Systematics of the genus *Cliffortia* L. [Rosaceae]

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Abstract

The Cape Floristic Region is the most diverse of the mediterranean climate floras. *Cliffortia* (Rosaceae) is one of the ten largest genera within the Cape Flora with 117 species currently recognised, 104 of which are endemic to the region. I investigate the processes that have driven the diversification of *Cliffortia* within the Cape Floristic Region by means of comparative analyses based on a phylogenetic hypothesis

Three separate datasets were used to reconstruct the phylogeny: nuclear (nuclear ribosomal 5S NTS) and chloroplast (*trn*L intron and *trn*L-*trn*F spacer, part of the *trn*K intron and *mat*K gene and the *psb*A-*trn*H spacer) sequences and morphology. Very strong incongruence between the nuclear and chloroplast trees suggest that hybridization is common between the species. Incongruence is found in the placement of 90 (61.6%) of the 146 accessions included in the analysis, 55 (37.7%) of which show strong incongruence (>80% jackknife support for alternate positions in the two genome trees). Reticulations are found across widely disparate parts of the tree and whole clades have incongruent positions. Therefore, hybridization is proposed as a major process in the evolution of *Cliffortia*.

A revised classification is constructed based upon the new phylogeny. Classifications that follow both the new PhyloCode and traditional Linnaean classification were attempted. The problems for classification caused by extensive reticulations are discussed. Four monophyletic subgenera are recognised, as well as 20 sections, many of which are paraphyletic due to the presence of reticulations. The difficulty in delimiting hybrid species and species complexes is also discussed.

The processes that have influenced speciation are investigated by mapping morphological and ecological characters onto a tree that best represents the reticulate phylogeny. Several different ecological shifts are indicated (e.g. altitude, water dependence and substrate) and these may have promoted speciation. In particular, diversification has occurred in lineages with clonal resprouting as the ancestral growth form. This is attributed to hybridization as a speciation process because hybrids are more commonly found in taxa with the ability to reproduce vegetatively. The possibility that hybridization may have been involved in the diversification of other Cape taxa is postulated.

Biogeographic analysis shows that five phytochoria can be recognised for *Cliffortia*, that correspond to those found in other Cape taxa. Seven areas of endemism can also be

recognised using parsimony analysis: North-western Mountains, South-western Mountains, Cape Peninsula and Cape Flats, Agulhas Plain, Southern Langeberg, Klein and Groot Swartberg, and Drakensberg. Only five species are currently found exclusively outside the existing reserve structure and these should be targeted in assessments for conservation planning.

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"After the marvellous creation of the World, a work so *unim*aginable, measureless and stupendous, the Earth was covered everywhere with Plants, those machines of the supreme craftsmanship; their mechanism so far exceeds our grasp that no craftsman's skill can pry into them or imitate them, even in a single detail."

Opening sentence from the dedication of Linnaeus's Hortus Cliffortianus
Translated by J.L. Heller in Taxon 17: 663–719 (1968)

1. Introduction

Ever since the first Europeans landed at the Cape and returned with some of the plants that they found there, the Cape Flora has gained a deserved reputation for being one of the most remarkable in the world. In more recent years, the early suspicions that an exceptional flora lay at the southernmost reaches of Africa have been supported by more empirical evidence. Good (1974) and Takhtajan (1986) regarded the Cape Flora as one of the six floral kingdoms of the world, based upon number of taxa and degree of endemism. It is by far the smallest kingdom, containing an estimated 9030 species, 69% of which are endemic, in an area of only 90,000 km^2 (Goldblatt & Manning, 2002). This remarkable concentration of species makes the Cape Flora, with its temperate mediterranean-type climate, comparable in terms of species diversity to many areas of the wet tropics.

The Cape Flora and mediterranean-climate floras:

While the Cape continues to be promoted as the smallest of the six floral kingdom (Cowling & Richardson, 1995), four other floras exist that have a similar mediterranean climate: South-west Australia, California, Chile and the Mediterranean Basin itself. They too are exceptionally rich botanically, compared to other areas on equal latitudes, and all rebut the general trend of decreasing diversity with increasing latitude (Cowling *et al.*, 1996). In addition, while the Cape Flora is considerably more speciose than the next richest mediterranean-climate flora (South-west Australia with 5710 species in an area over three times the size, Beard *et al.*, 2000), the number of endemic families and genera is not very different from some of Takhtajan's other phytogeographical regions (Cox, 2001). Therefore, Cox recommended that all these mediterranean-climate areas should be attributed an equal status and denoted as phytogeographical regions. Others have recommended that phytogeographical areas are non-hierarchical and all should be attributed equal status (White, 1971). Hence in this study the term Cape Floristic Region (CFR) is used for the area.

The mediterranean-climate regions are characterised by warm, dry summers and cool, wet winters. They also have in common relatively infertile soils, especially the CFR and South-west Australia, and except for Chile, susceptibility to fire (Cowling *et al.*, 1996). Amongst the five regions, increasing diversity has been associated with reduced nutrients and increased fire frequency (Cowling *et al.*, 1996). Many attempts have been made to explain the high diversity within each region by using comparative studies of different factors affecting local and regional diversity and endemism (e.g. Linder, 1991;

Cowling & Holmes, 1992a; Cowling *et al.*, 1992; Cowling *et al.*, 1994; McDonald & Cowling, 1995; Cowling & Lamont, 1998; Cowling & Lombard, 2002).

Explanations for species diversity can be sought by examining the reasons for differential speciation and extinction rates (Rosenzweig, 1995; Cowling & Lombard, 2002). Within the CFR and South-west Australia, high speciation rates are predominantly attributed to the relatively high fire frequencies, which cause local extinction and high lineage turnover, especially amongst non-sprouting lineages (Cowling, 1987; Cowling & Holmes, 1992a; Cowling & Lamont, 1998). In addition, short gene flow distances allow speciation across steep and strong differential selection gradients (Linder, 1985), while fire has also been invoked as a force that increases vicariance events by extinction of intervening populations (Cowling, 1987). On the other hand, overall extinction rates are considered to be reduced by more stable and mild Quaternary climates caused by maritime influences (Cowling *et al.*, 1996; Cowling & Lombard, 2002; Goldblatt & Manning, 2002).

However, these assessments are often based upon just a single taxonomic group, and in particular members of the Proteaceae (e.g. Cowling, 1987; Cowling & Lamont, 1998). Similar studies across a broader range of taxonomic lineages are needed to confirm whether observed patterns are universally applicable within these regions.

The Cape Flora and large genera:

While the Cape Flora is comparable to many wet tropical floras in terms of number of species per unit area and rate of species turn-over, it is comparatively poor in genera and families (Linder *et al.*, 1992). This is highlighted by the presence of only around half as many genera as Costa Rica, which has a similar number of species (Goldblatt & Manning, 2002). Consequently, the ