	0.15260	-			
97	QQ3 *D. pulchellum*	0.17594	0.17235	0.17235	0.16158	0.15978	0.15260	0.15260	0.00000	-		
98	PP1 *D. brevis*	0.17415	0.17415	0.17415	0.15978	0.15799	0.15440	0.15440	0.00539	0.00539	-	
99	PP3 *D. brevis*	0.17594	0.17774	0.17415	0.15799	0.15619	0.15440	0.15440	0.01257	0.01257	0.01077	-
100	NN2 *B. gudu*	0.20108	0.17056	0.17235	0.18133	0.18312	0.19390	0.19390	0.17235	0.17235	0.17056	0.17415
101	NN1 *B. gudu*	0.19749	0.16517	0.16697	0.17953	0.18133	0.18851	0.18851	0.17056	0.17056	0.16876	0.17235
102	OO3 *B. tugelae*	0.18851	0.16876	0.17056	0.17594	0.17774	0.18492	0.18492	0.16517	0.16517	0.16338	0.16697
103	OO2 *B. tugelae*	0.18851	0.16876	0.17056	0.17774	0.17953	0.18492	0.18492	0.16517	0.16517	0.16338	0.16697

Appendix 4.5. Continued. Uncorrected p-distances.

		100	101	102	103
100	NN2 *B. gudu*	-			
101	NN1 *B. gudu*	0.00539	-		
102	OO3 *B. tugelae*	0.02693	0.02154	-	
103	OO2 *B. tugelae*	0.02873	0.02334	0.00180	-

Appendix 4.6. Corrected GTR distances with alpha 1.64 between the 102 local notonemourid individuals of 39 species and one outgroup taxon sampled. The code to the left of the taxon name is the specimen field code. The exact localities are provided in Table 4.2. To calculate percentage difference, multiply the value by 100.

		1	2	3	4	5	6	7	8	9	10	11
1	IM1 *Notonemoura latipennis*	-										
2	CC8 *A. clavata*	0.27242	-									
3	CC9 *A. clavata*	0.26596	0.00544	-								
4	JJ3 *A. denticulata*	0.28426	0.21229	0.21637	-							
5	JJ4 *A. denticulata*	0.28363	0.21135	0.21530	0.00544	-						
6	JJ5 *A. denticulata*	0.28730	0.21106	0.21502	0.00545	0.00730	-					
7	MM4 *A. tabularis*	0.27494	0.20276	0.20986	0.08641	0.08400	0.08635	-				
8	EE6 *A. nigra*	0.31762	0.17254	0.17588	0.22383	0.21959	0.22045	0.22626	-			
9	EE7 *A. nigra*	0.31629	0.16602	0.16922	0.22265	0.21841	0.21927	0.22893	0.00912	-		
10	L4a *A. mclellani*	0.25989	0.21255	0.21687	0.22544	0.22832	0.22798	0.22713	0.23130	0.22364	-	
11	G2 *A. witsenbergensis*	0.25989	0.21255	0.21687	0.22544	0.22832	0.22798	0.22713	0.23130	0.22364	0.00000	-
12	I3 *A. longiloba*	0.25989	0.21255	0.21687	0.22544	0.22832	0.22798	0.22713	0.23130	0.22364	0.00000	0.00000
13	L3 *A. mclellani*	0.25989	0.21255	0.21687	0.22544	0.22832	0.22798	0.22713	0.23130	0.22364	0.00000	0.00000
14	G1 *A. witsenbergensis*	0.25989	0.21255	0.21687	0.22544	0.22832	0.22798	0.22713	0.23130	0.22364	0.00000	0.00000
15	P4 *A. incisura*	0.25924	0.21733	0.22156	0.22523	0.22807	0.22766	0.22700	0.22787	0.22039	0.00546	0.00546
16	P1 *A. incisura*	0.25989	0.21449	0.21873	0.22240	0.22523	0.22483	0.22415	0.22845	0.22089	0.00364	0.00364
17	P5 *A. incisura*	0.26288	0.21486	0.21918	0.22261	0.22544	0.22504	0.22444	0.22869	0.22104	0.00181	0.00181
18	I2 *A. longiloba*	0.26268	0.21329	0.21760	0.22262	0.22549	0.22516	0.22429	0.23192	0.22418	0.00181	0.00181
19	I1 *A. longiloba*	0.26685	0.21133	0.21559	0.22609	0.22892	0.22848	0.23322	0.22522	0.21767	0.00361	0.00361
20	J2 *A. swartbergensis*	0.26376	0.21601	0.22031	0.22535	0.22823	0.22790	0.23042	0.23500	0.22722	0.00735	0.00735
21	J1 *A. swartbergensis*	0.25984	0.21226	0.21650	0.22517	0.22805	0.22771	0.23018	0.23865	0.23075	0.00548	0.00548
22	E6 *A. mclellani*	0.25676	0.21783	0.22223	0.22204	0.22490	0.22454	0.22379	0.23380	0.22615	0.00362	0.00362
23	L5 *A. mclellani*	0.25676	0.21783	0.22223	0.22204	0.22490	0.22454	0.22379	0.23380	0.22615	0.00362	0.00362
24	E1 *A. mclellani*	0.25676	0.21783	0.22223	0.22204	0.22490	0.22454	0.22379	0.23380	0.22615	0.00362	0.00362
25	L2 *A. mclellani*	0.26056	0.22149	0.22595	0.21869	0.22821	0.22780	0.22707	0.23741	0.22964	0.00543	0.00543
26	P3 *A. incisura*	0.26250	0.22118	0.22567	0.22352	0.22630	0.22581	0.23625	0.23749	0.22954	0.00727	0.00727
27	B5 *A. zwicki*	0.27280	0.21837	0.22277	0.21970	0.22252	0.22212	0.22485	0.22714	0.21972	0.01470	0.01470
28	B1 *A. zwicki*	0.26881	0.21462	0.21896	0.21637	0.21919	0.21884	0.22150	0.22357	0.21628	0.01283	0.01283
29	B2 *A. zwicki*	0.26881	0.21462	0.21896	0.21637	0.21919	0.21884	0.22150	0.22357	0.21628	0.01283	0.01283
30	B4 *A. zwicki*	0.26491	0.21095	0.21523	0.21310	0.21592	0.21562	0.22120	0.22702	0.21963	0.01470	0.01470
31	DDD2 *A. swartbergensis*	0.25904	0.20821	0.21233	0.22159	0.22446	0.22417	0.22653	0.23437	0.22666	0.00915	0.00915
32	O1 *A. swartbergensis*	0.26243	0.21426	0.21843	0.22217	0.22500	0.22460	0.23263	0.23795	0.22989	0.00912	0.00912
33	O2 *A. swartbergensis*	0.26977	0.21788	0.22212	0.23113	0.23406	0.23372	0.23597	0.23931	0.23133	0.01099	0.01099
34	C2 *A. breviloba*	0.27261	0.22075	0.22504	0.22847	0.23131	0.23087	0.23558	0.23984	0.23202	0.00727	0.00727
35	C1 *A. breviloba*	0.27261	0.22075	0.22504	0.22847	0.23131	0.23087	0.23558	0.23984	0.23202	0.00727	0.00727
36	H3 *A. cederbergensis*	0.27154	0.20544	0.20955	0.21057	0.21331	0.21292	0.22631	0.21755	0.21039	0.01656	0.01656
37	H2 *A. cederbergensis*	0.26846	0.21000	0.21422	0.20696	0.20974	0.20949	0.22367	0.22190	0.21452	0.01470	0.01470
38	N2 *A. austrocapensis*	0.26897	0.21735	0.22164	0.21883	0.22167	0.22137	0.22125	0.21461	0.20536	0.02807	0.02807
39	CCC3 *A. austrocapensis*	0.26897	0.21735	0.22164	0.21883	0.22167	0.22137	0.22125	0.21461	0.20536	0.02807	0.02807
40	N3 *A. austrocapensis*	0.26897	0.21735	0.22164	0.21883	0.22167	0.22137	0.22125	0.21461	0.20536	0.02807	0.02807
41	N4 *A. austrocapensis*	0.26897	0.21735	0.22164	0.21883	0.22167	0.22137	0.22125	0.21461	0.20536	0.02807	0.02807
42	M2 *A. austrocapensis*	0.26924	0.21725	0.22156	0.22145	0.22435	0.22414	0.22383	0.21459	0.20539	0.02804	0.02804
43	CCC1 *A. austrocapensis*	0.26516	0.21389	0.21812	0.21564	0.21848	0.21822	0.21805	0.21128	0.20213	0.03007	0.03007
44	D4 *A. brevispina*	0.25780	0.20482	0.20889	0.21832	0.22119	0.21520	0.22329	0.23535	0.22801	0.03209	0.03209
45	D3 *A. brevispina*	0.26520	0.21161	0.21579	0.22136	0.22423	0.21819	0.22629	0.23524	0.22792	0.03208	0.03208
46	F4 *A. pickeri*	0.26594	0.20356	0.20764	0.22592	0.22571	0.22822	0.22439	0.22956	0.22906	0.02625	0.02625
47	F2 *A. pickeri*	0.27283	0.20940	0.21355	0.23211	0.23190	0.23446	0.23035	0.23580	0.23531	0.03016	0.03016
48	X3 *A. uncinata*	0.27882	0.20876	0.21287	0.23189	0.23480	0.23437	0.22985	0.22678	0.22649	0.02810	0.02810
49	X4 *A. uncinata*	0.27167	0.20977	0.21393	0.23579	0.23870	0.23823	0.23374	0.22759	0.22731	0.02816	0.02816
50	T2 *A. bovina*	0.28338	0.21630	0.22077	0.22246	0.21861	0.22464	0.22493	0.19779	0.19345	0.07337	0.07337
51	T1 *A. bovina*	0.28583	0.20602	0.21023	0.21574	0.21212	0.21808	0.21813	0.19087	0.18676	0.07352	0.07352
52	A2 *A. capensis*	0.27980	0.21903	0.21636	0.21455	0.21734	0.21709	0.25312	0.20777	0.20359	0.07168	0.07168
53	A1 *A. capensis*	0.27980	0.21903	0.21636	0.21455	0.21734	0.21709	0.25312	0.20777	0.20359	0.07168	0.07168
54	U3 *A. chanae*	0.27401	0.18856	0.19215	0.20423	0.20083	0.20665	0.20461	0.21639	0.20915	0.07083	0.07083
55	U4 *A. chanae*	0.27470	0.18282	0.18638	0.20437	0.20087	0.20671	0.20455	0.20796	0.20083	0.07079	0.07079
56	S3 *A. bicornis*	0.25925	0.18095	0.18450	0.20553	0.20821	0.20781	0.20920	0.20227	0.19533	0.10289	0.10289
57	S5 *A. bicornis*	0.25925	0.18095	0.18450	0.20553	0.20821	0.20781	0.20920	0.20227	0.19533	0.10289	0.10289
58	S4 *A. bicornis*	0.26596	0.18641	0.19000	0.20615	0.20878	0.20829	0.21499	0.20317	0.19613	0.10764	0.10764
59	W2 *A. lyrata*	0.26687	0.17909	0.18266	0.20987	0.21248	0.21188	0.21870	0.19223	0.18531	0.09840	0.09840
60	W3 *A. lyrata*	0.26687	0.17909	0.18266	0.20987	0.21248	0.21188	0.21870	0.19223	0.18531	0.09840	0.09840
61	II3 *A. spatulata*	0.29405	0.05830	0.06081	0.24065	0.23645	0.23912	0.23002	0.16212	0.15551	0.21133	0.21133
62	HH4 *A. securata*	0.29710	0.06028	0.06276	0.24327	0.23907	0.24178	0.23269	0.16451	0.15794	0.21081	0.21081
63	Y1 *A. barnardi*	0.29066	0.05628	0.05879	0.24073	0.23655	0.23922	0.23016	0.15953	0.15295	0.20843	0.20843
64	Y4 *A. barnardi*	0.29405	0.05830	0.06081	0.24065	0.23645	0.23912	0.23002	0.16212	0.15551	0.21133	0.21133
65	II5 *A. spatulata*	0.28744	0.06012	0.06255	0.24816	0.24382	0.24650	0.23739	0.15942	0.15287	0.21334	0.21334

Appendix 4.6. Continued. Corrected distances.

		1	2	3	4	5	6	7	8	9	10	11
66	HH1 *A. securata*	0.29872	0.06248	0.06501	0.25229	0.24789	0.25053	0.23406	0.15379	0.14742	0.20632	0.20632
67	AA2 *A. bullata*	0.28638	0.05795	0.06025	0.23778	0.23358	0.23623	0.23547	0.15959	0.15291	0.21159	0.21159
68	CC2 *A. clavata*	0.29108	0.05815	0.06054	0.24171	0.23747	0.24010	0.23085	0.15893	0.15253	0.21343	0.21343
69	CC7 *A. clavata*	0.29108	0.05418	0.05663	0.24171	0.23747	0.24010	0.23085	0.15893	0.15253	0.20866	0.20866
70	FF2 *A. quadrata*	0.28792	0.05613	0.05851	0.24159	0.23734	0.24003	0.23040	0.16709	0.16034	0.21288	0.21288
71	AA5 *A. bullata*	0.28409	0.06239	0.06477	0.24339	0.23919	0.24198	0.23250	0.16823	0.16124	0.21166	0.21166
72	GG1 *A. scutata*	0.28504	0.05370	0.05171	0.24266	0.24248	0.24126	0.22358	0.15983	0.14852	0.20668	0.20668
73	GG2 *A. scutata*	0.28903	0.06212	0.06005	0.25487	0.25469	0.25346	0.23344	0.16362	0.15450	0.21287	0.21287
74	AA6 *A. bullata*	0.28274	0.06612	0.06828	0.24201	0.23785	0.24056	0.22554	0.16360	0.15993	0.23007	0.23007
75	DD2 *A. flabellata*	0.30562	0.08387	0.08401	0.23476	0.23364	0.22825	0.22435	0.16928	0.16006	0.21759	0.21759
76	DD1 *A. flabellata*	0.30677	0.07938	0.07952	0.23841	0.23726	0.23185	0.22836	0.16655	0.15742	0.21847	0.21847
77	AA7 *A. bullata*	0.29123	0.09167	0.09460	0.23603	0.23469	0.23445	0.21931	0.18833	0.18109	0.19777	0.19777
78	BB1 *A. cassida*	0.30615	0.13760	0.13980	0.23046	0.22925	0.22904	0.23969	0.19400	0.19010	0.20493	0.20493
79	JC1 *A. cassida*	0.30345	0.14288	0.14068	0.22770	0.22653	0.22629	0.23684	0.19477	0.19073	0.20219	0.20219
80	JD2 *A. cassida*	0.29000	0.14163	0.13944	0.22133	0.22028	0.22004	0.22811	0.19529	0.19118	0.18832	0.18832
81	JA5 *A. cassida*	0.29968	0.15369	0.15447	0.23733	0.23596	0.23579	0.24158	0.17389	0.17004	0.19851	0.19851
82	AB1 *A. pauletteae*	0.30713	0.12373	0.12598	0.21699	0.22263	0.22252	0.21063	0.16489	0.15767	0.21682	0.21682
83	AB2 *A. pauletteae*	0.30302	0.12642	0.12867	0.22039	0.22611	0.22599	0.21401	0.16178	0.15470	0.22039	0.22039
84	Z1 *A. bifurcata*	0.27405	0.14546	0.14950	0.21848	0.21722	0.21703	0.21014	0.18037	0.18582	0.23659	0.23659
85	Z3 *A. bifurcata*	0.32191	0.14626	0.15044	0.24044	0.23913	0.23890	0.23490	0.19154	0.19019	0.24950	0.24950
86	JJ2 *A. denticulata*	0.28057	0.20897	0.21299	0.00181	0.00361	0.00364	0.08408	0.22038	0.21921	0.22216	0.22216
87	MM1 *A. tabularis*	0.27826	0.20037	0.20747	0.08187	0.08408	0.08181	0.00361	0.22688	0.22951	0.22119	0.22119
88	KK2 *A. hawaquae*	0.26061	0.19005	0.19082	0.14724	0.14980	0.14692	0.12257	0.21896	0.21469	0.17966	0.17966
89	KK1 *A. hawaquae*	0.27645	0.18364	0.18443	0.16857	0.16597	0.16295	0.15001	0.20328	0.19628	0.22667	0.22667
90	LL1 *A. outeniquae*	0.23657	0.21722	0.22098	0.16350	0.16909	0.16890	0.13684	0.21968	0.21834	0.20976	0.20976
91	LL2 *A. outeniquae*	0.23998	0.21755	0.22134	0.16953	0.16915	0.16890	0.13681	0.21303	0.21175	0.20314	0.20314
92	Q2 *A. amatolae*	0.24044	0.21965	0.22714	0.21285	0.21564	0.21538	0.20331	0.22672	0.22220	0.16349	0.16349
93	Q3 *A. amatolae*	0.24392	0.22300	0.23060	0.21601	0.21880	0.21849	0.20635	0.22309	0.21863	0.16655	0.16655
94	R2 *A. spinulata*	0.26766	0.20681	0.21156	0.24004	0.23510	0.24153	0.23175	0.24259	0.24177	0.16183	0.16183
95	R3 *A. spinulata*	0.26766	0.20681	0.21156	0.24004	0.23510	0.24153	0.23175	0.24259	0.24177	0.16183	0.16183
96	QQ1 *D. pulchellum*	0.31490	0.21774	0.22007	0.24072	0.23632	0.23947	0.21161	0.21246	0.20627	0.20200	0.20200
97	QQ3 *D. pulchellum*	0.31490	0.21774	0.22007	0.24072	0.23632	0.23947	0.21161	0.21246	0.20627	0.20200	0.20200
98	PP1 *D. brevis*	0.31189	0.22285	0.22513	0.23796	0.23351	0.23667	0.20901	0.21688	0.21062	0.19974	0.19974
99	PP3 *D. brevis*	0.30871	0.22555	0.22787	0.23785	0.23342	0.23656	0.21258	0.21323	0.21191	0.19504	0.19504
100	NN2 *B. gudu*	0.28232	0.22693	0.23694	0.22982	0.23970	0.23893	0.22537	0.23370	0.22618	0.23644	0.23644
101	NN1 *B. gudu*	0.27964	0.21814	0.22795	0.23563	0.24563	0.24489	0.21899	0.23250	0.22487	0.22761	0.22761
102	OO3 *B. tugelae*	0.27624	0.20773	0.21713	0.24146	0.25159	0.25082	0.22158	0.22449	0.21714	0.22761	0.22761
103	OO2 *B. tugelae*	0.27928	0.20727	0.21666	0.24132	0.25145	0.25068	0.22135	0.22415	0.21683	0.23024	0.23024

Appendix 4.6. Continued. Corrected distances.

		12	13	14	15	16	17	18	19	20	21	22
12	I3 *A. longiloba*	-										
13	L3 *A. mclellani*	0.00000	-									
14	G1 *A. witsenbergensis*	0.00000	0.00000	-								
15	P4 *A. incisura*	0.00546	0.00546	0.00546	-							
16	P1 *A. incisura*	0.00364	0.00364	0.00364	0.00181	-						
17	P5 *A. incisura*	0.00181	0.00181	0.00181	0.00362	0.00181	-					
18	I2 *A. longiloba*	0.00181	0.00181	0.00181	0.00730	0.00546	0.00362	-				
19	I1 *A. longiloba*	0.00361	0.00361	0.00361	0.00913	0.00730	0.00545	0.00543	-			
20	J2 *A. swartbergensis*	0.00735	0.00735	0.00735	0.01283	0.01099	0.00915	0.00918	0.01105	-		
21	J1 *A. swartbergensis*	0.00548	0.00548	0.00548	0.01095	0.00912	0.00729	0.00730	0.00915	0.00181	-	
22	E6 *A. mclellani*	0.00362	0.00362	0.00362	0.00914	0.00732	0.00545	0.00543	0.00726	0.01098	0.00911	-
23	L5 *A. mclellani*	0.00362	0.00362	0.00362	0.00914	0.00732	0.00545	0.00543	0.00726	0.01098	0.00911	0.00000
24	E1 *A. mclellani*	0.00362	0.00362	0.00362	0.00914	0.00732	0.00545	0.00543	0.00726	0.01098	0.00911	0.00000
25	L2 *A. mclellani*	0.00543	0.00543	0.00543	0.01096	0.00913	0.00726	0.00725	0.00909	0.01287	0.01098	0.00181
26	P3 *A. incisura*	0.00727	0.00727	0.00727	0.01285	0.01101	0.00913	0.00910	0.01098	0.01479	0.01288	0.01095
27	B5 *A. zwicki*	0.01470	0.01470	0.01470	0.01845	0.01655	0.01657	0.01661	0.01846	0.02239	0.02043	0.01469
28	B1 *A. zwicki*	0.01283	0.01283	0.01283	0.01657	0.01468	0.01470	0.01473	0.01657	0.02043	0.01850	0.01281
29	B2 *A. zwicki*	0.01283	0.01283	0.01283	0.01657	0.01468	0.01470	0.01473	0.01657	0.02043	0.01850	0.01281
30	B4 *A. zwicki*	0.01470	0.01470	0.01470	0.01844	0.01655	0.01657	0.01661	0.01846	0.02239	0.02043	0.01469
31	DDD2 *A. swartbergensis*	0.00915	0.00915	0.00915	0.01469	0.01284	0.01099	0.00918	0.01288	0.00544	0.00361	0.01282
32	O1 *A. swartbergensis*	0.00912	0.00912	0.00912	0.01471	0.01286	0.01098	0.00912	0.01284	0.00545	0.00364	0.01281
33	O2 *A. swartbergensis*	0.01099	0.01099	0.01099	0.01658	0.01473	0.01284	0.01100	0.01473	0.00727	0.00545	0.01468
34	C2 *A. breviloba*	0.00727	0.00727	0.00727	0.01288	0.01102	0.00913	0.00726	0.01096	0.01473	0.01283	0.01096
35	C1 *A. breviloba*	0.00727	0.00727	0.00727	0.01288	0.01102	0.00913	0.00726	0.01096	0.01473	0.01283	0.01096
36	H3 *A. cederbergensis*	0.01656	0.01656	0.01656	0.02034	0.01845	0.01846	0.01846	0.01660	0.02431	0.02234	0.02031
37	H2 *A. cederbergensis*	0.01470	0.01470	0.01470	0.01844	0.01656	0.01656	0.01659	0.01476	0.02245	0.02047	0.01841

Appendix 4.6. Continued. Corrected distances.

		12	13	14	15	16	17	18	19	20	21	22
38	N2 *A. austrocapensis*	0.02807	0.02807	0.02807	0.03192	0.02996	0.02999	0.03004	0.03199	0.03620	0.03412	0.02805
39	CCC3 *A. austrocapensis*	0.02807	0.02807	0.02807	0.03192	0.02996	0.02999	0.03004	0.03199	0.03620	0.03412	0.02805
40	N3 *A. austrocapensis*	0.02807	0.02807	0.02807	0.03192	0.02996	0.02999	0.03004	0.03199	0.03620	0.03412	0.02805
41	N4 *A. austrocapensis*	0.02807	0.02807	0.02807	0.03192	0.02996	0.02999	0.03004	0.03199	0.03620	0.03412	0.02805
42	M2 *A. austrocapensis*	0.02804	0.02804	0.02804	0.03190	0.02996	0.02997	0.02999	0.03196	0.03613	0.03407	0.02800
43	CCC1 *A. austrocapensis*	0.03007	0.03007	0.03007	0.03392	0.03195	0.03199	0.03205	0.03401	0.03830	0.03620	0.03006
44	D4 *A. brevispina*	0.03209	0.03209	0.03209	0.03601	0.03406	0.03406	0.03404	0.03609	0.04032	0.03822	0.03201
45	D3 *A. brevispina*	0.03208	0.03208	0.03208	0.03600	0.03406	0.03406	0.03403	0.03608	0.04031	0.03821	0.03201
46	F4 *A. pickeri*	0.02625	0.02625	0.02625	0.03008	0.02813	0.02815	0.02820	0.03016	0.03442	0.03234	0.03002
47	F2 *A. pickeri*	0.03016	0.03016	0.03016	0.03402	0.03206	0.03208	0.03212	0.03412	0.03842	0.03631	0.03396
48	X3 *A. uncinata*	0.02810	0.02810	0.02810	0.03198	0.03004	0.03005	0.03006	0.03204	0.03618	0.03412	0.02804
49	X4 *A. uncinata*	0.02816	0.02816	0.02816	0.03201	0.03007	0.03008	0.03012	0.03210	0.03633	0.03425	0.02813
50	T2 *A. bovina*	0.07337	0.07337	0.07337	0.07092	0.07118	0.07128	0.07556	0.07360	0.08275	0.08034	0.07752
51	T1 *A. bovina*	0.07352	0.07352	0.07352	0.07104	0.07131	0.07140	0.07571	0.07372	0.08289	0.08047	0.07768
52	A2 *A. capensis*	0.07168	0.07168	0.07168	0.07612	0.07395	0.07403	0.07387	0.07616	0.07595	0.07379	0.07138
53	A1 *A. capensis*	0.07168	0.07168	0.07168	0.07612	0.07395	0.07403	0.07387	0.07616	0.07595	0.07379	0.07138
54	U3 *A. chanae*	0.07083	0.07083	0.07083	0.07075	0.06864	0.06868	0.06873	0.07526	0.07531	0.07304	0.07504
55	U4 *A. chanae*	0.07079	0.07079	0.07079	0.07076	0.06865	0.06870	0.06869	0.07519	0.07534	0.07303	0.07496
56	S3 *A. bicornis*	0.10289	0.10289	0.10289	0.10024	0.10058	0.10069	0.10060	0.10768	0.11326	0.11057	0.10731
57	S5 *A. bicornis*	0.10289	0.10289	0.10289	0.10024	0.10058	0.10069	0.10060	0.10768	0.11326	0.11057	0.10731
58	S4 *A. bicornis*	0.10764	0.10764	0.10764	0.10494	0.10530	0.10542	0.10534	0.10803	0.11815	0.11543	0.11212
59	W2 *A. lyrata*	0.09840	0.09840	0.09840	0.09580	0.09612	0.09625	0.09873	0.10313	0.10871	0.10605	0.10279
60	W3 *A. lyrata*	0.09840	0.09840	0.09840	0.09580	0.09612	0.09625	0.09873	0.10313	0.10871	0.10605	0.10279
61	II3 *A. spatulata*	0.21133	0.21133	0.21133	0.21265	0.21315	0.21367	0.21186	0.21026	0.21454	0.21811	0.21675
62	HH4 *A. securata*	0.21081	0.21081	0.21081	0.21216	0.21268	0.21315	0.21137	0.20974	0.21404	0.21761	0.21620
63	Y1 *A. barnardi*	0.20843	0.20843	0.20843	0.20975	0.21027	0.21076	0.20899	0.20736	0.21166	0.21522	0.21380
64	Y4 *A. barnardi*	0.21133	0.21133	0.21133	0.21265	0.21315	0.21367	0.21186	0.21026	0.21454	0.21811	0.21675
65	II5 *A. spatulata*	0.21334	0.21334	0.21334	0.21470	0.21522	0.21569	0.21390	0.21228	0.21661	0.22019	0.21875
66	HH1 *A. securata*	0.20632	0.20632	0.20632	0.20777	0.20820	0.20870	0.20678	0.20540	0.20946	0.21291	0.21173
67	AA2 *A. bullata*	0.21159	0.21159	0.21159	0.21309	0.21351	0.21402	0.21204	0.20582	0.21471	0.21819	0.21710
68	CC2 *A. clavata*	0.21343	0.21343	0.21343	0.21481	0.21531	0.21583	0.21397	0.21244	0.21669	0.22025	0.21890
69	CC7 *A. clavata*	0.20866	0.20866	0.20866	0.20995	0.21048	0.21099	0.20923	0.20760	0.21193	0.21549	0.21404
70	FF2 *A. quadrata*	0.21288	0.21288	0.21288	0.21430	0.21475	0.21530	0.21337	0.21195	0.21608	0.21960	0.21839
71	AA5 *A. bullata*	0.21166	0.21166	0.21166	0.21294	0.21346	0.21400	0.21221	0.21059	0.21492	0.21850	0.21708
72	GG1 *A. scutata*	0.20668	0.20668	0.20668	0.20813	0.20862	0.20908	0.20721	0.20575	0.21700	0.21334	0.21209
73	GG2 *A. scutata*	0.21287	0.21287	0.21287	0.21432	0.21474	0.21526	0.21332	0.21190	0.22315	0.21947	0.21839
74	AA6 *A. bullata*	0.23007	0.23007	0.23007	0.22649	0.22679	0.22739	0.23038	0.22940	0.22976	0.23320	0.23062
75	DD2 *A. flabellata*	0.21759	0.21759	0.21759	0.21413	0.21448	0.21502	0.21498	0.21680	0.22413	0.22055	0.22330
76	DD1 *A. flabellata*	0.21847	0.21847	0.21847	0.21501	0.21541	0.21594	0.21585	0.21759	0.22511	0.22148	0.22415
77	AA7 *A. bullata*	0.19777	0.19777	0.19777	0.19950	0.19972	0.20023	0.19803	0.19717	0.20720	0.20387	0.20326
78	BB1 *A. cassida*	0.20493	0.20493	0.20493	0.20156	0.20191	0.20239	0.20529	0.20423	0.21469	0.21121	0.21049
79	JC1 *A. cassida*	0.20219	0.20219	0.20219	0.19883	0.19912	0.19965	0.20250	0.20155	0.21180	0.20837	0.20777
80	JD2 *A. cassida*	0.18832	0.18832	0.18832	0.18507	0.18539	0.18586	0.18865	0.18766	0.19772	0.19437	0.19371
81	JA5 *A. cassida*	0.19851	0.19851	0.19851	0.19520	0.19564	0.19613	0.19896	0.19285	0.20846	0.20490	0.20389
82	AB1 *A. pauletteae*	0.21682	0.21682	0.21682	0.21693	0.21412	0.21432	0.21402	0.21594	0.22007	0.21650	0.22235
83	AB2 *A. pauletteae*	0.22039	0.22039	0.22039	0.21719	0.21769	0.21789	0.21756	0.21945	0.22370	0.22007	0.22593
84	Z1 *A. bifurcata*	0.23659	0.23659	0.23659	0.23283	0.23339	0.23391	0.23717	0.23036	0.23630	0.23998	0.24243
85	Z3 *A. bifurcata*	0.24950	0.24950	0.24950	0.24568	0.24643	0.24696	0.25027	0.24817	0.24879	0.25274	0.25530
86	JJ2 *A. denticulata*	0.22216	0.22216	0.22216	0.22193	0.21913	0.21933	0.21936	0.22276	0.22208	0.22190	0.21878
87	MM1 *A. tabularis*	0.22119	0.22119	0.22119	0.22108	0.21827	0.21855	0.21839	0.22719	0.22444	0.22421	0.21788
88	KK2 *A. hawaquae*	0.17966	0.17966	0.17966	0.18447	0.18180	0.18203	0.17702	0.18042	0.18258	0.18253	0.17937
89	KK1 *A. hawaquae*	0.22667	0.22667	0.22667	0.23150	0.22874	0.22928	0.22716	0.23300	0.23364	0.22991	0.22633
90	LL1 *A. outeniquae*	0.20976	0.20976	0.20976	0.21240	0.20976	0.20729	0.20715	0.21571	0.20890	0.21229	0.20617
91	LL2 *A. outeniquae*	0.20314	0.20314	0.20314	0.20578	0.20314	0.20070	0.20054	0.20896	0.20236	0.20564	0.19958
92	Q2 *A. amatolae*	0.16349	0.16349	0.16349	0.16303	0.16349	0.16116	0.16590	0.16283	0.16966	0.16649	0.16844
93	Q3 *A. amatolae*	0.16655	0.16655	0.16655	0.16606	0.16655	0.16422	0.16897	0.16586	0.17282	0.16959	0.17152
94	R2 *A. spinulata*	0.16183	0.16183	0.16183	0.16432	0.16183	0.15953	0.15937	0.15647	0.16500	0.16182	0.16206
95	R3 *A. spinulata*	0.16183	0.16183	0.16183	0.16432	0.16183	0.15953	0.15937	0.15647	0.16500	0.16182	0.16206
96	QQ1 *D. pulchellum*	0.20200	0.20200	0.20200	0.20585	0.20660	0.20427	0.20282	0.20806	0.20929	0.21302	0.20450
97	QQ3 *D. pulchellum*	0.20200	0.20200	0.20200	0.20585	0.20660	0.20427	0.20282	0.20806	0.20929	0.21302	0.20450
98	PP1 *D. brevis*	0.19974	0.19974	0.19974	0.20351	0.20427	0.20197	0.20057	0.20576	0.19961	0.20322	0.20224
99	PP3 *D. brevis*	0.19504	0.19504	0.19504	0.19900	0.19964	0.19730	0.19574	0.20095	0.20208	0.20569	0.19749
100	NN2 *B. gudu*	0.23644	0.23644	0.23644	0.23599	0.23644	0.23378	0.23914	0.23755	0.22874	0.23220	0.24226
101	NN1 *B. gudu*	0.22761	0.22761	0.22761	0.22719	0.22761	0.22502	0.23025	0.22870	0.22000	0.22340	0.23333
102	OO3 *B. tugelae*	0.22761	0.22761	0.22761	0.22719	0.22761	0.22503	0.23021	0.22675	0.21990	0.22332	0.23333
103	OO2 *B. tugelae*	0.23024	0.23024	0.23024	0.22982	0.23024	0.22761	0.23014	0.22942	0.22252	0.22594	0.23602

Appendix 4.6. Continued. Corrected distances.

		23	24	25	26	27	28	29	30	31	32	33
23	L5 *A. mclellani*	-										
24	E1 *A. mclellani*	0.00000	-									
25	L2 *A. mclellani*	0.00181	0.00181	-								
26	P3 *A. incisura*	0.01095	0.01095	0.01281	-							
27	B5 *A. zwicki*	0.01469	0.01469	0.01658	0.02227	-						
28	B1 *A. zwicki*	0.01281	0.01281	0.01469	0.02036	0.00181	-					
29	B2 *A. zwicki*	0.01281	0.01281	0.01469	0.02036	0.00181	0.00000	-				
30	B4 *A. zwicki*	0.01469	0.01469	0.01658	0.02227	0.00363	0.00181	0.00181	-			
31	DDD2 *A. swartbergensis*	0.01282	0.01282	0.01471	0.01665	0.02426	0.02230	0.02230	0.02426	-		
32	O1 *A. swartbergensis*	0.01281	0.01281	0.01468	0.01288	0.02421	0.02227	0.02227	0.02420	0.00361	-	
33	O2 *A. swartbergensis*	0.01468	0.01468	0.01657	0.01853	0.02616	0.02420	0.02420	0.02616	0.00544	0.00544	-
34	C2 *A. breviloba*	0.01096	0.01096	0.01279	0.01102	0.02229	0.02039	0.02039	0.02228	0.01289	0.01283	0.01472
35	C1 *A. breviloba*	0.01096	0.01096	0.01279	0.01102	0.02229	0.02039	0.02039	0.02228	0.01289	0.01283	0.01472
36	H3 *A. cederbergensis*	0.02031	0.02031	0.02221	0.02421	0.01659	0.01469	0.01469	0.01659	0.02228	0.02615	0.02812
37	H2 *A. cederbergensis*	0.01841	0.01841	0.02031	0.02229	0.01473	0.01283	0.01283	0.01473	0.02039	0.02423	0.02620
38	N2 *A. austrocapensis*	0.02805	0.02805	0.03005	0.03594	0.02043	0.01849	0.01849	0.01657	0.03810	0.03797	0.04004
39	CCC3 *A. austrocapensis*	0.02805	0.02805	0.03005	0.03594	0.02043	0.01849	0.01849	0.01657	0.03810	0.03797	0.04004
40	N3 *A. austrocapensis*	0.02805	0.02805	0.03005	0.03594	0.02043	0.01849	0.01849	0.01657	0.03810	0.03797	0.04004
41	N4 *A. austrocapensis*	0.02805	0.02805	0.03005	0.03594	0.02043	0.01849	0.01849	0.01657	0.03810	0.03797	0.04004
42	M2 *A. austrocapensis*	0.02800	0.02800	0.02999	0.03593	0.02038	0.01845	0.01845	0.01654	0.03806	0.03796	0.04002
43	CCC1 *A. austrocapensis*	0.03006	0.03006	0.03208	0.03799	0.02240	0.02043	0.02043	0.01849	0.04020	0.04005	0.04214
44	D4 *A. brevispina*	0.03201	0.03201	0.03404	0.04013	0.02832	0.02630	0.02630	0.02832	0.03812	0.04219	0.04429
45	D3 *A. brevispina*	0.03201	0.03201	0.03403	0.04013	0.02832	0.02630	0.02630	0.02831	0.03811	0.04219	0.04428
46	F4 *A. pickeri*	0.03002	0.03002	0.03203	0.03412	0.02633	0.02433	0.02433	0.02632	0.03218	0.03616	0.03824
47	F2 *A. pickeri*	0.03396	0.03396	0.03600	0.03404	0.03024	0.02822	0.02822	0.03024	0.03615	0.04019	0.04229
48	X3 *A. uncinata*	0.02804	0.02804	0.03003	0.03395	0.02434	0.02236	0.02236	0.02433	0.03812	0.03805	0.04010
49	X4 *A. uncinata*	0.02813	0.02813	0.03014	0.03397	0.02447	0.02247	0.02247	0.02446	0.03825	0.03812	0.04020
50	T2 *A. bovina*	0.07752	0.07752	0.07982	0.08230	0.07323	0.07549	0.07549	0.07777	0.08484	0.08462	0.08700
51	T1 *A. bovina*	0.07768	0.07768	0.07998	0.08249	0.07343	0.07568	0.07568	0.07796	0.08500	0.08481	0.08718
52	A2 *A. capensis*	0.07138	0.07138	0.07350	0.07596	0.07166	0.06953	0.06953	0.07164	0.07827	0.07378	0.07593
53	A1 *A. capensis*	0.07138	0.07138	0.07350	0.07596	0.07166	0.06953	0.06953	0.07164	0.07827	0.07378	0.07593
54	U3 *A. chanae*	0.07504	0.07504	0.07727	0.07972	0.07536	0.07313	0.07313	0.07322	0.07077	0.07519	0.07743
55	U4 *A. chanae*	0.07496	0.07496	0.07722	0.07964	0.07524	0.07297	0.07297	0.07306	0.07072	0.07512	0.07740
56	S3 *A. bicornis*	0.10731	0.10731	0.10985	0.11251	0.10850	0.10587	0.10587	0.10845	0.10783	0.11270	0.11533
57	S5 *A. bicornis*	0.10731	0.10731	0.10985	0.11251	0.10850	0.10587	0.10587	0.10845	0.10783	0.11270	0.11533
58	S4 *A. bicornis*	0.11212	0.11212	0.11469	0.11740	0.11338	0.11072	0.11072	0.11332	0.11265	0.11759	0.12025
59	W2 *A. lyrata*	0.10279	0.10279	0.10531	0.10790	0.10392	0.10132	0.10132	0.10388	0.10330	0.10811	0.10639
60	W3 *A. lyrata*	0.10279	0.10279	0.10531	0.10790	0.10392	0.10132	0.10132	0.10388	0.10330	0.10811	0.10639
61	II3 *A. spatulata*	0.21675	0.21675	0.22030	0.21709	0.21956	0.21591	0.21591	0.21234	0.21394	0.21716	0.22364
62	HH4 *A. securata*	0.21620	0.21620	0.21975	0.21659	0.21908	0.21544	0.21544	0.21186	0.21349	0.21676	0.22319
63	Y1 *A. barnardi*	0.21380	0.21380	0.21733	0.21416	0.21960	0.21597	0.21597	0.21240	0.21108	0.21432	0.22075
64	Y4 *A. barnardi*	0.21675	0.21675	0.22030	0.21709	0.21956	0.21591	0.21591	0.21234	0.21394	0.21716	0.22364
65	II5 *A. spatulata*	0.21875	0.21875	0.22230	0.21911	0.22167	0.21802	0.21802	0.21443	0.21605	0.21934	0.22578
66	HH1 *A. securata*	0.21173	0.21173	0.21515	0.21197	0.21786	0.21428	0.21428	0.21076	0.20890	0.21201	0.21838
67	AA2 *A. bullata*	0.21710	0.21710	0.22055	0.21735	0.21980	0.21626	0.21626	0.21280	0.21413	0.21724	0.22371
68	CC2 *A. clavata*	0.21890	0.21890	0.22243	0.21917	0.22178	0.21814	0.21814	0.21457	0.21608	0.21929	0.22583
69	CC7 *A. clavata*	0.21404	0.21404	0.21757	0.21433	0.21692	0.21328	0.21328	0.20971	0.21132	0.21454	0.22099
70	FF2 *A. quadrata*	0.21839	0.21839	0.22187	0.21860	0.22116	0.21757	0.21757	0.21405	0.21545	0.21858	0.22512
71	AA5 *A. bullata*	0.21708	0.21708	0.22063	0.21735	0.21994	0.21628	0.21628	0.21269	0.21428	0.21750	0.22401
72	GG1 *A. scutata*	0.21209	0.21209	0.21555	0.21243	0.21491	0.21134	0.21134	0.21480	0.20929	0.21249	0.21889
73	GG2 *A. scutata*	0.21839	0.21839	0.22190	0.21874	0.22101	0.21743	0.21743	0.22093	0.21536	0.21851	0.22501
74	AA6 *A. bullata*	0.23062	0.23062	0.23409	0.23561	0.23368	0.23012	0.23012	0.22663	0.22907	0.23202	0.23879
75	DD2 *A. flabellata*	0.22330	0.22330	0.22677	0.22361	0.22579	0.22226	0.22226	0.21880	0.22397	0.21993	0.22658
76	DD1 *A. flabellata*	0.22415	0.22415	0.22767	0.22450	0.22668	0.22310	0.22310	0.21958	0.22495	0.22086	0.22752
77	AA7 *A. bullata*	0.20326	0.20326	0.20646	0.20330	0.20372	0.20048	0.20048	0.19730	0.20011	0.20292	0.20263
78	BB1 *A. cassida*	0.21049	0.21049	0.21383	0.21419	0.21609	0.21271	0.21271	0.20938	0.21415	0.21066	0.20989
79	JC1 *A. cassida*	0.20777	0.20777	0.21107	0.21136	0.21332	0.20998	0.20998	0.20670	0.21121	0.20776	0.20706
80	JD2 *A. cassida*	0.19371	0.19371	0.19692	0.19727	0.19911	0.19585	0.19585	0.19265	0.19720	0.19386	0.19312
81	JA5 *A. cassida*	0.20389	0.20389	0.20729	0.20782	0.20936	0.20590	0.20590	0.20251	0.20789	0.20442	0.20334
82	AB1 *A. pauletteae*	0.22235	0.22235	0.21884	0.22257	0.21969	0.21614	0.21614	0.21591	0.21973	0.21580	0.22227
83	AB2 *A. pauletteae*	0.22593	0.22593	0.22235	0.22619	0.22331	0.21969	0.21969	0.21947	0.22336	0.21936	0.22589
84	Z1 *A. bifurcata*	0.24243	0.24243	0.24613	0.24331	0.25312	0.24915	0.24915	0.25296	0.23565	0.23933	0.23836
85	Z3 *A. bifurcata*	0.25530	0.25530	0.25930	0.25646	0.26246	0.25821	0.25821	0.26226	0.25627	0.25212	0.24567
86	JJ2 *A. denticulata*	0.21878	0.21878	0.22204	0.22019	0.21644	0.21316	0.21316	0.20994	0.21837	0.21890	0.22781
87	MM1 *A. tabularis*	0.21788	0.21788	0.22112	0.23016	0.21893	0.21563	0.21563	0.21534	0.22060	0.22660	0.22990
88	KK2 *A. hawaquae*	0.17937	0.17937	0.18238	0.18774	0.18196	0.17894	0.17894	0.18191	0.18531	0.18467	0.18773
89	KK1 *A. hawaquae*	0.22633	0.22633	0.22988	0.23076	0.23260	0.22898	0.22898	0.23252	0.22572	0.22680	0.23041

Appendix 4.6. Continued. Corrected distances.

		23	24	25	26	27	28	29	30	31	32	33
90	LL1 *A. outeniquae*	0.20617	0.20617	0.20280	0.21164	0.21772	0.21425	0.21425	0.21765	0.21557	0.21156	0.21807
91	LL2 *A. outeniquae*	0.19958	0.19958	0.20286	0.20500	0.21097	0.20761	0.20761	0.21091	0.20879	0.20488	0.21126
92	Q2 *A. amatolae*	0.16844	0.16844	0.17152	0.16562	0.16773	0.16467	0.16467	0.16167	0.17196	0.16861	0.17437
93	Q3 *A. amatolae*	0.17152	0.17152	0.17465	0.16868	0.17077	0.16766	0.16766	0.16461	0.17511	0.17172	0.17752
94	R2 *A. spinulata*	0.16206	0.16206	0.16519	0.17288	0.16994	0.16673	0.16673	0.16358	0.16493	0.16415	0.16727
95	R3 *A. spinulata*	0.16206	0.16206	0.16519	0.17288	0.16994	0.16673	0.16673	0.16358	0.16493	0.16415	0.16727
96	QQ1 *D. pulchellum*	0.20450	0.20450	0.20814	0.20171	0.20224	0.20578	0.20578	0.20938	0.21652	0.21249	0.21115
97	QQ3 *D. pulchellum*	0.20450	0.20450	0.20814	0.20171	0.20224	0.20578	0.20578	0.20938	0.21652	0.21249	0.21115
98	PP1 *D. brevis*	0.20224	0.20224	0.20587	0.19945	0.19996	0.20350	0.20350	0.20710	0.20660	0.20269	0.20146
99	PP3 *D. brevis*	0.19749	0.19749	0.20100	0.19469	0.19535	0.19877	0.19877	0.20225	0.20901	0.20506	0.20393
100	NN2 *B. gudu*	0.24226	0.24226	0.23862	0.24587	0.24740	0.25103	0.25103	0.25473	0.23841	0.23763	0.24115
101	NN1 *B. gudu*	0.23333	0.23333	0.22977	0.23688	0.23834	0.24191	0.24191	0.24554	0.22949	0.22871	0.23217
102	OO3 *B. tugelae*	0.23333	0.23333	0.22976	0.23167	0.23829	0.24188	0.24188	0.24552	0.22941	0.22337	0.23208
103	OO2 *B. tugelae*	0.23602	0.23602	0.23244	0.23431	0.24100	0.24458	0.24458	0.24823	0.22685	0.22085	0.22947

Appendix 4.6. Continued. Corrected distances.

		34	35	36	37	38	39	40	41	42	43	44
34	C2 *A. breviloba*	-										
35	C1 *A. breviloba*	0.00000	-									
36	H3 *A. cederbergensis*	0.02037	0.02037	-								
37	H2 *A. cederbergensis*	0.02227	0.02227	0.00544	-							
38	N2 *A. austrocapensis*	0.03591	0.03591	0.03009	0.02819	-						
39	CCC3 *A. austrocapensis*	0.03591	0.03591	0.03009	0.02819	0.00000	-					
40	N3 *A. austrocapensis*	0.03591	0.03591	0.03009	0.02819	0.00000	0.00000	-				
41	N4 *A. austrocapensis*	0.03591	0.03591	0.03009	0.02819	0.00000	0.00000	0.00000	-			
42	M2 *A. austrocapensis*	0.03589	0.03589	0.03010	0.02818	0.00362	0.00362	0.00362	0.00362	-		
43	CCC1 *A. austrocapensis*	0.03794	0.03794	0.03212	0.03022	0.00181	0.00181	0.00181	0.00181	0.00543	-	
44	D4 *A. brevispina*	0.04005	0.04005	0.03021	0.02825	0.03002	0.03002	0.03002	0.03002	0.03006	0.03203	-
45	D3 *A. brevispina*	0.04005	0.04005	0.03021	0.02825	0.03002	0.03002	0.03002	0.03002	0.03005	0.03202	0.00363
46	F4 *A. pickeri*	0.03405	0.03405	0.02431	0.02242	0.02804	0.02804	0.02804	0.02804	0.02804	0.03003	0.01282
47	F2 *A. pickeri*	0.03399	0.03399	0.02821	0.02630	0.03196	0.03196	0.03196	0.03196	0.03198	0.03398	0.01283
48	X3 *A. uncinata*	0.03599	0.03599	0.03016	0.02822	0.02608	0.02608	0.02608	0.02608	0.02609	0.02805	0.01850
49	X4 *A. uncinata*	0.03602	0.03602	0.03023	0.02832	0.02614	0.02614	0.02614	0.02614	0.02612	0.02813	0.01848
50	T2 *A. bovina*	0.08216	0.08216	0.07116	0.06905	0.06910	0.06910	0.06910	0.06910	0.06916	0.07136	0.08921
51	T1 *A. bovina*	0.08235	0.08235	0.07132	0.06918	0.06930	0.06930	0.06930	0.06930	0.06938	0.07157	0.08949
52	A2 *A. capensis*	0.07395	0.07395	0.06971	0.07207	0.07385	0.07385	0.07385	0.07385	0.07409	0.07600	0.08332
53	A1 *A. capensis*	0.07395	0.07395	0.06971	0.07207	0.07385	0.07385	0.07385	0.07385	0.07409	0.07600	0.08332
54	U3 *A. chanae*	0.07296	0.07296	0.06448	0.06668	0.06660	0.06660	0.06660	0.06660	0.06675	0.06878	0.07800
55	U4 *A. chanae*	0.07300	0.07300	0.06432	0.06647	0.06643	0.06643	0.06643	0.06643	0.06652	0.06863	0.07758
56	S3 *A. bicornis*	0.10085	0.10085	0.10316	0.10541	0.10792	0.10792	0.10792	0.10792	0.10793	0.10536	0.11230
57	S5 *A. bicornis*	0.10085	0.10085	0.10316	0.10541	0.10792	0.10792	0.10792	0.10792	0.10793	0.10536	0.11230
58	S4 *A. bicornis*	0.10559	0.10559	0.10350	0.10571	0.11277	0.11277	0.11277	0.11277	0.11279	0.11017	0.11724
59	W2 *A. lyrata*	0.10083	0.10083	0.09869	0.10089	0.10340	0.10340	0.10340	0.10340	0.10344	0.10086	0.10760
60	W3 *A. lyrata*	0.10083	0.10083	0.09869	0.10089	0.10340	0.10340	0.10340	0.10340	0.10344	0.10086	0.10760
61	II3 *A. spatulata*	0.21456	0.21456	0.20378	0.21313	0.21370	0.21370	0.21370	0.21370	0.21363	0.21034	0.20795
62	HH4 *A. securata*	0.21408	0.21408	0.20331	0.21265	0.21320	0.21320	0.21320	0.21320	0.21314	0.20985	0.20747
63	Y1 *A. barnardi*	0.21168	0.21168	0.20380	0.21317	0.21377	0.21377	0.21377	0.21377	0.21372	0.21042	0.20808
64	Y4 *A. barnardi*	0.21456	0.21456	0.20378	0.21313	0.21370	0.21370	0.21370	0.21370	0.21363	0.21034	0.20795
65	II5 *A. spatulata*	0.21665	0.21665	0.20582	0.21519	0.21089	0.21089	0.21089	0.21089	0.21074	0.20752	0.21001
66	HH1 *A. securata*	0.20943	0.20943	0.20216	0.21142	0.20718	0.20718	0.20718	0.20718	0.20703	0.20388	0.21326
67	AA2 *A. bullata*	0.21465	0.21465	0.19945	0.20861	0.20911	0.20911	0.20911	0.20911	0.20904	0.20585	0.20875
68	CC2 *A. clavata*	0.21662	0.21662	0.20588	0.21528	0.21093	0.21093	0.21093	0.21093	0.21082	0.20758	0.21029
69	CC7 *A. clavata*	0.21192	0.21192	0.20112	0.21044	0.21093	0.21093	0.21093	0.21093	0.21082	0.20758	0.20528
70	FF2 *A. quadrata*	0.21600	0.21600	0.20540	0.21473	0.21052	0.21052	0.21052	0.21052	0.21042	0.20721	0.20989
71	AA5 *A. bullata*	0.21491	0.21491	0.20405	0.21342	0.20911	0.20911	0.20911	0.20911	0.20894	0.20575	0.21328
72	GG1 *A. scutata*	0.21464	0.21464	0.20423	0.20858	0.21114	0.21114	0.21114	0.21114	0.21108	0.20775	0.19723
73	GG2 *A. scutata*	0.22078	0.22078	0.21033	0.21474	0.21715	0.21715	0.21715	0.21715	0.21708	0.21372	0.20480
74	AA6 *A. bullata*	0.23290	0.23290	0.22311	0.23266	0.22033	0.22033	0.22033	0.22033	0.22040	0.21705	0.21744
75	DD2 *A. flabellata*	0.22602	0.22602	0.22218	0.21960	0.21557	0.21557	0.21557	0.21557	0.21570	0.21228	0.21524
76	DD1 *A. flabellata*	0.22691	0.22691	0.21611	0.22044	0.21631	0.21631	0.21631	0.21631	0.21640	0.21297	0.21617
77	AA7 *A. bullata*	0.20053	0.20053	0.18909	0.19781	0.20082	0.20082	0.20082	0.20082	0.20081	0.19771	0.20125
78	BB1 *A. cassida*	0.20772	0.20772	0.20746	0.21009	0.21216	0.21216	0.21216	0.21216	0.21232	0.21547	0.20420
79	JC1 *A. cassida*	0.20488	0.20488	0.20474	0.20737	0.20955	0.20955	0.20955	0.20955	0.20971	0.21281	0.20160
80	JD2 *A. cassida*	0.19593	0.19593	0.19585	0.19332	0.19536	0.19536	0.19536	0.19536	0.19550	0.19854	0.18763
81	JA5 *A. cassida*	0.20620	0.20620	0.20087	0.19851	0.20518	0.20518	0.20518	0.20518	0.20523	0.20856	0.20180

Appendix 4.6. Continued. Corrected distances.

		34	35	36	37	38	39	40	41	42	43	44
82	AB1 *A. pauletteae*	0.22524	0.22524	0.21584	0.21335	0.21203	0.21203	0.21203	0.21203	0.21193	0.20871	0.21228
83	AB2 *A. pauletteae*	0.22888	0.22888	0.21936	0.21686	0.21549	0.21549	0.21549	0.21549	0.21538	0.21211	0.21576
84	Z1 *A. bifurcata*	0.23973	0.23973	0.23418	0.23680	0.23888	0.23888	0.23888	0.23888	0.24181	0.23523	0.21711
85	Z3 *A. bifurcata*	0.25306	0.25306	0.24830	0.25087	0.24783	0.24783	0.24783	0.24783	0.24781	0.24410	0.23083
86	JJ2 *A. denticulata*	0.22513	0.22513	0.20740	0.20388	0.21564	0.21564	0.21564	0.21564	0.21828	0.21249	0.21514
87	MM1 *A. tabularis*	0.22953	0.22953	0.22039	0.21779	0.21538	0.21538	0.21538	0.21538	0.21793	0.21223	0.21740
88	KK2 *A. hawaquae*	0.18743	0.18743	0.18294	0.18058	0.17958	0.17958	0.17958	0.17958	0.18177	0.17658	0.18143
89	KK1 *A. hawaquae*	0.23492	0.23492	0.22545	0.22277	0.21680	0.21680	0.21680	0.21680	0.21903	0.21339	0.21995
90	LL1 *A. outeniquae*	0.21801	0.21801	0.21585	0.22005	0.21186	0.21186	0.21186	0.21186	0.21428	0.20863	0.21564
91	LL2 *A. outeniquae*	0.21124	0.21124	0.20935	0.21338	0.20544	0.20544	0.20544	0.20544	0.20789	0.20230	0.20906
92	Q2 *A. amatolae*	0.16897	0.16897	0.16882	0.17122	0.16521	0.16521	0.16521	0.16521	0.16523	0.16221	0.17251
93	Q3 *A. amatolae*	0.17209	0.17209	0.17193	0.17433	0.16825	0.16825	0.16825	0.16825	0.16826	0.16521	0.17554
94	R2 *A. spinulata*	0.16960	0.16960	0.16341	0.16730	0.16001	0.16001	0.16001	0.16001	0.15995	0.15704	0.15737
95	R3 *A. spinulata*	0.16960	0.16960	0.16341	0.16730	0.16001	0.16001	0.16001	0.16001	0.15995	0.15704	0.15737
96	QQ1 *D. pulchellum*	0.21009	0.21009	0.20774	0.21279	0.19944	0.19944	0.19944	0.19944	0.19925	0.19600	0.19349
97	QQ3 *D. pulchellum*	0.21009	0.21009	0.20774	0.21279	0.19944	0.19944	0.19944	0.19944	0.19925	0.19600	0.19349
98	PP1 *D. brevis*	0.20779	0.20779	0.21277	0.21048	0.19716	0.19716	0.19716	0.19716	0.19694	0.19371	0.19115
99	PP3 *D. brevis*	0.20294	0.20294	0.20787	0.20555	0.19800	0.19800	0.19800	0.19800	0.19782	0.19462	0.18686
100	NN2 *B. gudu*	0.24605	0.24605	0.23933	0.24619	0.25456	0.25456	0.25456	0.25456	0.25457	0.25091	0.24879
101	NN1 *B. gudu*	0.23702	0.23702	0.23043	0.23717	0.24535	0.24535	0.24535	0.24535	0.24536	0.24176	0.24672
102	OO3 *B. tugelae*	0.23692	0.23692	0.23555	0.24237	0.24525	0.24525	0.24525	0.24525	0.24529	0.24566	0.24112
103	OO2 *B. tugelae*	0.23434	0.23434	0.23825	0.24506	0.24800	0.24800	0.24800	0.24800	0.24808	0.24841	0.24390

Appendix 4.6. Continued. Corrected distances.

		45	46	47	48	49	50	51	52	53	54	55
45	D3 *A. brevispina*	-										
46	F4 *A. pickeri*	0.01282	-									
47	F2 *A. pickeri*	0.01283	0.00361	-								
48	X3 *A. uncinata*	0.01850	0.01674	0.01664	-							
49	X4 *A. uncinata*	0.01848	0.01664	0.01658	0.00361	-						
50	T2 *A. bovina*	0.08452	0.08232	0.08688	0.08449	0.08457	-					
51	T1 *A. bovina*	0.08480	0.08254	0.08712	0.08476	0.08480	0.00726	-				
52	A2 *A. capensis*	0.08331	0.08093	0.08553	0.07400	0.07381	0.08436	0.07992	-			
53	A1 *A. capensis*	0.08331	0.08093	0.08553	0.07400	0.07381	0.08436	0.07992	0.00000	-		
54	U3 *A. chanae*	0.07799	0.07102	0.07546	0.07319	0.07314	0.08381	0.08387	0.08139	0.08139	-	
55	U4 *A. chanae*	0.07757	0.07074	0.07515	0.07742	0.07740	0.07939	0.07939	0.08573	0.08573	0.00726	-
56	S3 *A. bicornis*	0.10729	0.10502	0.10984	0.10983	0.11246	0.11149	0.11148	0.11270	0.11270	0.08943	0.08751
57	S5 *A. bicornis*	0.10729	0.10502	0.10984	0.10983	0.11246	0.11149	0.11148	0.11270	0.11270	0.08943	0.08751
58	S4 *A. bicornis*	0.11217	0.10984	0.11472	0.11472	0.11738	0.11210	0.11207	0.11761	0.11761	0.09399	0.09202
59	W2 *A. lyrata*	0.10263	0.10040	0.10516	0.10513	0.10776	0.11259	0.11253	0.10781	0.10781	0.09288	0.09103
60	W3 *A. lyrata*	0.10263	0.10040	0.10516	0.10513	0.10776	0.11259	0.11253	0.10781	0.10781	0.09288	0.09103
61	II3 *A. spatulata*	0.21473	0.20138	0.20718	0.21178	0.21279	0.20885	0.20534	0.22158	0.22158	0.19591	0.19017
62	HH4 *A. securata*	0.21425	0.20091	0.20671	0.21136	0.21237	0.20823	0.20480	0.22109	0.22109	0.19552	0.18973
63	Y1 *A. barnardi*	0.21485	0.20140	0.20723	0.21186	0.21283	0.20587	0.20243	0.21862	0.21862	0.19314	0.18740
64	Y4 *A. barnardi*	0.21473	0.20138	0.20718	0.21178	0.21279	0.20885	0.20534	0.22158	0.22158	0.19591	0.19017
65	II5 *A. spatulata*	0.21681	0.20340	0.20921	0.21389	0.21489	0.20907	0.20556	0.22660	0.22660	0.19619	0.19048
66	HH1 *A. securata*	0.21316	0.19972	0.20549	0.21021	0.21112	0.20226	0.19888	0.21970	0.21970	0.19002	0.18441
67	AA2 *A. bullata*	0.21536	0.20183	0.20763	0.21237	0.21320	0.19945	0.19604	0.22195	0.22195	0.19180	0.18620
68	CC2 *A. clavata*	0.21705	0.20346	0.20932	0.20909	0.21499	0.20775	0.20436	0.22367	0.22367	0.19341	0.18771
69	CC7 *A. clavata*	0.21204	0.19872	0.20449	0.20909	0.21009	0.20775	0.20436	0.21877	0.21877	0.19341	0.18771
70	FF2 *A. quadrata*	0.21657	0.20298	0.20881	0.21353	0.21440	0.21034	0.20685	0.22314	0.22314	0.19294	0.18734
71	AA5 *A. bullata*	0.22008	0.20642	0.21230	0.21205	0.21306	0.21383	0.21034	0.22675	0.22675	0.19625	0.19056
72	GG1 *A. scutata*	0.20366	0.19039	0.19606	0.20080	0.20156	0.20490	0.19477	0.21960	0.21960	0.19853	0.19260
73	GG2 *A. scutata*	0.20475	0.19643	0.19734	0.20182	0.20270	0.21235	0.20212	0.23586	0.23586	0.20946	0.20346
74	AA6 *A. bullata*	0.22412	0.21539	0.22137	0.22925	0.22990	0.21356	0.21007	0.22933	0.22933	0.20163	0.19604
75	DD2 *A. flabellata*	0.21819	0.21474	0.22073	0.21867	0.21939	0.22188	0.21139	0.23006	0.23006	0.20038	0.19466
76	DD1 *A. flabellata*	0.21917	0.21571	0.22171	0.21939	0.22021	0.21591	0.20556	0.22922	0.22922	0.20109	0.19532
77	AA7 *A. bullata*	0.20121	0.19328	0.19890	0.20313	0.20380	0.20776	0.20432	0.21322	0.21322	0.19207	0.19045
78	BB1 *A. cassida*	0.20697	0.21019	0.21099	0.20911	0.20615	0.20169	0.19822	0.19300	0.19300	0.19580	0.19433
79	JC1 *A. cassida*	0.20431	0.20746	0.20822	0.20636	0.20339	0.19914	0.19561	0.19049	0.19049	0.19297	0.19149
80	JD2 *A. cassida*	0.19030	0.19340	0.19416	0.19545	0.19252	0.18525	0.18485	0.19406	0.19406	0.18699	0.18262
81	JA5 *A. cassida*	0.20513	0.20847	0.20942	0.20227	0.19948	0.19857	0.19515	0.20108	0.20108	0.19562	0.19416
82	AB1 *A. pauletteae*	0.21220	0.20400	0.20983	0.20561	0.20631	0.20511	0.20464	0.20353	0.20353	0.18063	0.17290
83	AB2 *A. pauletteae*	0.21566	0.20744	0.21332	0.20896	0.20972	0.20192	0.20146	0.20664	0.20664	0.18375	0.17595
84	Z1 *A. bifurcata*	0.22005	0.22176	0.22791	0.23060	0.23132	0.22058	0.22608	0.25355	0.25355	0.21026	0.20882
85	Z3 *A. bifurcata*	0.23443	0.23699	0.24332	0.23824	0.23931	0.21994	0.22550	0.24117	0.24117	0.23060	0.22944

Appendix 4.6. Continued. Corrected distances.

		45	46	47	48	49	50	51	52	53	54	55
86	JJ2 *A. denticulata*	0.21813	0.22258	0.22872	0.22851	0.23235	0.21920	0.21259	0.21148	0.21148	0.20118	0.20128
87	MM1 *A. tabularis*	0.22037	0.21852	0.22439	0.22384	0.22767	0.22570	0.21876	0.24684	0.24684	0.20515	0.20514
88	KK2 *A. hawaquae*	0.18749	0.18602	0.19160	0.19044	0.19099	0.18103	0.17455	0.20305	0.20305	0.18260	0.18045
89	KK1 *A. hawaquae*	0.22697	0.22074	0.22691	0.23221	0.23631	0.22507	0.21793	0.21371	0.21371	0.20076	0.18947
90	LL1 *A. outeniquae*	0.22243	0.21722	0.22319	0.22122	0.22190	0.21112	0.21708	0.23004	0.23004	0.22032	0.20881
91	LL2 *A. outeniquae*	0.21565	0.21060	0.21644	0.21463	0.21524	0.20495	0.21071	0.22337	0.22337	0.21366	0.20233
92	Q2 *A. amatolae*	0.16938	0.17060	0.17613	0.17243	0.17289	0.17068	0.16416	0.17847	0.17847	0.16419	0.15907
93	Q3 *A. amatolae*	0.17236	0.17368	0.17926	0.17543	0.17594	0.17387	0.16726	0.17532	0.17532	0.16731	0.16215
94	R2 *A. spinulata*	0.15432	0.15442	0.15975	0.15638	0.15689	0.16075	0.16687	0.17622	0.17622	0.15083	0.14829
95	R3 *A. spinulata*	0.15432	0.15442	0.15975	0.15638	0.15689	0.16075	0.16687	0.17622	0.17622	0.15083	0.14829
96	QQ1 *D. pulchellum*	0.19680	0.20459	0.20794	0.19744	0.19858	0.21637	0.20904	0.21113	0.21113	0.22461	0.22531
97	QQ3 *D. pulchellum*	0.19680	0.20459	0.20794	0.19744	0.19858	0.21637	0.20904	0.21113	0.21113	0.22461	0.22531
98	PP1 *D. brevis*	0.19446	0.20227	0.20562	0.19513	0.19630	0.22130	0.21369	0.22081	0.22081	0.22220	0.22293
99	PP3 *D. brevis*	0.19003	0.19764	0.20082	0.19065	0.19167	0.21452	0.20708	0.21970	0.21970	0.22103	0.22178
100	NN2 *B. gudu*	0.25586	0.25394	0.26047	0.24470	0.24435	0.25169	0.24685	0.26744	0.26744	0.26681	0.26495
101	NN1 *B. gudu*	0.25391	0.24475	0.25114	0.23846	0.23536	0.24263	0.23783	0.26367	0.26367	0.25737	0.25552
102	OO3 *B. tugelae*	0.24834	0.23939	0.24570	0.23807	0.23499	0.24377	0.23612	0.25038	0.25038	0.24946	0.24801
103	OO2 *B. tugelae*	0.25112	0.24205	0.24841	0.24083	0.23770	0.24643	0.23880	0.25327	0.25327	0.24928	0.24784

Appendix 4.6. Continued. Corrected distances.

		56	57	58	59	60	61	62	63	64	65	66
56	S3 *A. bicornis*	-										
57	S5 *A. bicornis*	0.00000	-									
58	S4 *A. bicornis*	0.00361	0.00361	-								
59	W2 *A. lyrata*	0.01282	0.01282	0.01286	-							
60	W3 *A. lyrata*	0.01282	0.01282	0.01286	0.00000	-						
61	II3 *A. spatulata*	0.18153	0.18153	0.18690	0.17953	0.17953	-					
62	HH4 *A. securata*	0.18094	0.18094	0.18631	0.17896	0.17896	0.00181	-				
63	Y1 *A. barnardi*	0.17867	0.17867	0.18402	0.17669	0.17669	0.00180	0.00362	-			
64	Y4 *A. barnardi*	0.18153	0.18153	0.18690	0.17953	0.17953	0.00000	0.00181	0.00180	-		
65	II5 *A. spatulata*	0.17908	0.17908	0.18439	0.17708	0.17708	0.00543	0.00727	0.00726	0.00543	-	
66	HH1 *A. securata*	0.17306	0.17306	0.17831	0.17103	0.17103	0.00729	0.00912	0.00911	0.00729	0.00544	-
67	AA2 *A. bullata*	0.17531	0.17531	0.17589	0.17324	0.17324	0.01097	0.01286	0.01280	0.01097	0.00909	0.01096
68	CC2 *A. clavata*	0.17336	0.17336	0.17863	0.17141	0.17141	0.00913	0.01102	0.01097	0.00913	0.00729	0.00909
69	CC7 *A. clavata*	0.17631	0.17631	0.18163	0.17429	0.17429	0.00543	0.00727	0.00725	0.00543	0.00729	0.00909
70	FF2 *A. quadrata*	0.17630	0.17630	0.18160	0.17423	0.17423	0.01279	0.01467	0.01462	0.01279	0.01091	0.01282
71	AA5 *A. bullata*	0.17922	0.17922	0.18456	0.17718	0.17718	0.01845	0.02038	0.02030	0.01845	0.01651	0.01845
72	GG1 *A. scutata*	0.17742	0.17742	0.18275	0.17538	0.17538	0.02612	0.02807	0.02801	0.02612	0.02413	0.02615
73	GG2 *A. scutata*	0.18586	0.18586	0.19128	0.18362	0.18362	0.04164	0.04366	0.04361	0.04164	0.03960	0.03764
74	AA6 *A. bullata*	0.18263	0.18263	0.18803	0.18042	0.18042	0.02826	0.03032	0.03016	0.02826	0.02627	0.02621
75	DD2 *A. flabellata*	0.19764	0.19764	0.20329	0.20695	0.20695	0.06886	0.07103	0.07087	0.06886	0.07096	0.07137
76	DD1 *A. flabellata*	0.19824	0.19824	0.20389	0.20766	0.20766	0.06451	0.06664	0.06650	0.06451	0.06660	0.06698
77	AA7 *A. bullata*	0.18968	0.18968	0.19514	0.18746	0.18746	0.06035	0.06236	0.06040	0.06035	0.06462	0.06259
78	BB1 *A. cassida*	0.18099	0.18099	0.18638	0.17902	0.17902	0.12515	0.12739	0.12291	0.12515	0.13038	0.13096
79	JC1 *A. cassida*	0.18162	0.18162	0.18701	0.17964	0.17964	0.12578	0.12798	0.12354	0.12578	0.13104	0.13168
80	JD2 *A. cassida*	0.17280	0.17280	0.17813	0.17082	0.17082	0.12939	0.13165	0.12708	0.12939	0.13474	0.13519
81	JA5 *A. cassida*	0.19632	0.19632	0.19713	0.19138	0.19138	0.14031	0.14260	0.13805	0.14031	0.14563	0.14639
82	AB1 *A. pauletteae*	0.17017	0.17017	0.17544	0.17870	0.17870	0.11709	0.11938	0.11935	0.11709	0.11444	0.10969
83	AB2 *A. pauletteae*	0.16736	0.16736	0.17258	0.17577	0.17577	0.11450	0.11679	0.11676	0.11450	0.11188	0.10718
84	Z1 *A. bifurcata*	0.20358	0.20358	0.20431	0.20152	0.20152	0.11705	0.11926	0.11938	0.11705	0.11984	0.12292
85	Z3 *A. bifurcata*	0.21687	0.21687	0.21590	0.20359	0.20359	0.12309	0.12525	0.12546	0.12309	0.13036	0.12793
86	JJ2 *A. denticulata*	0.20241	0.20241	0.20298	0.20660	0.20660	0.23710	0.23973	0.23719	0.23710	0.24454	0.24857
87	MM1 *A. tabularis*	0.20350	0.20350	0.20920	0.21284	0.21284	0.22790	0.23052	0.22800	0.22790	0.23530	0.23197
88	KK2 *A. hawaquae*	0.20520	0.20520	0.20613	0.21047	0.21047	0.19634	0.19873	0.19349	0.19634	0.20014	0.20023
89	KK1 *A. hawaquae*	0.19492	0.19492	0.20066	0.18829	0.18829	0.18449	0.18695	0.18173	0.18449	0.18802	0.18810
90	LL1 *A. outeniquae*	0.21146	0.21146	0.21737	0.21687	0.21687	0.21050	0.21030	0.20741	0.21050	0.21303	0.22302
91	LL2 *A. outeniquae*	0.20928	0.20928	0.21517	0.21487	0.21487	0.20413	0.20389	0.20106	0.20413	0.20657	0.21632
92	Q2 *A. amatolae*	0.15898	0.15898	0.16431	0.16531	0.16531	0.21649	0.21578	0.21333	0.21649	0.21424	0.21348
93	Q3 *A. amatolae*	0.16214	0.16214	0.16752	0.16856	0.16856	0.21987	0.21916	0.21670	0.21987	0.21766	0.21685
94	R2 *A. spinulata*	0.16315	0.16315	0.16387	0.16149	0.16149	0.23180	0.23128	0.22885	0.23180	0.23602	0.22926
95	R3 *A. spinulata*	0.16315	0.16315	0.16387	0.16149	0.16149	0.23180	0.23128	0.22885	0.23180	0.23602	0.22926
96	QQ1 *D. pulchellum*	0.19984	0.19984	0.20558	0.20074	0.20074	0.20954	0.21194	0.21243	0.20954	0.21635	0.21334
97	QQ3 *D. pulchellum*	0.19984	0.19984	0.20558	0.20074	0.20074	0.20954	0.21194	0.21243	0.20954	0.21635	0.21334
98	PP1 *D. brevis*	0.19756	0.19756	0.20326	0.19836	0.19836	0.21432	0.21668	0.21723	0.21432	0.22124	0.21821
99	PP3 *D. brevis*	0.20356	0.20356	0.20935	0.20435	0.20435	0.20988	0.21228	0.21280	0.20988	0.21676	0.21373

Appendix 4.6. Continued. Corrected distances.

		56	57	58	59	60	61	62	63	64	65	66
100	NN2 *B. gudu*	0.23144	0.23144	0.23235	0.23328	0.23328	0.23683	0.23950	0.23995	0.23683	0.23681	0.24100
101	NN1 *B. gudu*	0.22524	0.22524	0.22611	0.22698	0.22698	0.22792	0.23054	0.23101	0.22792	0.22792	0.23203
102	OO3 *B. tugelae*	0.22792	0.22792	0.23391	0.22972	0.22972	0.21142	0.21397	0.21448	0.21142	0.21460	0.21837
103	OO2 *B. tugelae*	0.22768	0.22768	0.23368	0.22891	0.22891	0.21086	0.21341	0.21389	0.21086	0.21399	0.21778

Appendix 4.6. Continued. Corrected distances.

		67	68	69	70	71	72	73	74	75	76	77
67	AA2 *A. bullata*	-										
68	CC2 *A. clavata*	0.01286	-									
69	CC7 *A. clavata*	0.01286	0.00364	-								
70	FF2 *A. quadrata*	0.00914	0.01277	0.01277	-							
71	AA5 *A. bullata*	0.01480	0.01841	0.01841	0.00920	-						
72	GG1 *A. scutata*	0.02609	0.02802	0.02802	0.02794	0.03386	-					
73	GG2 *A. scutata*	0.03374	0.04362	0.04362	0.03964	0.04165	0.02028	-				
74	AA6 *A. bullata*	0.02478	0.02626	0.02626	0.02440	0.03035	0.03779	0.04976	-			
75	DD2 *A. flabellata*	0.07338	0.07096	0.07096	0.07341	0.08005	0.07324	0.08416	0.07526	-		
76	DD1 *A. flabellata*	0.06896	0.06660	0.06660	0.06900	0.07554	0.06883	0.07962	0.07084	0.00361	-	
77	AA7 *A. bullata*	0.06234	0.06463	0.06258	0.06029	0.06657	0.07323	0.08174	0.06841	0.08946	0.08488	-
78	BB1 *A. cassida*	0.12511	0.12581	0.12347	0.12525	0.13256	0.13480	0.12944	0.13179	0.12819	0.12756	0.10244
79	JC1 *A. cassida*	0.12570	0.12649	0.12417	0.12586	0.13315	0.13537	0.13003	0.13240	0.12589	0.12524	0.10268
80	JD2 *A. cassida*	0.12942	0.13004	0.12762	0.12966	0.13701	0.13449	0.12922	0.13623	0.12510	0.12449	0.11171
81	JA5 *A. cassida*	0.13566	0.14103	0.13870	0.14024	0.14321	0.14843	0.14308	0.14703	0.14328	0.13801	0.11655
82	AB1 *A. pauletteae*	0.11248	0.11197	0.11197	0.11298	0.12009	0.11415	0.11096	0.11641	0.11433	0.10946	0.12203
83	AB2 *A. pauletteae*	0.10990	0.10944	0.10944	0.11036	0.11744	0.11163	0.10849	0.11385	0.11176	0.10693	0.11937
84	Z1 *A. bifurcata*	0.11573	0.11522	0.11522	0.11757	0.11493	0.12267	0.12948	0.11332	0.13925	0.13999	0.13909
85	Z3 *A. bifurcata*	0.13045	0.12565	0.12140	0.12806	0.12530	0.13335	0.14011	0.12296	0.15709	0.15162	0.14180
86	JJ2 *A. denticulata*	0.23428	0.23812	0.23812	0.23809	0.23995	0.23909	0.25123	0.23852	0.23135	0.23496	0.23244
87	MM1 *A. tabularis*	0.23326	0.22874	0.22874	0.22820	0.23021	0.21772	0.22739	0.22332	0.22211	0.22615	0.21711
88	KK2 *A. hawaquae*	0.19418	0.19726	0.19726	0.19986	0.19658	0.18432	0.18867	0.20581	0.18423	0.18478	0.20394
89	KK1 *A. hawaquae*	0.19294	0.18208	0.18493	0.19757	0.19918	0.17417	0.18970	0.20354	0.16664	0.17197	0.19761
90	LL1 *A. outeniquae*	0.21725	0.21628	0.21149	0.21230	0.21403	0.22151	0.22927	0.21848	0.21978	0.21386	0.23734
91	LL2 *A. outeniquae*	0.21083	0.20978	0.20504	0.20599	0.20776	0.21488	0.22260	0.21202	0.21332	0.20754	0.23019
92	Q2 *A. amatolae*	0.21377	0.22394	0.22394	0.21483	0.21826	0.21043	0.20847	0.21408	0.21750	0.22323	0.22109
93	Q3 *A. amatolae*	0.21715	0.22740	0.22740	0.21824	0.22173	0.21370	0.21171	0.21741	0.22083	0.22661	0.22449
94	R2 *A. spinulata*	0.22395	0.23611	0.23611	0.23330	0.23718	0.23050	0.21738	0.21813	0.21926	0.21320	0.22450
95	R3 *A. spinulata*	0.22395	0.23611	0.23611	0.23330	0.23718	0.23050	0.21738	0.21813	0.21926	0.21320	0.22450
96	QQ1 *D. pulchellum*	0.20914	0.21350	0.21063	0.20741	0.19484	0.19685	0.19438	0.21512	0.21518	0.20918	0.20063
97	QQ3 *D. pulchellum*	0.20914	0.21350	0.21063	0.20741	0.19484	0.19685	0.19438	0.21512	0.21518	0.20918	0.20063
98	PP1 *D. brevis*	0.21385	0.21834	0.21552	0.21209	0.19956	0.20137	0.19896	0.21970	0.21275	0.21386	0.20528
99	PP3 *D. brevis*	0.20949	0.21390	0.21098	0.20785	0.19528	0.19713	0.19462	0.21254	0.20864	0.20959	0.19814
100	NN2 *B. gudu*	0.23149	0.23112	0.23066	0.22795	0.22771	0.22672	0.23484	0.24222	0.23804	0.23162	0.24586
101	NN1 *B. gudu*	0.22272	0.22509	0.22186	0.21921	0.21895	0.21796	0.22590	0.23315	0.22908	0.22279	0.23677
102	OO3 *B. tugelae*	0.21433	0.21174	0.20859	0.21282	0.21256	0.20474	0.21234	0.21417	0.20896	0.20307	0.22250
103	OO2 *B. tugelae*	0.21380	0.21113	0.20803	0.21227	0.21195	0.20430	0.21197	0.21362	0.20888	0.20299	0.22199

Appendix 4.6. Continued. Corrected distances.

		78	79	80	81	82	83	84	85	86	87	88
78	BB1 *A. cassida*	-										
79	JC1 *A. cassida*	0.00361	-									
80	JD2 *A. cassida*	0.01465	0.01094	-								
81	JA5 *A. cassida*	0.03424	0.03025	0.03392	-							
82	AB1 *A. pauletteae*	0.12841	0.13062	0.12548	0.13765	-						
83	AB2 *A. pauletteae*	0.12580	0.12801	0.12293	0.13496	0.00181	-					
84	Z1 *A. bifurcata*	0.15346	0.15420	0.15124	0.16940	0.16891	0.16574	-				
85	Z3 *A. bifurcata*	0.14738	0.14806	0.14518	0.16172	0.15772	0.15465	0.04620	-			
86	JJ2 *A. denticulata*	0.22704	0.22434	0.21804	0.23380	0.22039	0.22386	0.21498	0.23672	-		
87	MM1 *A. tabularis*	0.23740	0.23456	0.22591	0.23925	0.20840	0.21176	0.20788	0.23246	0.07957	-	
88	KK2 *A. hawaquae*	0.21636	0.21053	0.19648	0.20782	0.19649	0.19980	0.19329	0.20823	0.14460	0.11770	-
89	KK1 *A. hawaquae*	0.19002	0.18443	0.18244	0.18883	0.18850	0.19168	0.19221	0.19173	0.16571	0.14477	0.11232
90	LL1 *A. outeniquae*	0.23234	0.22897	0.21032	0.22707	0.20547	0.20882	0.21370	0.20071	0.16650	0.13449	0.13571
91	LL2 *A. outeniquae*	0.22979	0.22639	0.20786	0.22721	0.21232	0.21580	0.20712	0.19418	0.16656	0.13447	0.13574
92	Q2 *A. amatolae*	0.22861	0.23292	0.22289	0.25377	0.21731	0.21381	0.22164	0.23728	0.20974	0.19773	0.18989
93	Q3 *A. amatolae*	0.22512	0.22937	0.22639	0.25007	0.22086	0.21731	0.22528	0.24108	0.21285	0.20072	0.19292
94	R2 *A. spinulata*	0.20319	0.19751	0.18945	0.20363	0.18052	0.18378	0.21777	0.23855	0.23632	0.23306	0.20856
95	R3 *A. spinulata*	0.20319	0.19751	0.18945	0.20363	0.18052	0.18378	0.21777	0.23855	0.23632	0.23306	0.20856

Appendix 4.6. Continued. Corrected distances.

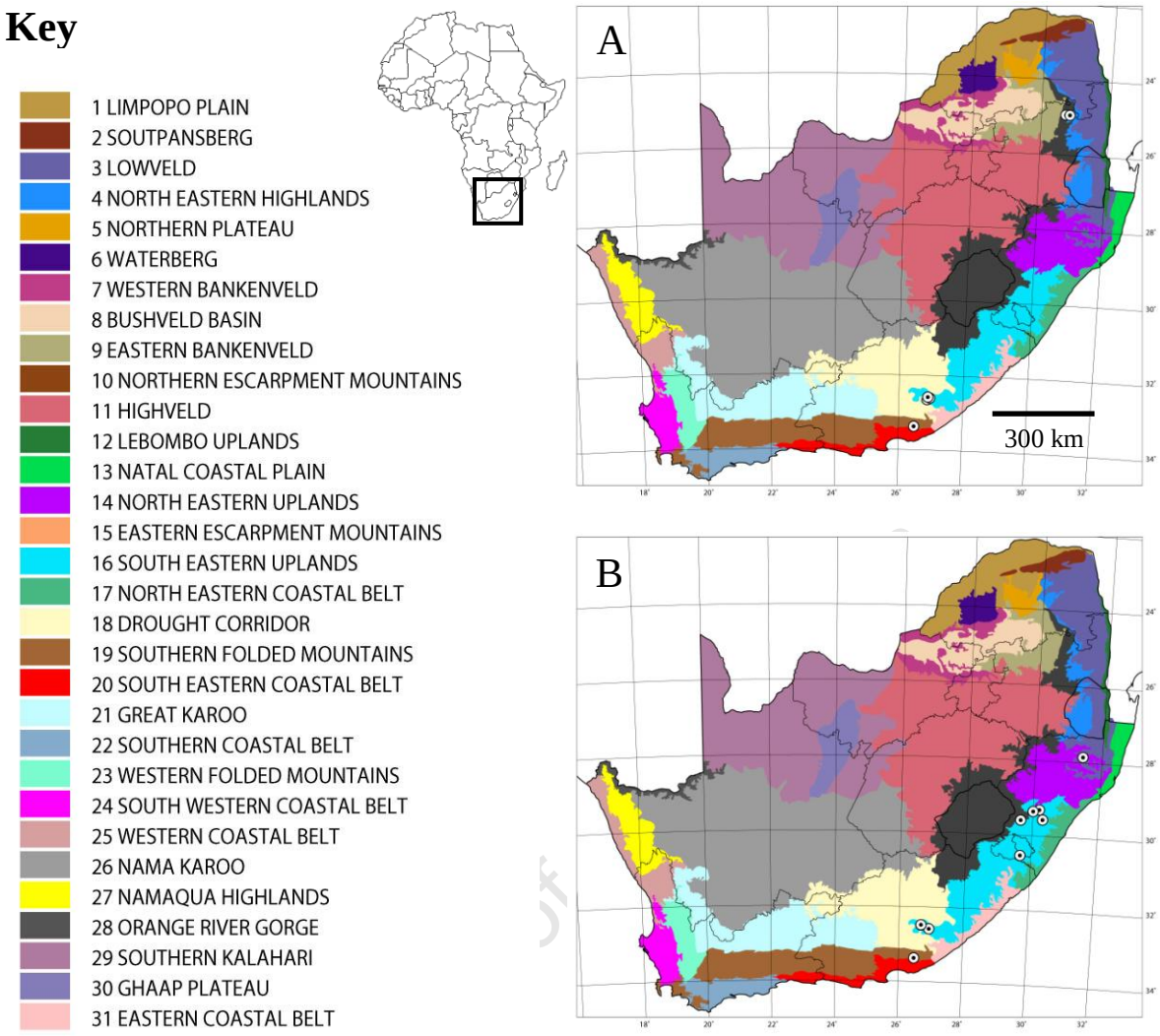
		78	79	80	81	82	83	84	85	86	87	88
96	QQ1 *D. pulchellum*	0.19451	0.19394	0.19200	0.19394	0.18682	0.18358	0.18445	0.18160	0.23714	0.21235	0.22928
97	QQ3 *D. pulchellum*	0.19451	0.19394	0.19200	0.19394	0.18682	0.18358	0.18445	0.18160	0.23714	0.21235	0.22928
98	PP1 *D. brevis*	0.19838	0.19767	0.19589	0.19791	0.19133	0.18798	0.18201	0.18566	0.23438	0.20979	0.22673
99	PP3 *D. brevis*	0.20795	0.20719	0.20544	0.20740	0.19386	0.19052	0.17560	0.17907	0.23427	0.21352	0.23104
100	NN2 *B. gudu*	0.27067	0.26914	0.25951	0.27931	0.22517	0.22171	0.22416	0.23037	0.23343	0.21932	0.25130
101	NN1 *B. gudu*	0.26967	0.26801	0.25819	0.27864	0.21650	0.21309	0.22262	0.22900	0.23926	0.21306	0.24988
102	OO3 *B. tugelae*	0.25262	0.25123	0.24629	0.26539	0.20206	0.19888	0.21851	0.21898	0.24516	0.21564	0.25007
103	OO2 *B. tugelae*	0.25200	0.25062	0.24572	0.26488	0.20190	0.19871	0.21799	0.21861	0.24501	0.21541	0.25012

Appendix 4.6. Continued. Corrected distances.

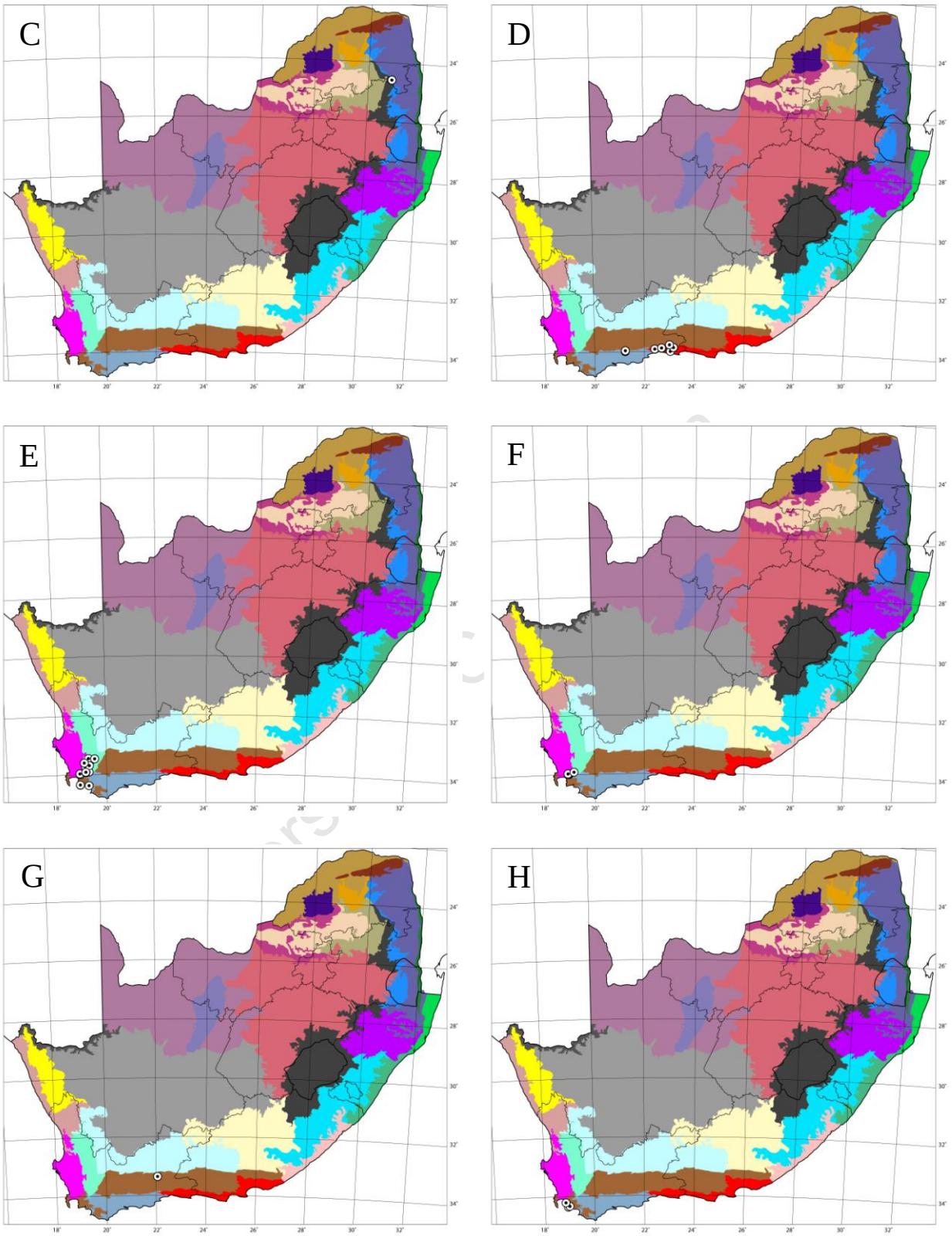
		89	90	91	92	93	94	95	96	97	98	99
89	KK1 *A. hawaquae*	-										
90	LL1 *A. outeniquae*	0.16353	-									
91	LL2 *A. outeniquae*	0.16348	0.00726	-								
92	Q2 *A. amatolae*	0.21805	0.19022	0.18180	-							
93	Q3 *A. amatolae*	0.21456	0.19336	0.18488	0.00181	-						
94	R2 *A. spinulata*	0.24890	0.21146	0.20948	0.13456	0.13749	-					
95	R3 *A. spinulata*	0.24890	0.21146	0.20948	0.13456	0.13749	0.00000	-				
96	QQ1 *D. pulchellum*	0.22788	0.22449	0.22467	0.21209	0.20841	0.19527	0.19527	-			
97	QQ3 *D. pulchellum*	0.22788	0.22449	0.22467	0.21209	0.20841	0.19527	0.19527	0.00000	-		
98	PP1 *D. brevis*	0.22511	0.22898	0.22922	0.20977	0.20609	0.19996	0.19996	0.00544	0.00544	-	
99	PP3 *D. brevis*	0.22819	0.23614	0.22911	0.20585	0.20224	0.19983	0.19983	0.01285	0.01285	0.01099	-
100	NN2 *B. gudu*	0.27852	0.21939	0.22381	0.23951	0.24299	0.26606	0.26606	0.22544	0.22544	0.22275	0.22970
101	NN1 *B. gudu*	0.27086	0.21088	0.21522	0.23764	0.24117	0.25659	0.25659	0.22364	0.22364	0.22102	0.22807
102	OO3 *B. tugelae*	0.25496	0.21696	0.22156	0.23109	0.23456	0.24580	0.24580	0.21241	0.21241	0.20987	0.21667
103	OO2 *B. tugelae*	0.25436	0.21692	0.22153	0.23369	0.23716	0.24568	0.24568	0.21199	0.21199	0.20946	0.21625

Appendix 4.6. Continued. Corrected distances.

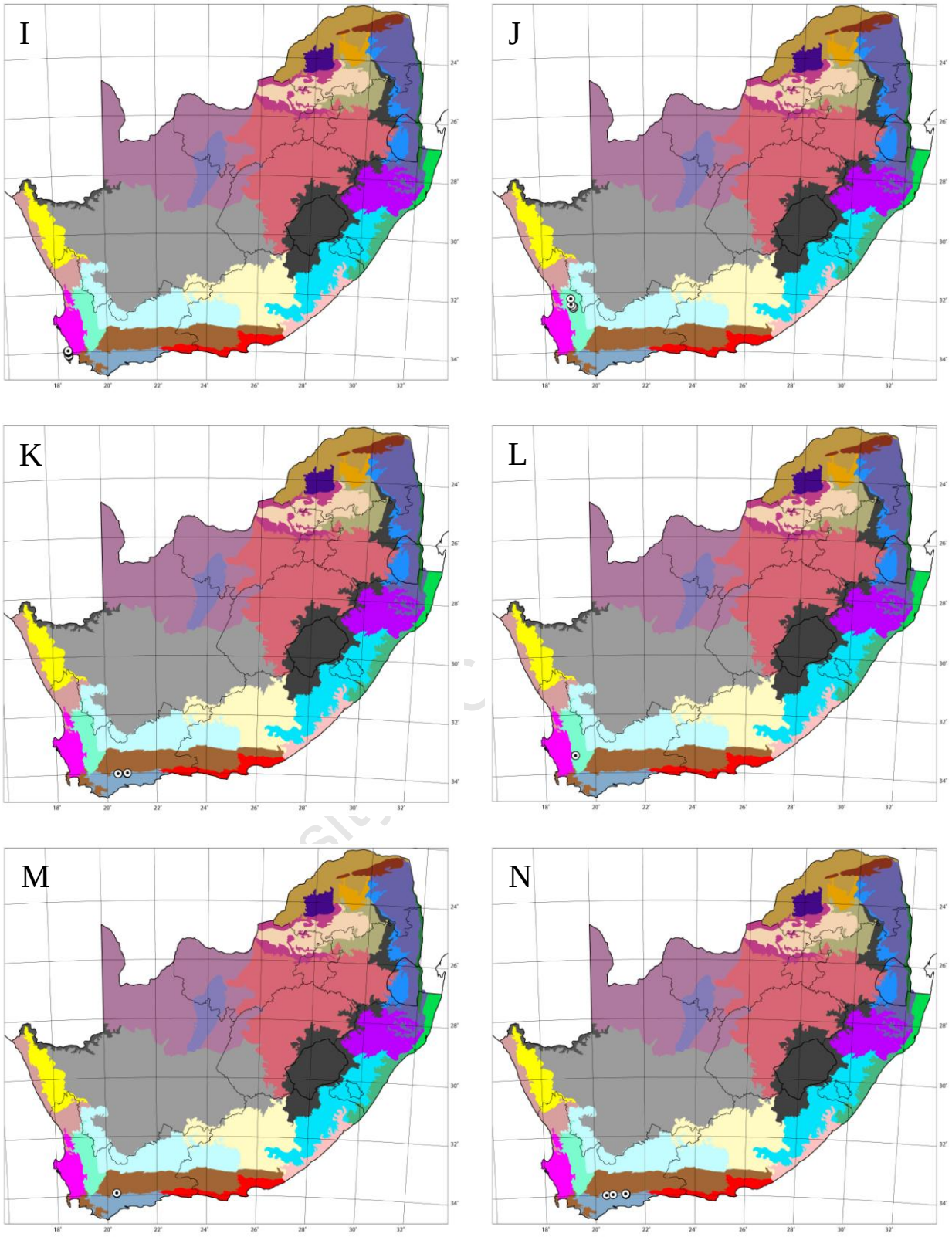
		100	101	102	103
100	NN2 *B. gudu*	-			
101	NN1 *B. gudu*	0.00543	-		
102	OO3 *B. tugelae*	0.02805	0.02226	-	
103	OO2 *B. tugelae*	0.03003	0.02421	0.00181	-



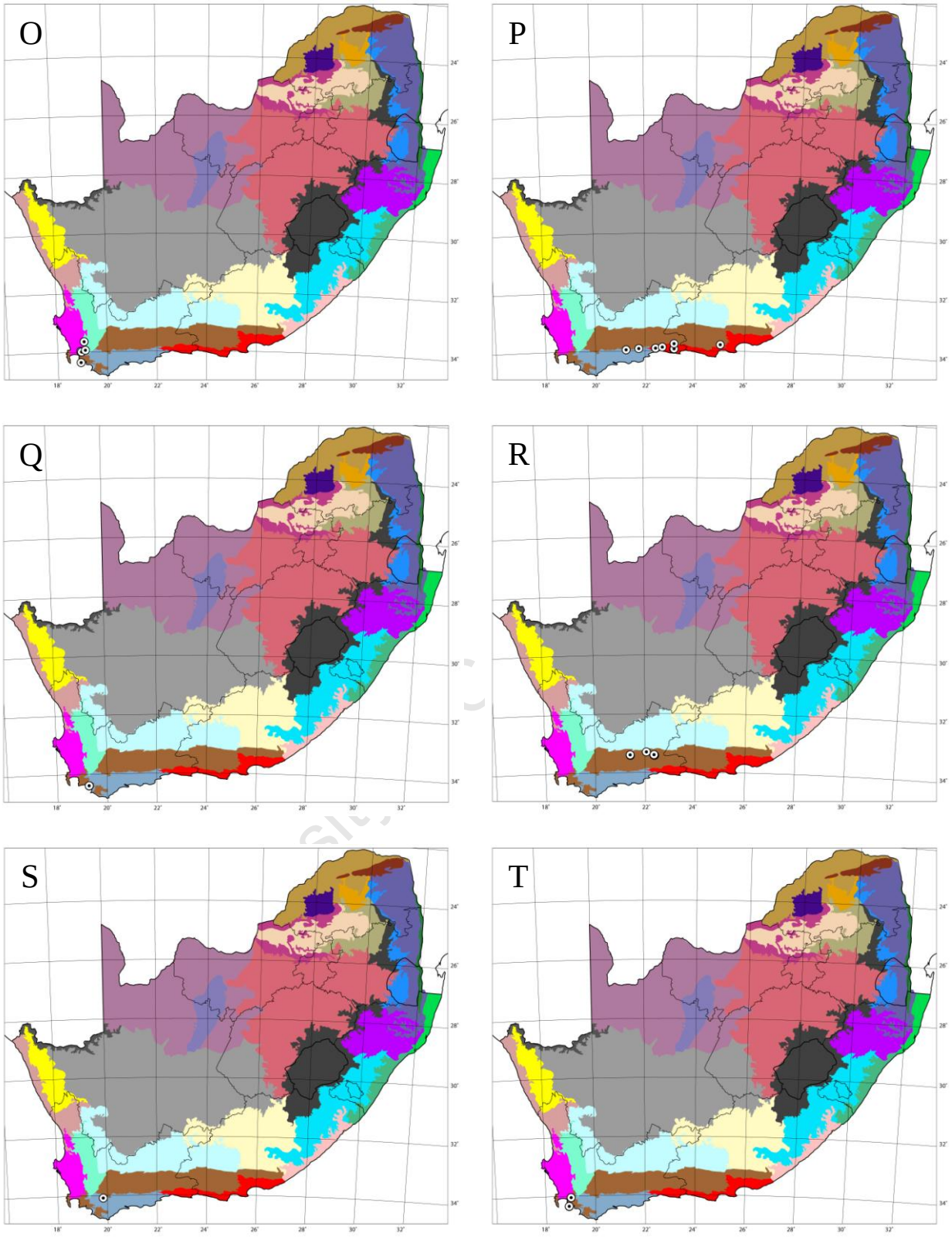
Appendix 4.7 A-RR. Species distributions and provincial boundaries overlaid on Level 1 River Ecoregions of South Africa, Lesotho and Swaziland (Kleynhans *et al*. 2005). Distance scale bar of 300 km is provided in **A**. **A**, *Afronemoura amatolae*; **B**, *Afronemoura spinulata*; **C**, *Afronemoura stuckenbergi*; **D**, *Aphanicerca austrocapensis* sp. n.; **E**, *Aphanicerca bicornis*; **F**, *Aphanicerca bovina*; **G**, *Aphanicerca breviloba* sp. n.; **H**, *Aphanicerca brevispina* sp. n.; **I**, *Aphanicerca capensis*; **J**, *Aphanicerca cederbergensis* sp. n.; **K**, *Aphanicerca chanae*; **L**, *Aphanicerca gnua*; **M**, *Aphanicerca incisura* sp. n.; **N**, *Aphanicerca longiloba* sp. n.; **O**, *Aphanicerca lyrata*; **P**, *Aphanicerca mclellani* sp. n.; **Q**, *Aphanicerca pickeri* sp. n.; **R**, *Aphanicerca swartbergensis* sp. n.; **S**, *Aphanicerca tereta*; **T**, *Aphanicerca uncinata*; **U**, *Aphanicerca witsenbergensis* sp. n.; **V**, *Aphanicerca zwicki* sp. n.; **W**, *Aphanicercella barnardi*; **X**, *Aphanicercella bifurcata*; **Y**, *Aphanicercella bullata*; **Z**, *Aphanicercella cassida*; **AA**, *Aphanicercella clavata*; **BB**, *Aphanicercella flabellata*; **CC**, *Aphanicercella namaquaensis* sp. n.; **DD**, *Aphanicercella nigra*; **EE**, *Aphanicercella pauletteae* sp. n.; **FF**, *Aphanicercella quadrata*; **GG**, *Aphanicercella scutata*; **HH**, *Aphanicercella securata*; **II**, *Aphanicercella spatulata*; **JJ**, *Aphanicercopsis denticulata*; **KK**, *Aphanicercopsis hawaquae*; **LL**, *Aphanicercopsis outeniquae*; **MM**, *Aphanicercopsis tabularis*; **NN**, *Balinskycercella fontium*; **OO**, *Balinskycercella gudu*; **PP**, *Balinskycercella tugelae*; **QQ**, *Desmonemoura brevis*; **RR**, *Desmonemoura pulchellum*. Inset shows South Africa's geographical position.



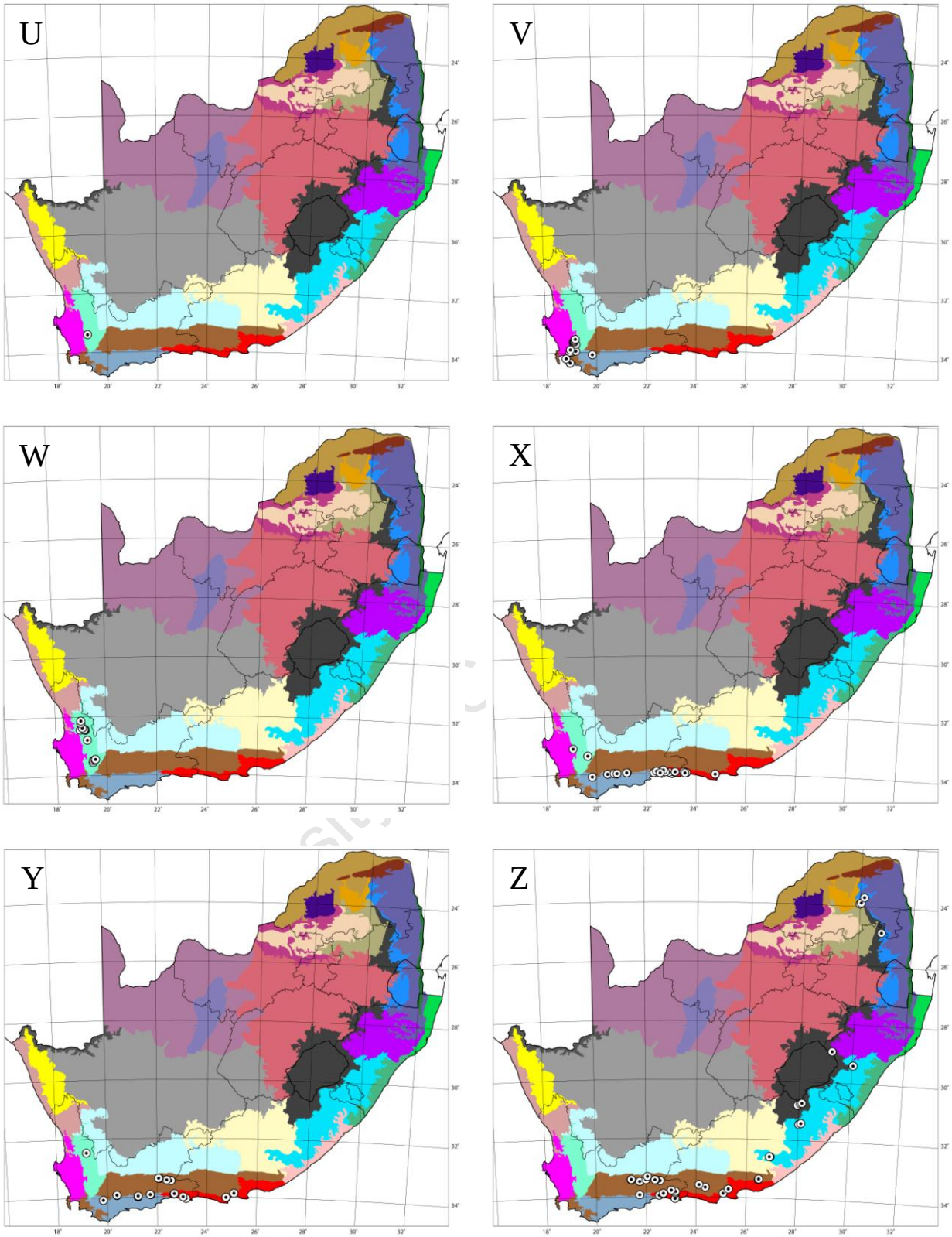
Appendix 4.7. Continued.



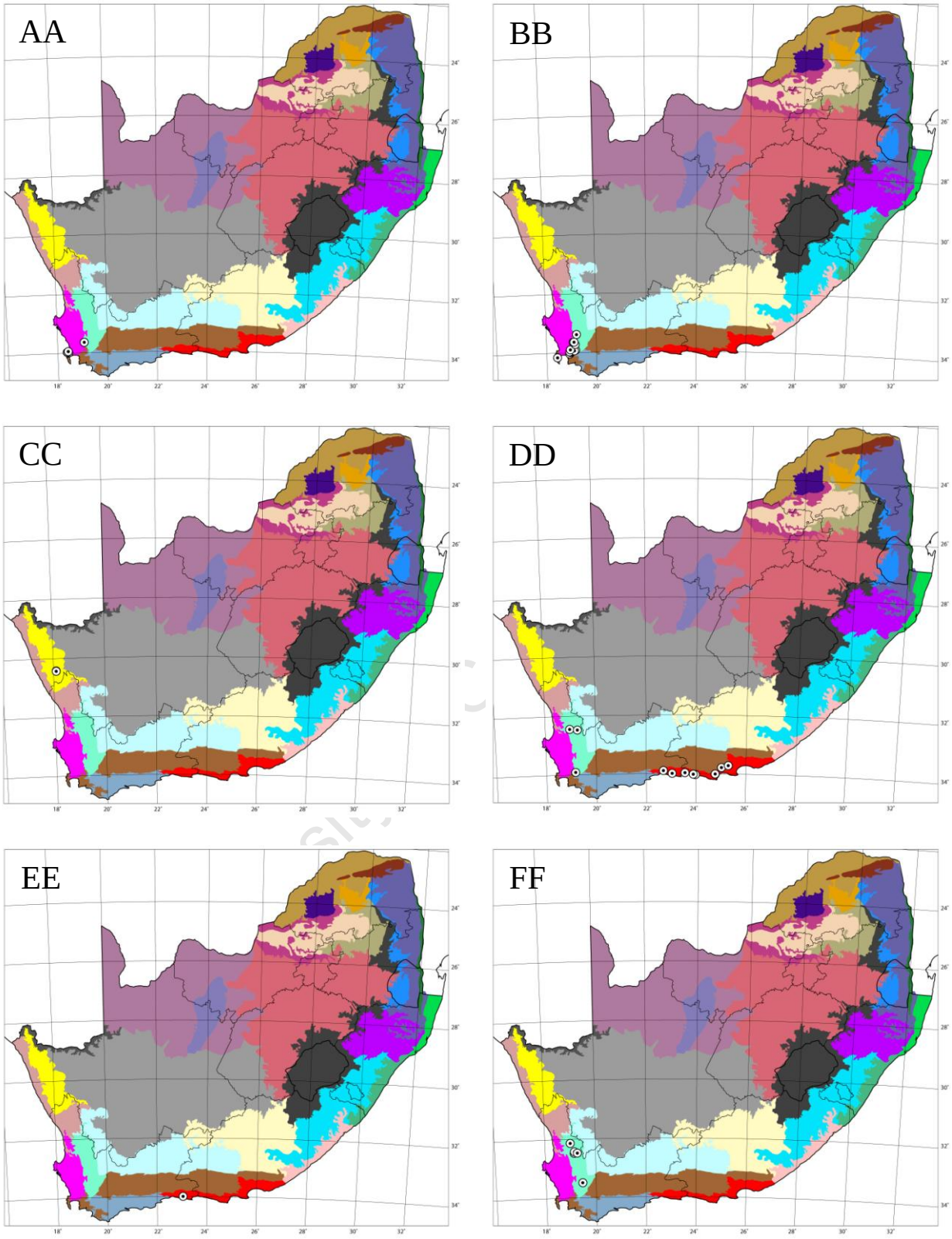
Appendix 4.7. Continued.



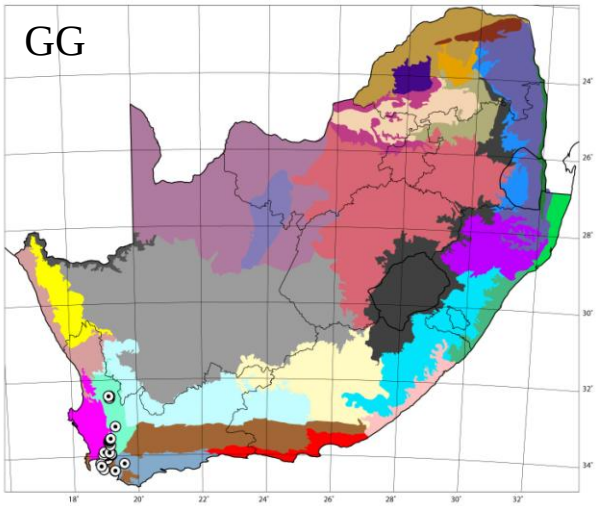
Appendix 4.7. Continued.

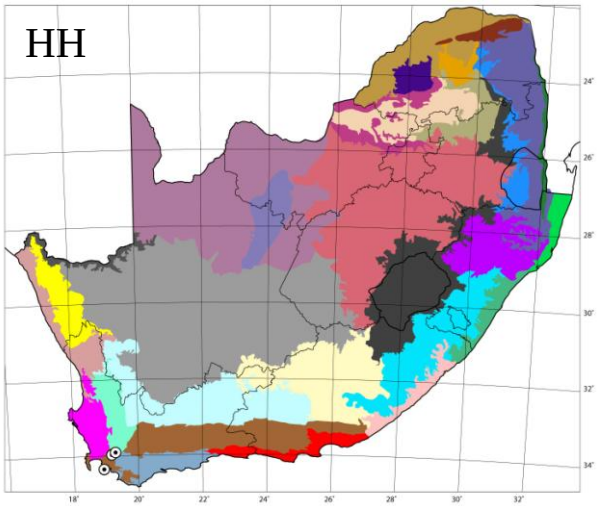


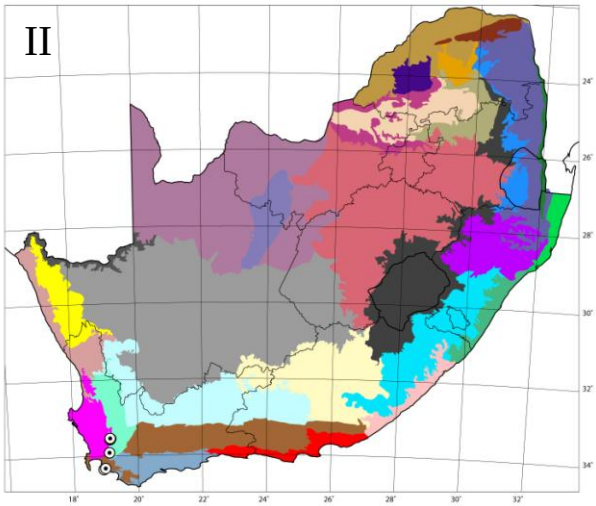
Appendix 4.7. Continued.

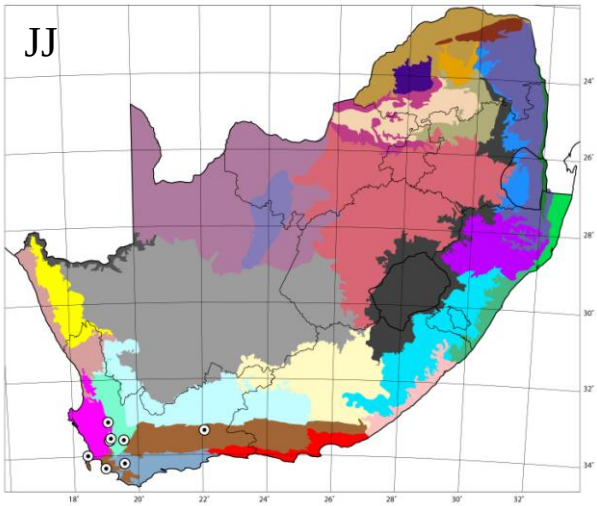


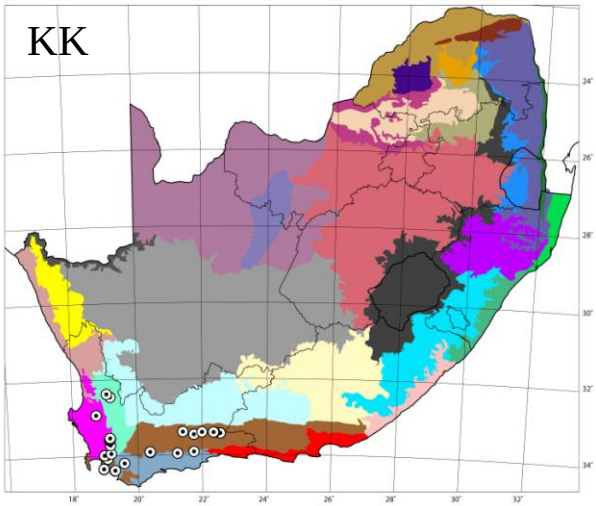
Appendix 4.7. Continued.

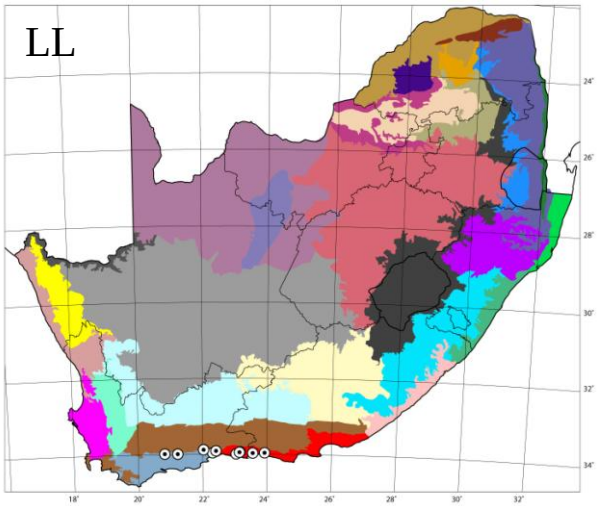




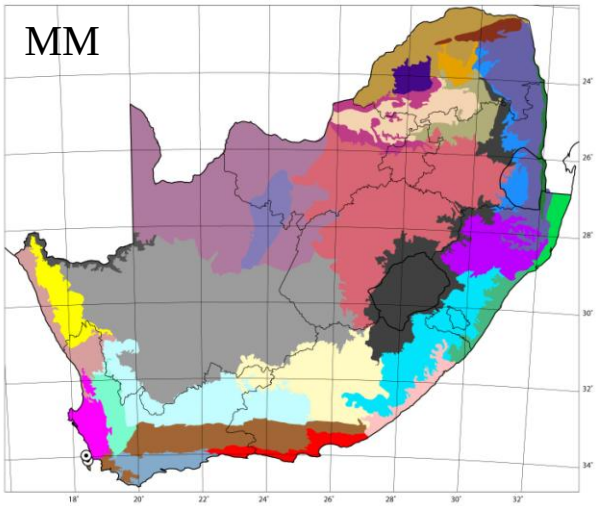


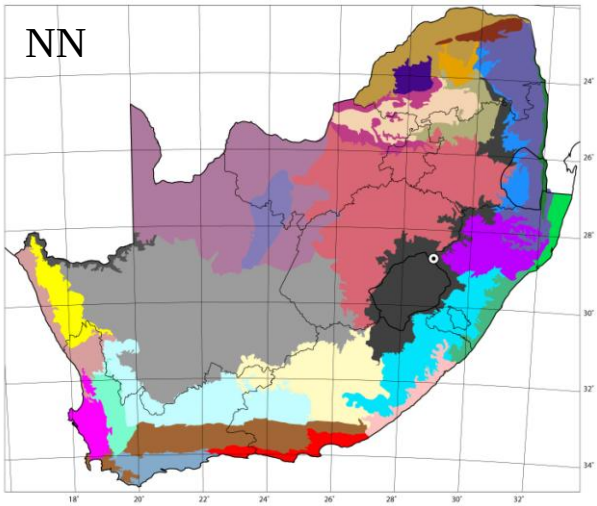


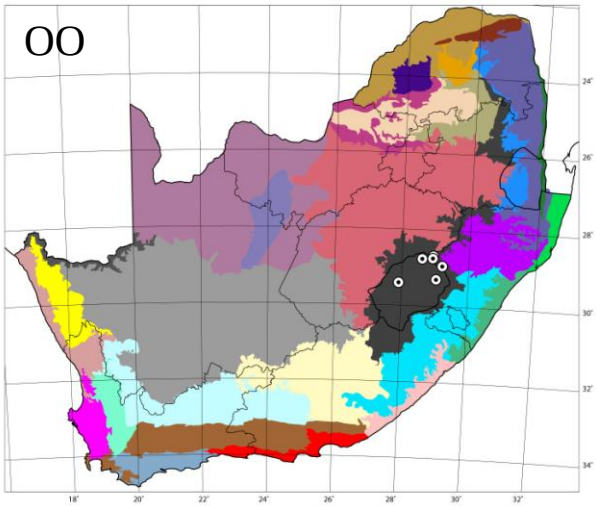


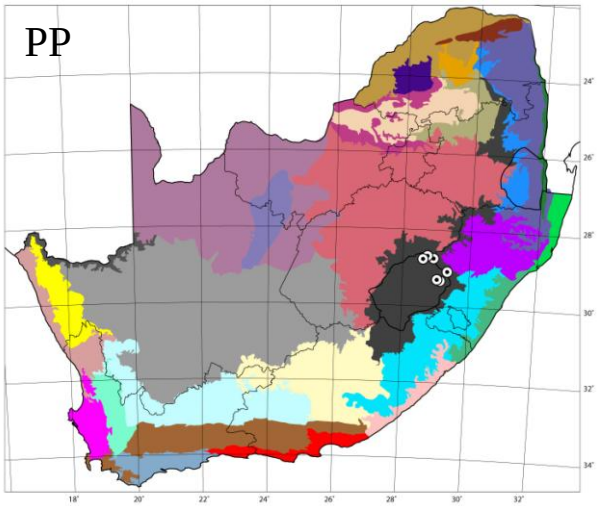


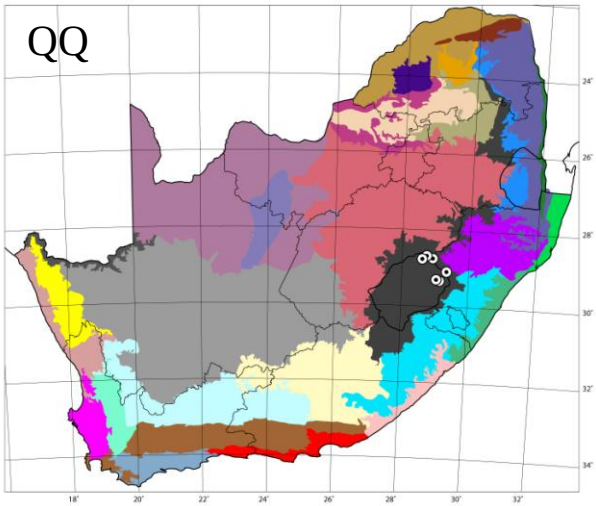
Appendix 4.7. Continued.

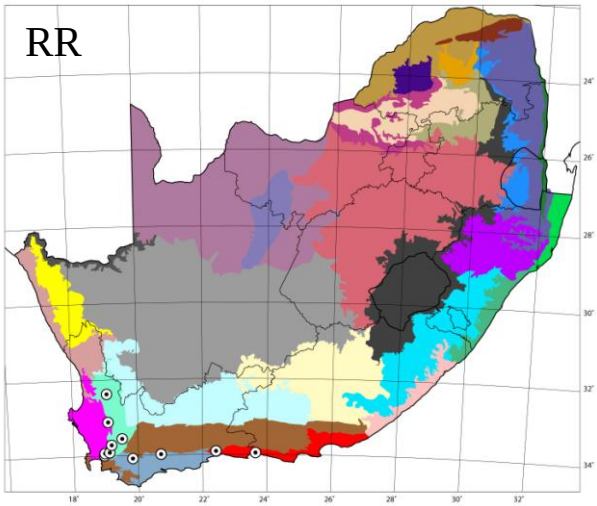




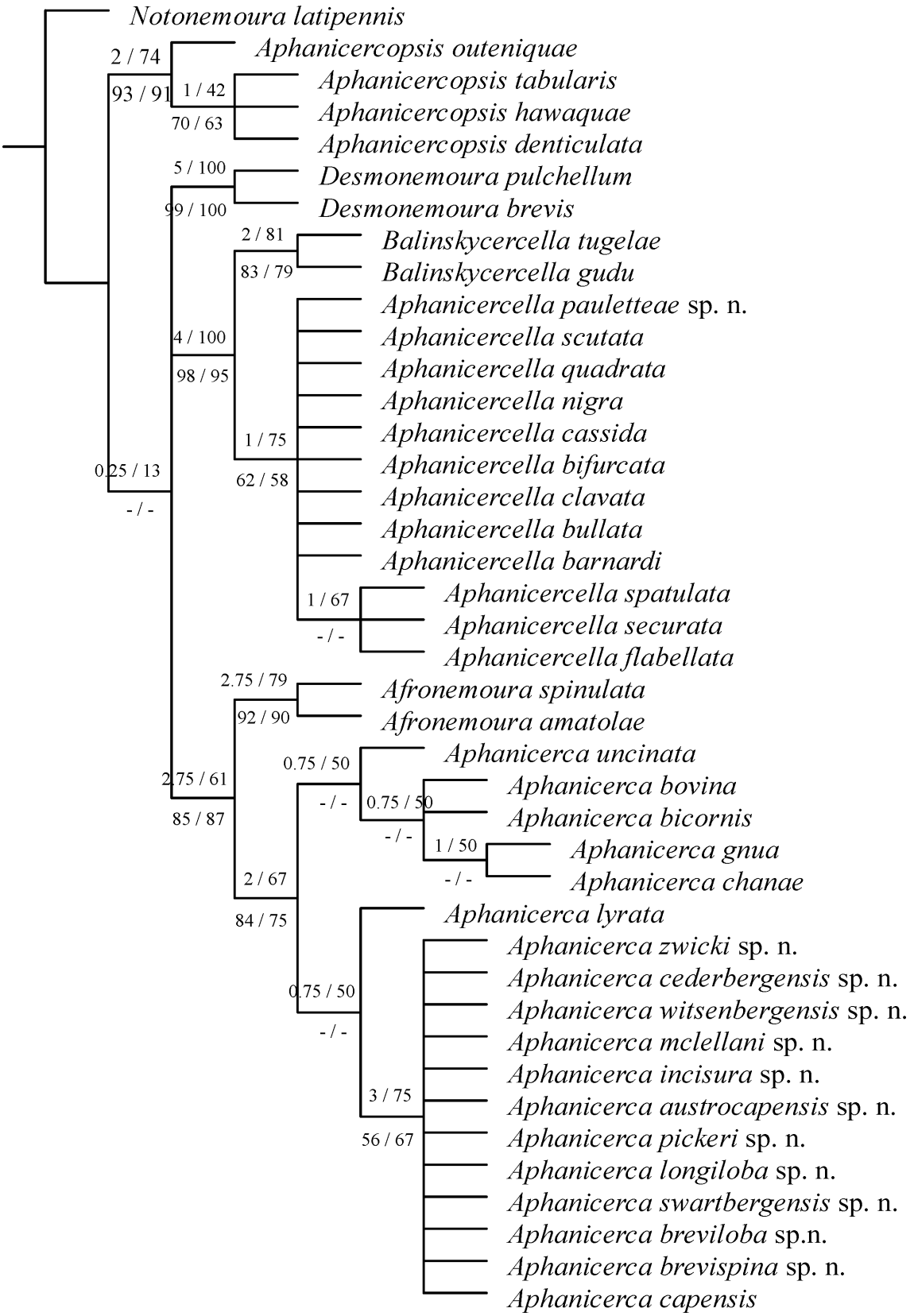




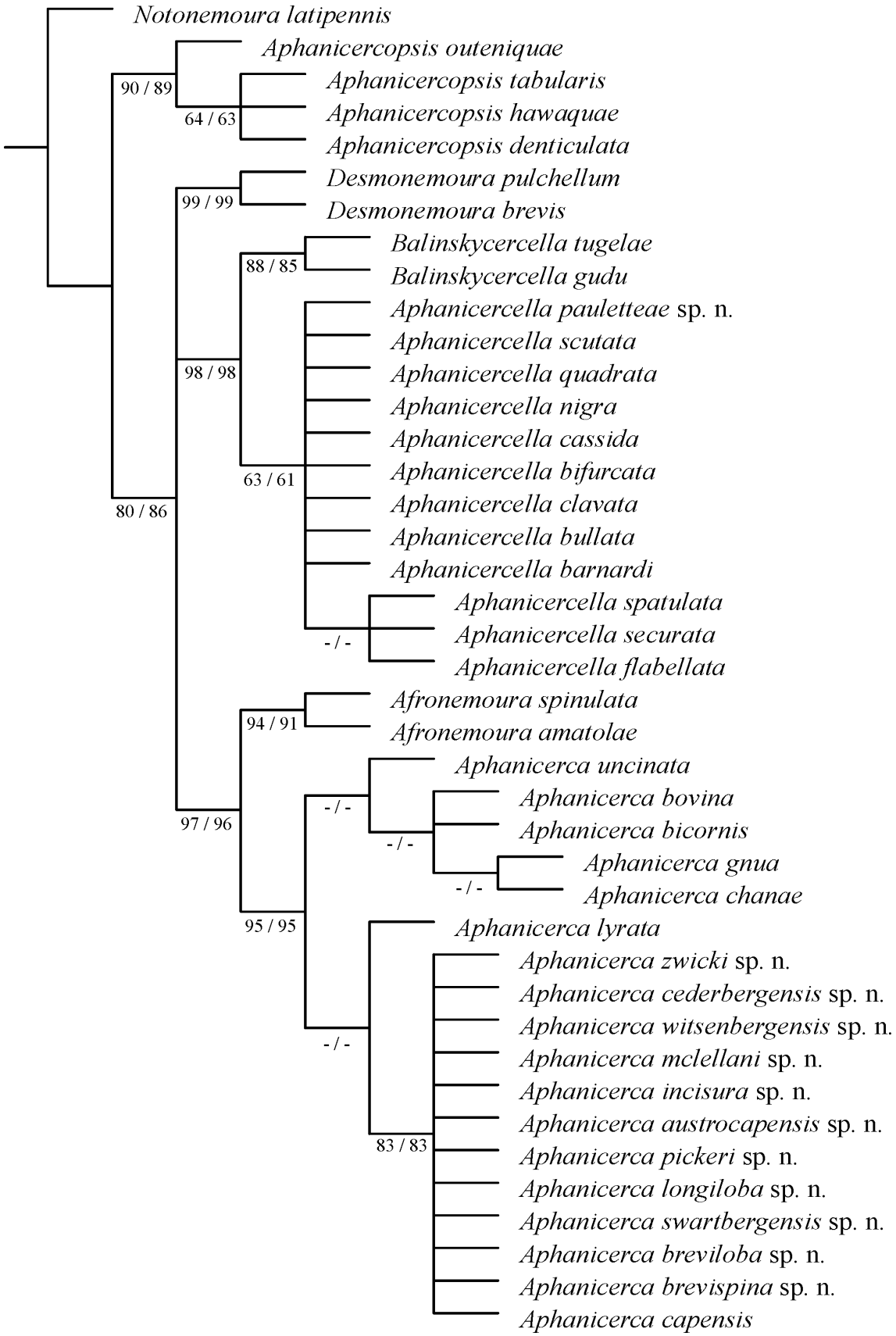




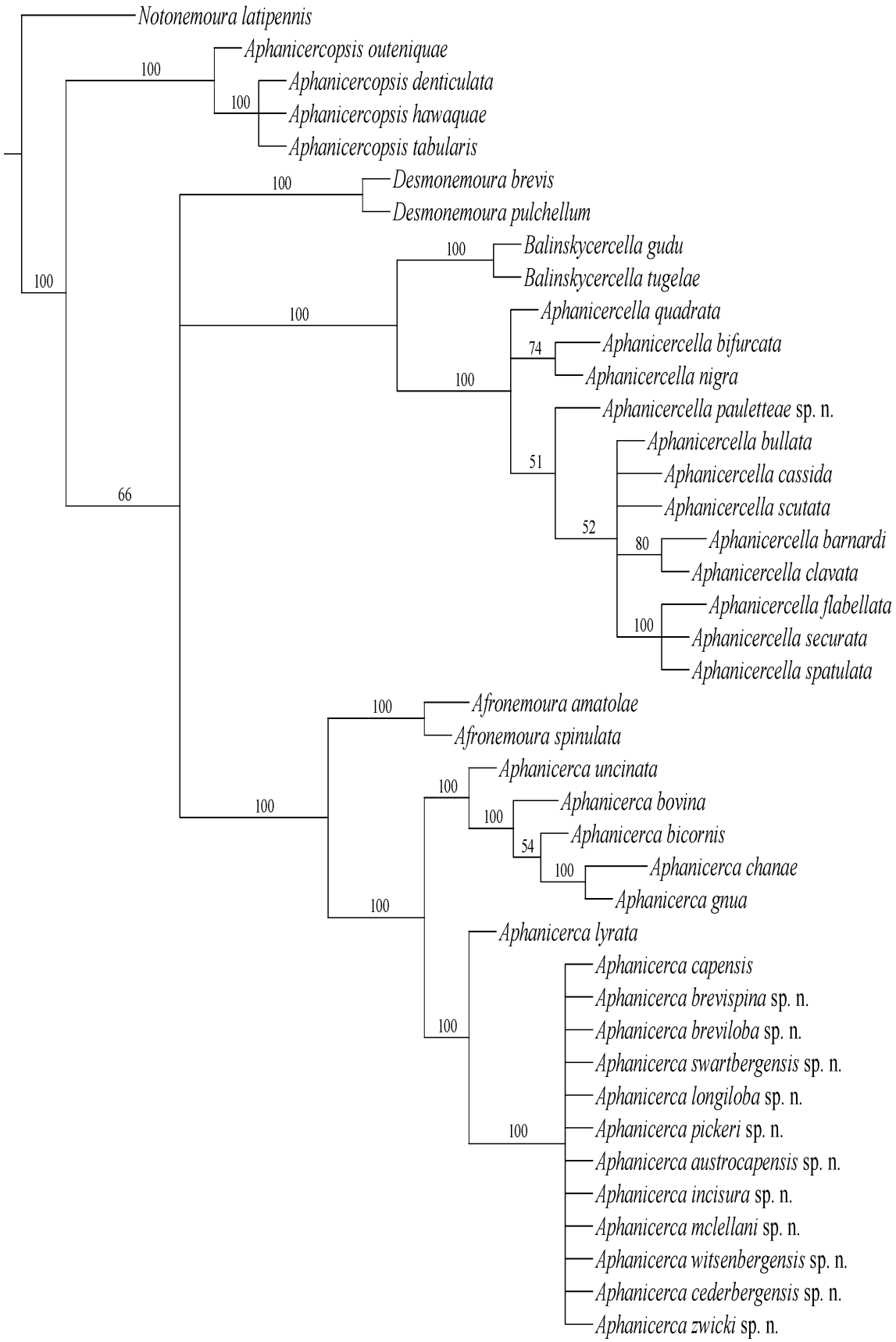
Appendix 4.7. Continued.



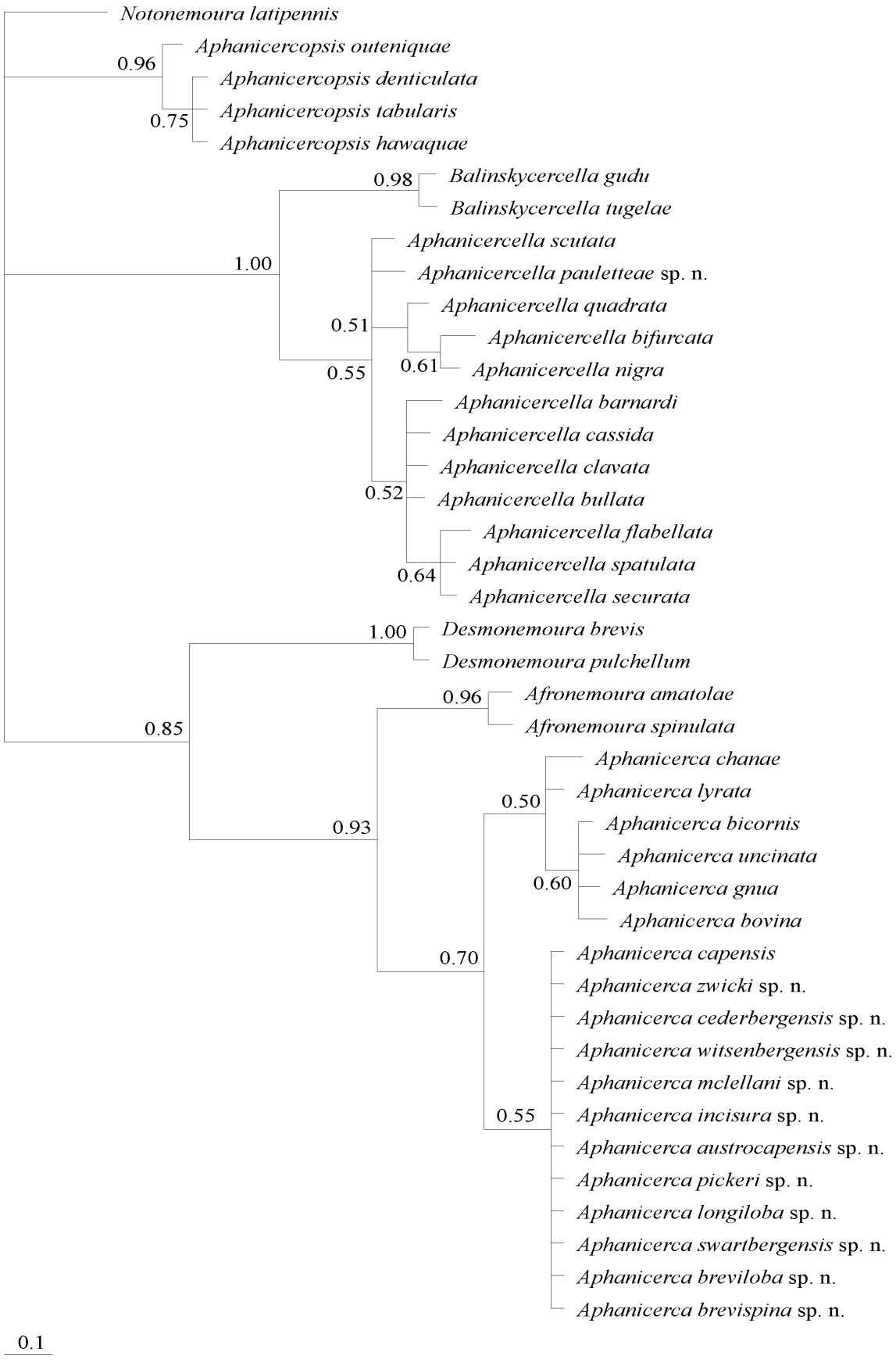
Appendix 4.8. Strict consensus tree of 254 most parsimonious cladograms using morphological characters under self weighting with k = 3. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A dash indicates failure to recover the node.



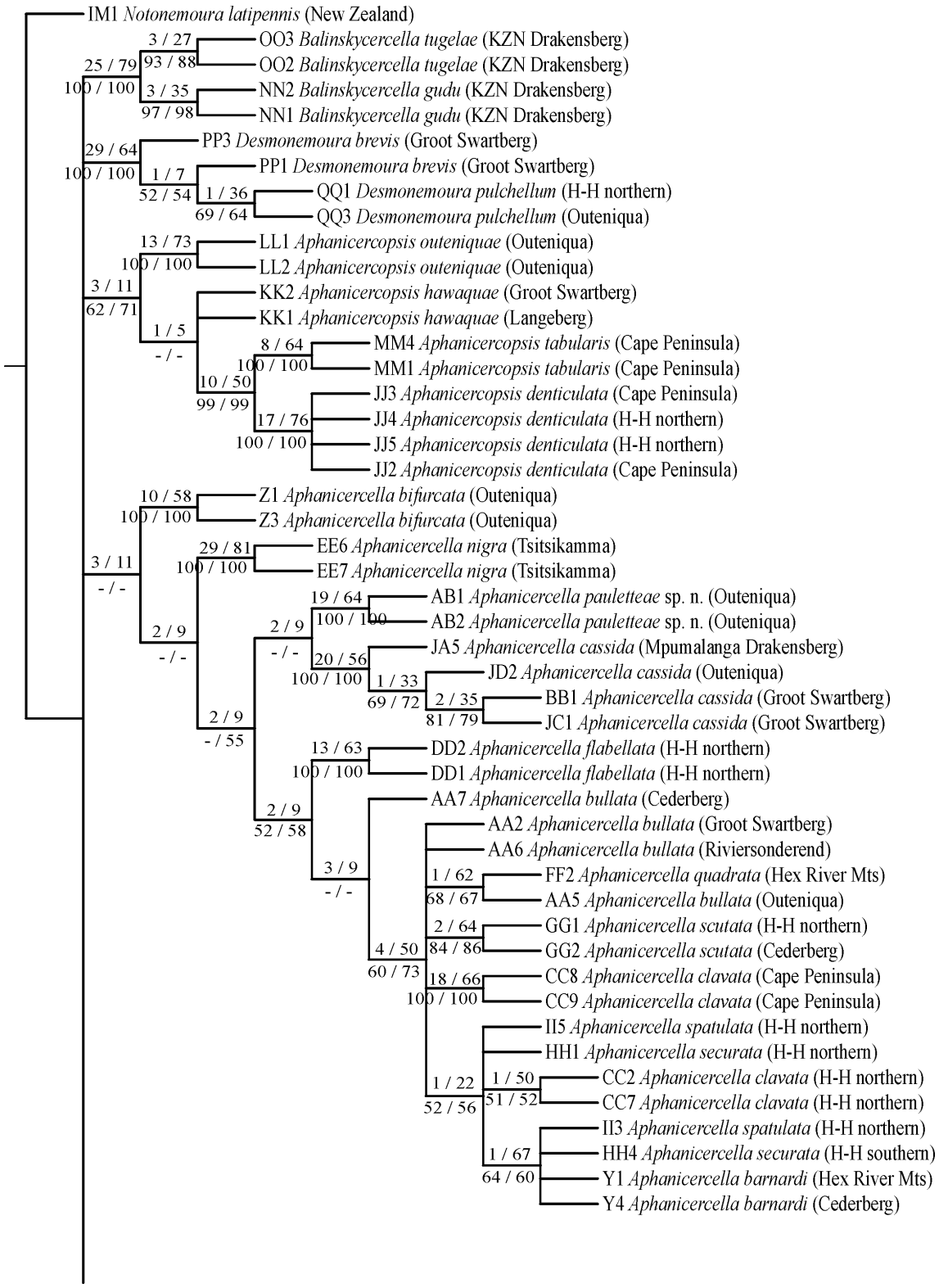
Appendix 4.9. Strict consensus tree of 248 most parsimonious cladograms using morphological characters under successive approximations weighting. Bootstrap (left) and jackknife percentages are given below the branches. A dash indicates failure to recover the node.



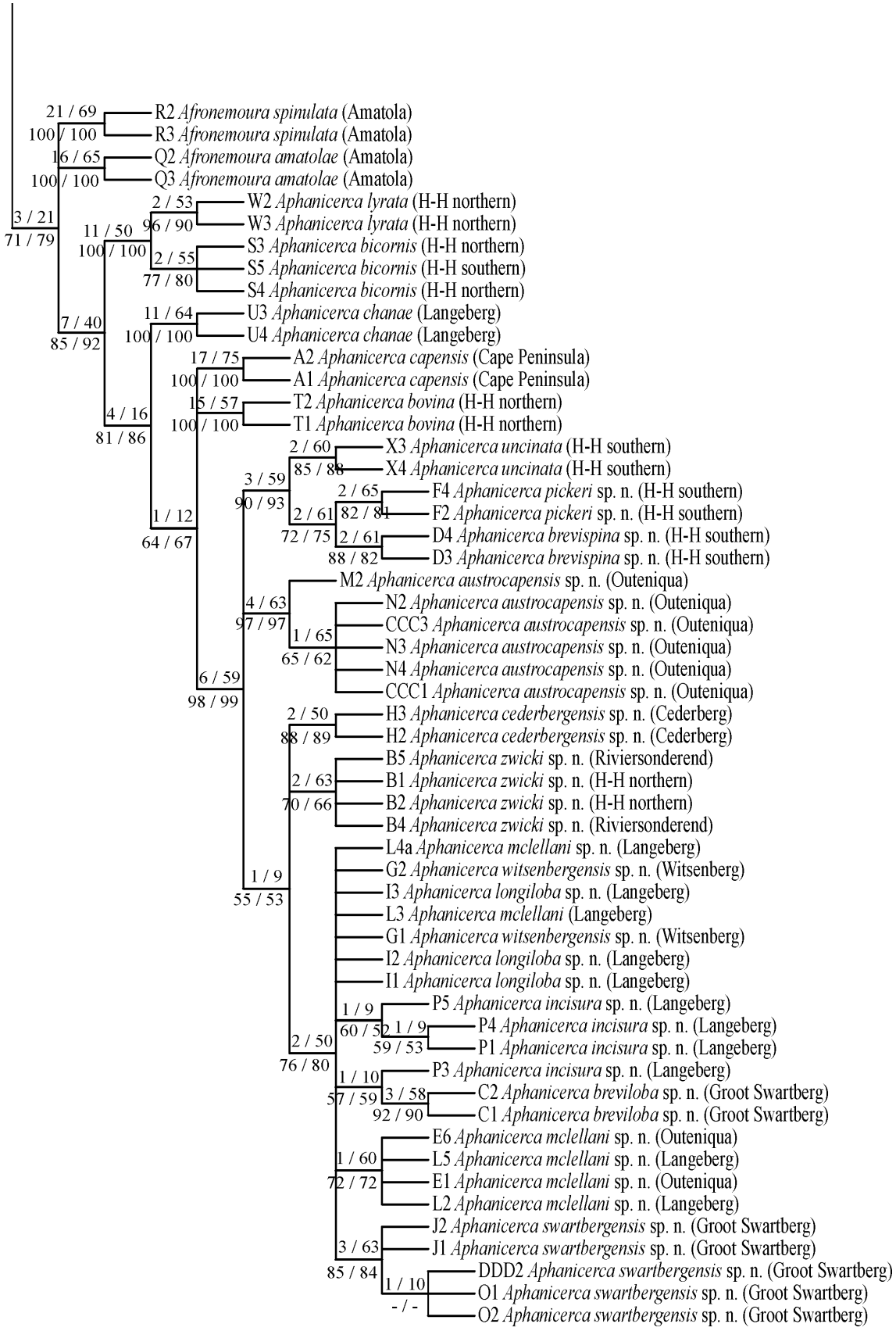
Appendix 4.10. Majority rule consensus tree (L = 87, CI = 85, RI = 96) of 372 most parsimonious cladograms (L = 84, CI = 88, RI = 97) using morphological characters under equal weighting. Numbers above branches indicate the percentage of cladograms that recovered the branch.



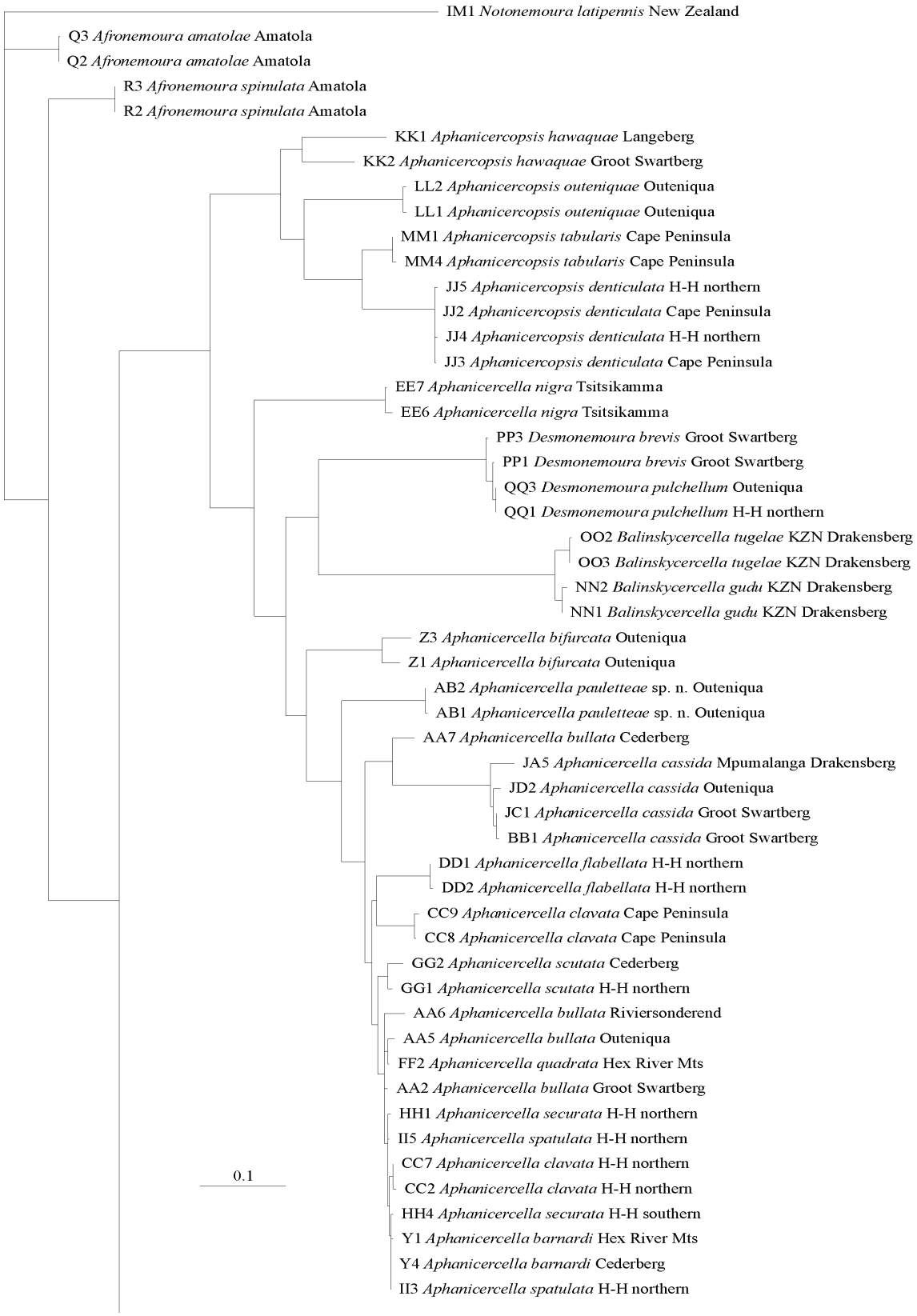
Appendix 4.11. Bayesian Inference majority rule phylogram of morphological data of 39 species of southern African Notonemouridae. Posterior probabilities are given at nodes. The scale bar indicates proportion of character state changes.



Appendix 4.12. Strict consensus tree (L = 1169, CI = 30, RI = 83) of 63 most parsimonious cladograms (L = 1106, CI = 32, RI = 85) using 557 COI bases as characters under equal weighting. Bremer support (left) and relative Bremer support above the branches, and bootstrap (left) and jackknife percentages below. A support value represented by a dash indicates that the node was not recovered. Taxon names are preceded by the sample field code. The mountain range indicates only the sample locality and not the entire range of the species. H-H = Hottentots Holland; KZN = KwaZulu-Natal. Appendix 4.12 continued overleaf.

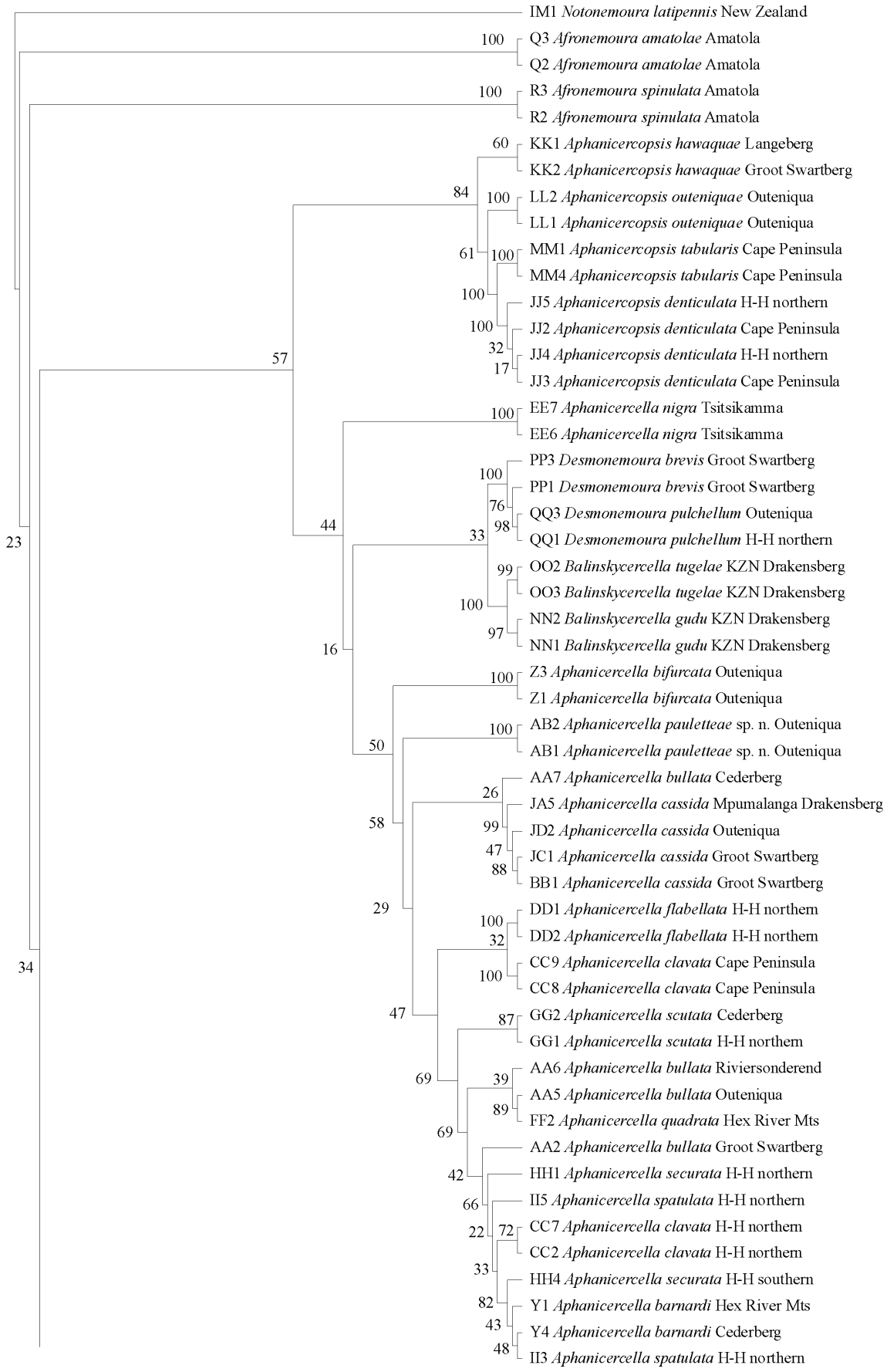


Appendix 4.12. Continued.

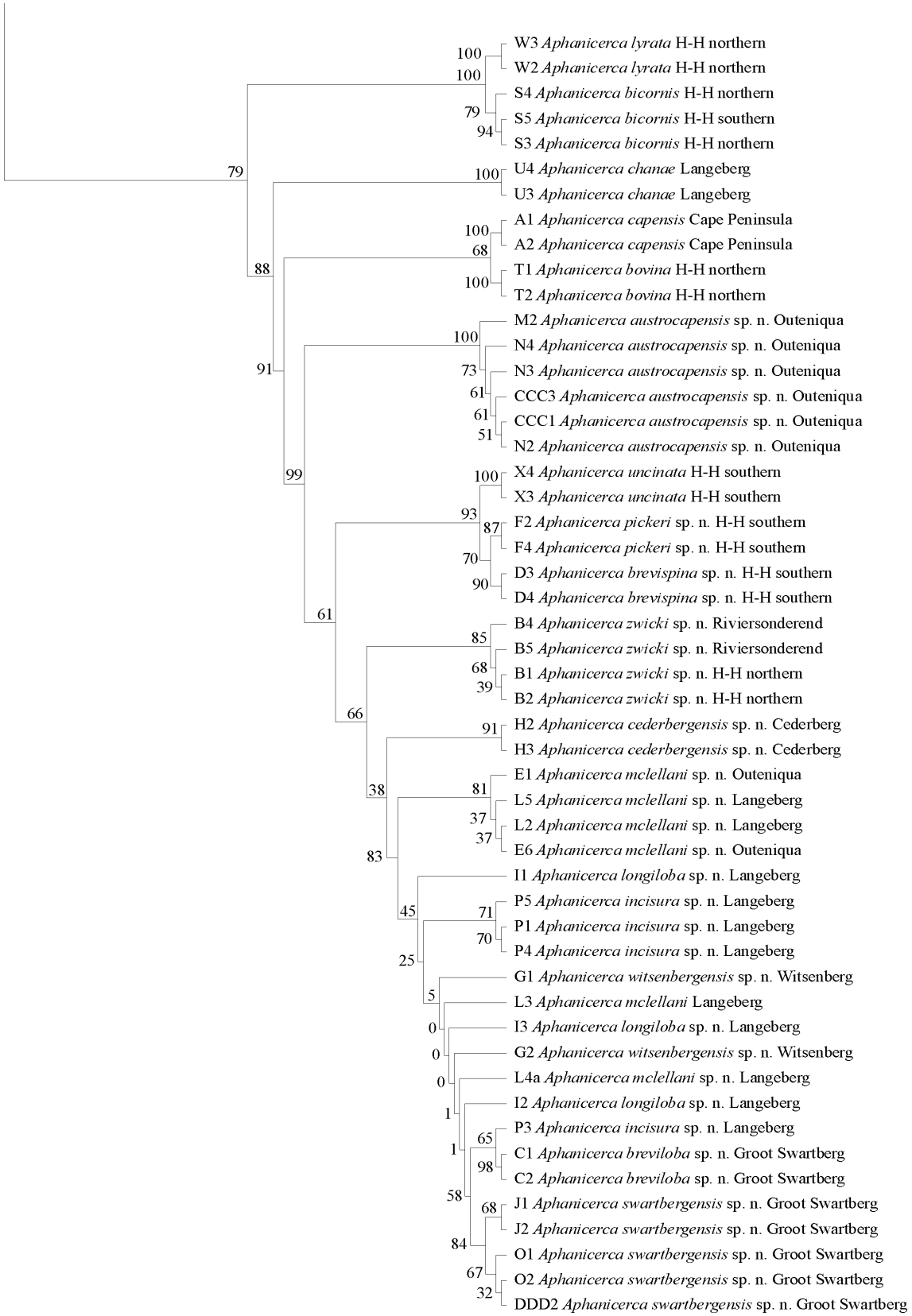


Appendix 4.13. Maximum likelihood tree of COI data of the 39 species of southern African Notonemouridae. *Notonemoura latipennis* is the outgroup. The model of nucleotide substitution used was GTR + I + gamma. The specimen code is to the left of the species name and the locality mountain range of that specimen only is given to the right. H-H = Hottentots Holland. The scale bar indicates 0.1 substitutions per site. Appendix 4.13 continued overleaf.

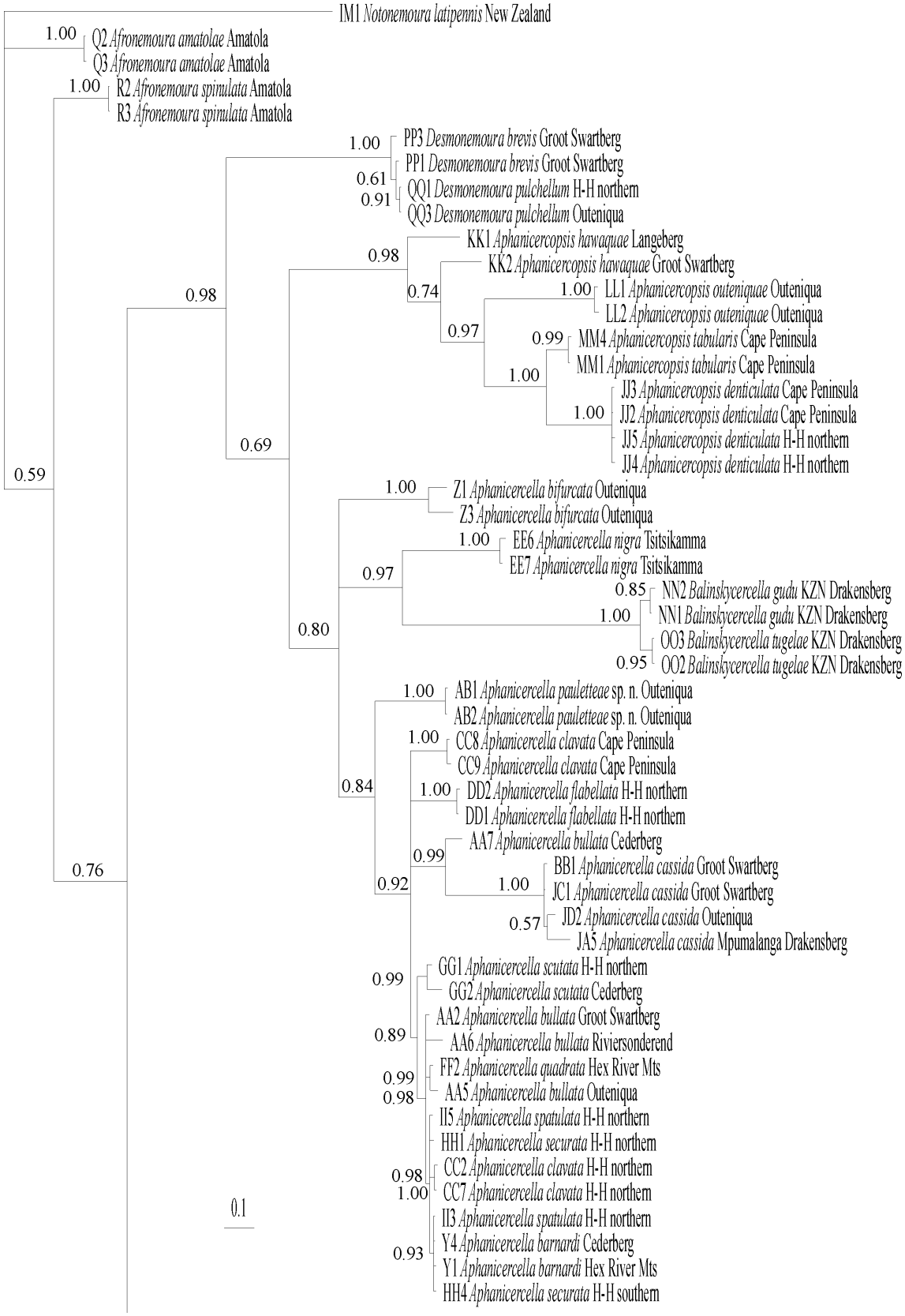
Appendix 4.13. Continued.



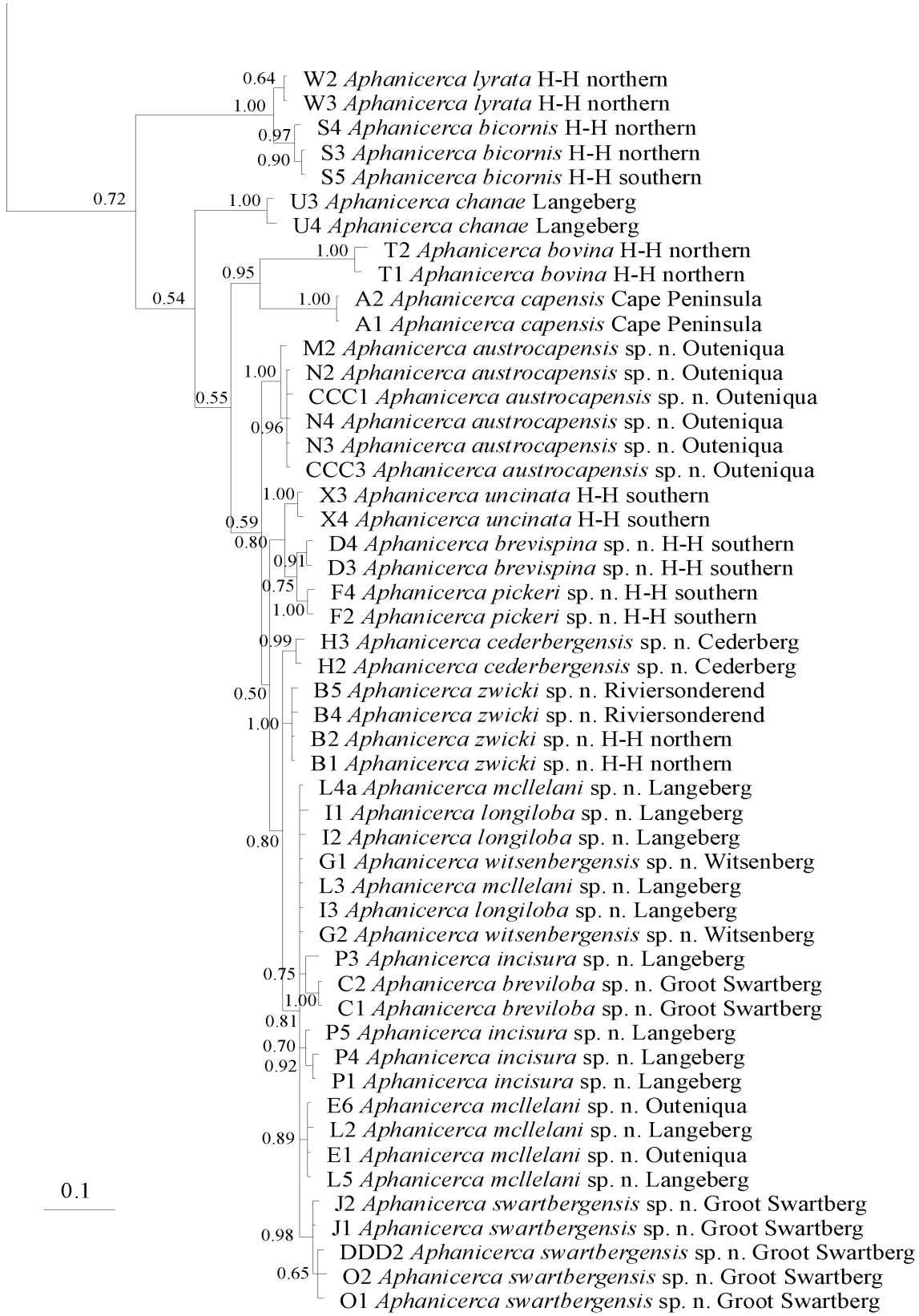
Appendix 4.14. Maximum likelihood cladogram of the phylogram in Appendix 4.13. Bootstrap values are shown for each branch. Appendix 4.14 continued overleaf.



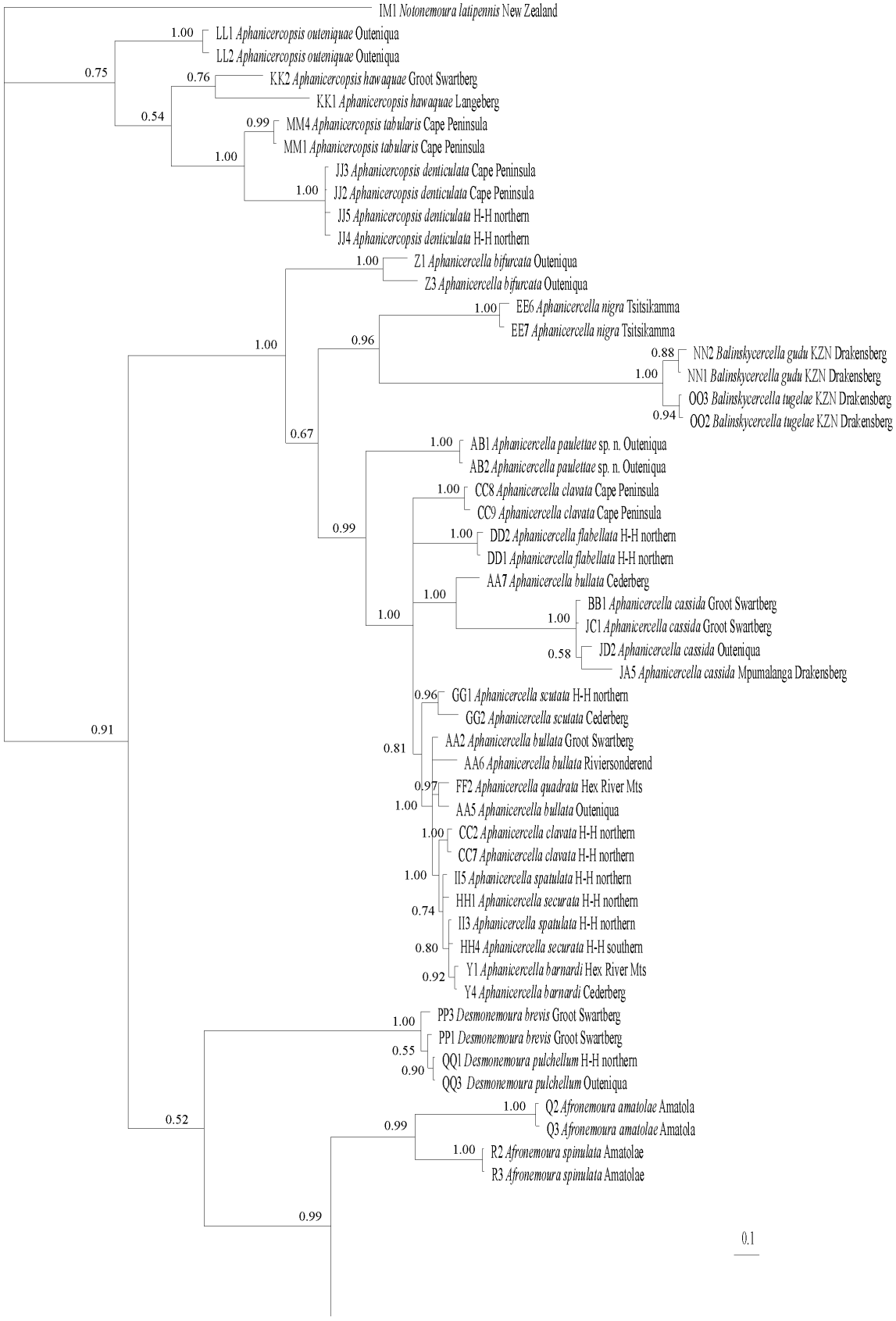
Appendix 4.14. Continued.



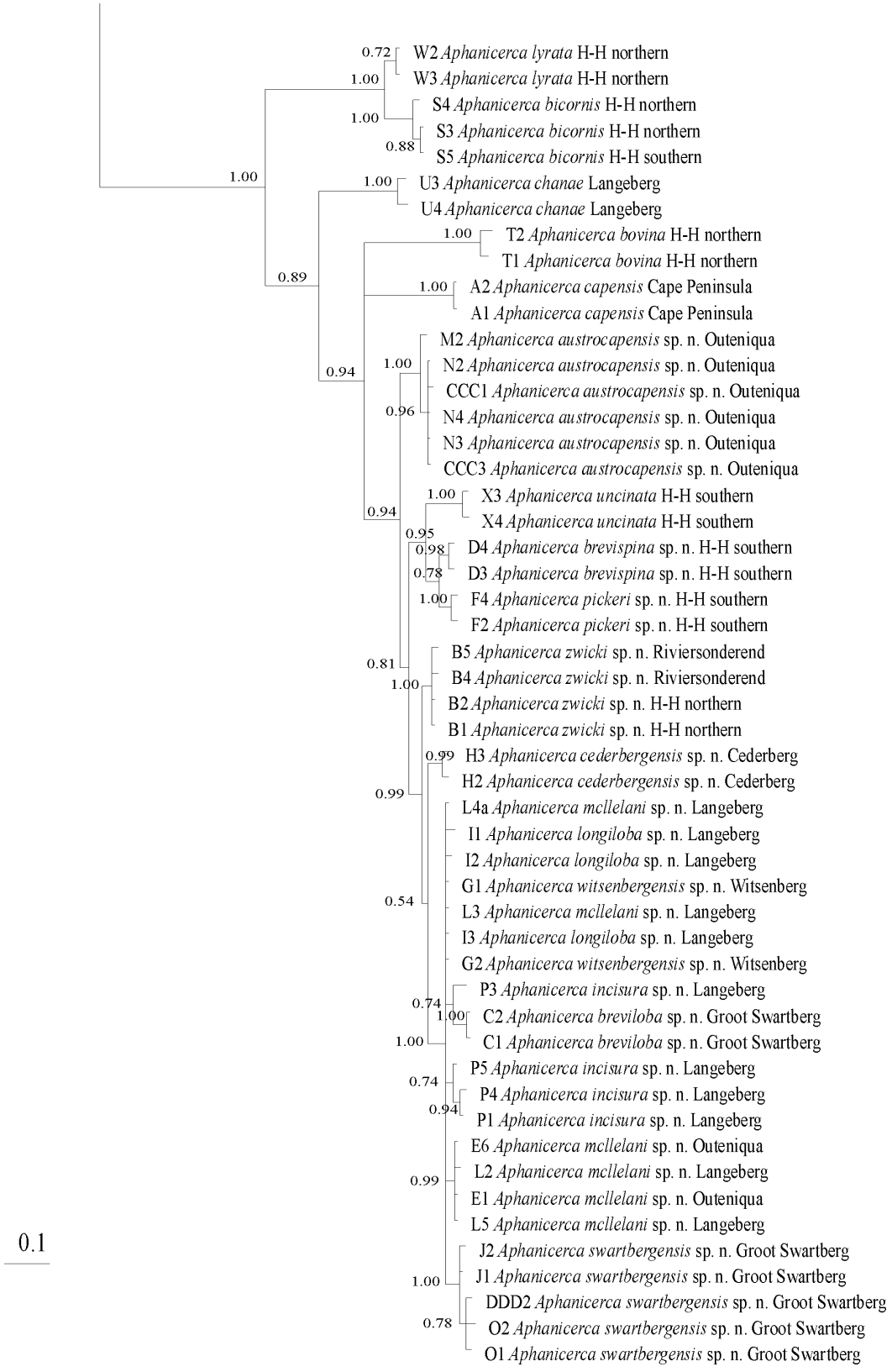
Appendix 4.15. Bayesian Inference majority rule phylogram of COI (mtDNA) of 39 species of southern African Notonemouridae. Posterior probabilities are given at nodes. Taxon names are preceded by the specimen field code. The mountain range indicates only the sample locality and not the entire range of the species. H-H = Hottentots Holland Mountains, KZN = KwaZulu-Natal. The scale bar indicates 0.1 substitutions per site. Appendix 4.15 continued overleaf.



Appendix 4.15. Continued.



Appendix 4.16. Bayesian Inference majority rule phylogram of combined COI (mtDNA) and morphology partitions of 39 species of southern African Notonemouridae. Posterior probabilities are given at nodes. Taxon names are preceded by the specimen field code. The mountain range indicates only the sample locality and not the entire range of the species. H-H = Hottentots Holland Mountains, KZN = KwaZulu-Natal. The scale bar indicates 0.1 substitutions per site. Appendix 4.16 continued overleaf.



Appendix 4.16. Continued.

Summary

TAXONOMY

The taxonomic platform (from Stevens & Picker 1995, 1999; Picker and Stevens 1997, 1999) which formed the foundation for the systematic study provided morphological features for later evaluation as potential cladistic characters for the phylogenetic analyses. A new genus, *Balinskycercella* Stevens & Picker, was described and *Aphanicercella barnardi* confirmed to be a species complex of five species using morphology and mate choice data. Four new species in other genera were described, one each in *Desmonemoura* and *Afronemoura*, and two in *Aphanicerca*. Larval taxonomy produced characters for separating all genera and some species.

CRYPTIC SPECIATION: RESOLUTION OF THE *APHANICERCA CAPENSIS* SPECIES COMPLEX

Morphometric Analysis

- Multivariate analysis of variance (MANOVA) showed that all morphogroups were significantly different from each other with respect to the nine variables used in this analysis.
- All variables were significantly different between morphogroups. Every morphogroup differed from every other morphogroup in at least two variables (Tukey HSD test; Table 3.3).
- Principal Components Analysis showed clearly that the summarized morphometric variables conferred a grouping structure on the various metapopulations or morphogroups of the *A. capensis* species complex (Fig. 3.6).
- The Discriminant Function Analysis resulted in overall highly significant discrimination between morphogroups (Fig. 3.7).
- Genitalic variables were found to be more important than body size variables in discriminatory power.
- Examining the number of times a variable distinguished between two groups in the MANOVA, the PC1 and PC2 component loadings and the DFA partial Wilks' lambdas, the variables with the greatest discriminating power among the methods were: length of the dorsal process of tergite 9, length of spinous part of the tergite 9 dorsal process, and width of separation of the apices of the tergite 9 dorsal process lobes.
- A standard discriminant function formula could be applied to future data to categorise new specimens to one of the existing morphogroups.

Distribution

- There was found to be no correlation between morphological dissimilarity and geographic distance (Mantel test), which suggests the existence of multiple non-interbreeding morphospecies in the groups sampled (Figs 3.8-3.9).
- Mountain range morphogroup endemics were found to be the rule. Current distribution records categorized all 12 morphogroups as endemic to their respective montane regions (Figs 3.1, 3.8).
- Most syntopic and sympatric morphogroups were morphometrically more similar to other morphogroups than to each other (Tables 3.3, 3.9; Figs 3.6-3.9).
- Syntopic morphogroups were: ***P*** and ***S*** in the Outeniqua Mountains; ***P***, ***S*** and ***L*** in the Langeberg; and ***B*** and ***Z*** in the southern Hottentots Holland Mountains (Figs 3.1, 3.8; Table 3.9).
- Sympatric morphogroups were: ***P*** and ***S*** in the Outeniqua Mountains; ***P***, ***S***, ***R*** and ***L*** in the Langeberg; ***B***, ***N*** and ***Z*** in the southern Hottentots Holland Mountains, and ***E*** and ***G*** in the Groot Swartberg (Figs 3.1, 3.8; Table 3.9).
- Most pairs of mountain ranges that clustered together by morphologically similar stoneflies were geographically disjunct (Fig. 3.9).

Mate Choice experiments

- Cape Peninsula ***C*** males preferentially paired with Cape Peninsula females over Stellenbosch ***Z*** females, Bain's Kloof ***Z*** females, and Cederberg ***W*** females (Table 3.10).
- The Stellenbosch ***Z*** males, Bain's Kloof ***Z*** males, and Cederberg ***W*** males however, showed no discrimination between morphogroups (Table 3.10).

Mitochondrial DNA

- The first codon position had the most uniform nucleotide composition. The second position had a thymine bias (43.5%), and the third position was A-T rich, as found in many insect orders.
- The 40 *A. capensis* species complex individuals sampled comprised 27 haplotypes, of which 20 were unique, and seven were shared.
- All haplotypes were unique to their morphogroups except for haplotype 10 which was shared by three morphogroups, namely ***L***, ***S*** and ***T***, of which the first two were syntopic at Kristalkloof in the Langeberg range (Tables 3.13-3.14).
- Analysis of genetic structure showed that 91% of genetic variation was due to among population (morphogroup) haplotype differences, and 9% within population.

- Population pairwise F_{ST} values showed significant differentiation for 29 out of 66 morphogroup pairs (Table 3.16).
- Genetic distance was not correlated to morphology (matrix correlation analysis Mantel test). However, it showed a significant positive correlation to geographic distance.
- The statistical parsimony network excluded morphogroups ***C*** (Cape Peninsula), ***B*** (Betty's Bay, southern Hottentots Holland), and ***N*** (Hermanus, southern Hottentots Holland) at the 95% confidence level (Fig. 3.14).
- Most of the morphogroups formed monophyletic clades, albeit with short branch lengths, with the exception of ***R***, ***S***, and ***L*** (Figs 3.10-3.14). ***T*** was represented by only one haplotype which was shared with ***S*** and ***L*** (Table 3.14).
- All the phylogenetic trees showed a sister group relationship between morphogroup ***C*** (*A. capensis sensu strictu*) and *A. bovina*; also, *A. uncinata* from the Hottentots Holland Mountains was the sympatric sister group to the morphogroups ***B*** and ***N*** (Figs 3.10-3.13).
- Nested Clade Phylogeographic Analysis results suggested that allopatric fragmentation had occurred in two clades (2-3 and 4-1), with haplotype 10 being ancestral (Fig. 3.14; Table 3.18).
- Past gradual range expansion followed by fragmentation was the pattern inferred for clade 3-3, and restricted gene flow with isolation by distance for clade 4-2 (Fig. 3.14; Table 3.18).

Synthesis

Data analysis and synthesis provided:

- Lines of evidence used to infer separately evolving metapopulation lineages of notonemourids in the *Aphanicerca capensis* species complex. These included: allopatric fragmentation, genetic structure, intrinsic reproductive isolation in syntopic morphogroups, intrinsic reproductive isolation in sympatric morphogroups, intrinsic reproductive isolation inferred by complete premating isolation in experimental trials, intrinsic reproductive isolation inferred by unidirectional or incomplete premating isolation in experimental trials, phenetic distinctiveness, morphological diagnosability, reciprocal monophyly, and monophyly (Table 3.20).
- Evidence to support recognition of 12 independently evolving species within the *Aphanicerca capensis* species complex (with additional species likely to be described following further collections of morphogroups too poorly represented to be of use in this analysis).
- Hypotheses for speciation processes in the species complex.

- Support for the controversial role of reinforcement as a force in speciation following secondary contact subsequent to allopatric speciation.
- Recognition of the non-congruency of the *Aphanicerca* COI gene tree and species tree.
- Further evidence of the inappropriate sole use of genetic distance in species delimitation (especially in recently diverged entities).
- Evidence that COI failed as a DNA barcode to unambiguously delimit all species within the genus *Aphanicerca* without reference to other lines of evidence, and therefore by inference failed in the southern African Notonemouridae as a whole.
- Evidence that reproductive cohesion appeared to be incomplete in the recently separated allopatric species of the *A. capensis* complex, but species unity was maintained in sympatric situations.
- Evidence that rates of change in mate recognition systems in the *A. capensis* complex may lag behind those of morphological and genetic divergence in vicariant speciation.
- Evidence of random spatial distribution of morphological types (i.e. not a cline) within this species complex across the CFM.
- Evidence of mitochondrial introgression (possibly historical) or incomplete lineage sorting (or both) within the species complex.
- Evidence of a centre of origin of the species complex in the central region of the Southern Folded Mountains (*sensu* Kleynhans *et al*. 2005) (possibly the Langeberg region).

A MORPHOLOGICAL AND MOLECULAR PHYLOGENY OF THE SOUTHERN AFRICAN NOTONEMOURIDAE

Morphology

- As no prior morphological phylogenies exist, all 48 morphological characters were newly devised and scored across 40 of the 44 possible species in producing the morphological and combined cladograms (Appendices 4.2-4.3). All five morphology maximum parsimony (MP) weighting schemes (equal (Figs 4.4-4.5), *a priori* (Figs 4.7-4.8), successive approximations (Appendix 4.9), implied (Fig. 4.10) and self (Appendix 4.8)) and the Bayesian Inference (BI) morphology cladogram (Appendix 4.11) were consistent on the monophyly of the genera, the clade (*Aphanicercella, Balinskycercella*), and the clade (*Afronemoura*, *Aphanicerca*).
- Male paraproct glands were described for the first time in Plecoptera, and possibly in Insecta (Figs 4.1-4.2).

- Unusual paired reproductive tract structures (probably spermathecae), not previously described in Plecoptera but with possible homology in *Capnioneura* (*Capniidae*), were described in female *Aphanicercopsis* excepting *A. outeniquae* (Fig. 4.3).
- Some important and phylogenetically useful characters were: the degree of fusion of the ventral nerve cord abdominal ganglia (Fig. 4.3), male paraproct glands (occurrence and form), and accessory glands of the male seminal vesicle (Figs 4.1-4.2).
- The unambiguous synapomorphic character states that defined the monophyletic clade (*Balinskycercella*, *Aphanicercella*) in the equal weighting (EW) strict consensus cladogram (Fig. 4.4) were:
    i. Male sternite 9 short
    ii. Male pleurites 10 large and mobile relative to lateral dorsal plates
    iii. Median dorsal plate of male tergite 10 subtriangular (crescentic)
    iv. Male paraproct glands short and thick with a single loop
    v. Male paraproct membranous apex folded over
    vi. Seventh sternite (subgenital plate) bears female genital pore
    vii. Female subgenital plate is not produced caudad to the attachment to the membranous part of the sternite
- These, with the exception of ii, iii, and iv are the same as for the *a priori* (*AP*) analysis (Fig. 4.7).
- The unambiguous synapomorphic character states that defined the monophyletic clade (*Afronemoura*, *Aphanicerca*) in the EW strict consensus cladogram (Fig. 4.4) were:
    i. Male tergite 10 lateral dorsal plates arise from the posterior margin of the tergite
    ii. Lateral supporting sclerite of male paraproct is a robust, short, broad plate
    iii. Medial supporting sclerite of male paraproct is a flat subrectangular plate, parallel to and shorter than lateral sclerite
    iv. Male paraproct membranous tip is not acute apically
- These, with the exception of ii, were the same as for the *AP* analysis (Fig. 4.7).
- The only unambiguously optimized synapomorphic character state that defined the monophyletic clade (*Desmonemoura* (*Afronemoura*, *Aphanicerca*)) in the *AP* MP analysis was increased fusion of ventral nerve cord ganglia, a double weighted character (Fig. 4.7).
- Unambiguous synapomorphies of the *AP* cladogram clade ((*Aphanicercella*, *Balinskycercella*), (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*))) were the presence of male paraproct glands, and the presence of bilateral accessory glands of the male seminal vesicle (Fig. 4.7).

Mitochondrial DNA and combined analyses

- The model based analyses of both the mtDNA partition (ML and BI) (Appendices 4.13 and 4.15 respectively) and combined analyses (BI) (Appendix 4.16) are regarded as less reliable than parsimony in light of the recovery of nonmonophyly of two genera.
- The generic relationships under the parsimony criterion were divided into those that are stable and those that are unstable. Stable clades were common to all trees of all parsimony methods used (Figs 4.4-4.12; Appendices 4.8-4.10, 4.12). These were: (*Aphanicercella*, *Balinskycercella*), and (*Afronemoura*, *Aphanicerca*).
- The most conservative consensus was found to be a polytomy of four clades, namely (*Aphanicercopsis*, *Desmonemoura*, (*Aphanicercella*, *Balinskycercella*), and (*Afronemoura*, *Aphanicerca*)) (Figs 4.4-4.45); when better resolved consensus cladograms recovered *Aphanicercopsis* as the sister group to the remaining genera, then *Desmonemoura* either formed part of the remaining tritomy (Fig. 4.10; Appendices 4.8-4.9), or became sister to (*Afronemoura*, *Aphanicerca*) (Figs 4.7-4.9).
- The combined *a priori* morphology and molecular consensus cladogram (*Aphanicercopsis* ((*Aphanicercella*, *Balinskycercella*), (*Desmonemoura*, (*Afronemoura*, *Aphanicerca*)))) is favoured because at generic level it is fully resolved (Fig. 4.12).

Biogeography

- Distribution maps were provided for all species, and distributions were discussed in relation to major mountain ranges (Figs 4.16-4.17; Appendix 4.7).
- The distribution of the notonemourid genera and species (Fig. 4.16; Appendix 4.7) divided the region into three zones. Two major zones were the Cape Folded Mountains (CFM) (which comprise the Western Folded Mountains and Southern Folded Mountains) and the Eastern Highlands (Fig. 4.13). The third was a minor zone, the Namaqua Highlands.
- The cluster analysis dendrogram showed that mountains ranges had a more similar species composition to geographically proximate mountains than they had to more distant mountains (Fig. 4.15). The low percentage similarity between mountains indicates that local endemism, at mountain range scale, was common. Almost 41% of the species were endemic to a single mountain range group.
- Endemism within the CFM, at 80% (i.e. 20 of the total of 25 endemic species across all ecoregions), was a striking result (Table 4.3).
- Numerous species were found to be endemic to single streams or very restricted areas, and a few were widespread, the most notable being *Aphanicercella cassida*.
- A hypothesis forwarded for the evolution of the South African Notonemouridae proposes that the common ancestor of the six genera dispersed from a CFM origin, to

become widespread across the montane areas of the southern tip of the African continent after the separation from Gondwanaland, including the CFM, Amatola and Drakensberg regions. Because allopatric speciation is believed to be far more prevalent than sympatric speciation, and because there are four genera present in the CFM and usually multiple genera within one stream, it is likely that populations of this most recent common ancestor of these genera became separated by vicariant events (or surrogates such as topographical complexity) within the CFM, allowing the genera to evolve. Species within these genera subsequently underwent cycles of range expansion and speciation in allopatry. Secondary contact would ultimately have occurred resulting in generic sympatry.

- The populations of the common ancestor of *Aphanicerca* and *Afronemoura* became isolated from each other resulting in the evolution of these genera in allopatry. The vicariant event in this case is unknown, but separation of the Amatola Mountains from the Cape Folded Mountains by the formation of the Great Fish River valley during the uplifts and erosions of the mid-Miocene and late-Pliocene is a possible cause. Climatic factors such as increasing aridity followed by expansion of and subsequent contraction of the winter rainfall region may also have been causative.
- The most recent common ancestor of the *Aphanicercella*, *Balinskycercella* clade must have dispersed to become widespread over the entire region from a CFM origin. *Balinskycercella* may be specifically adapted to high altitude habitats.

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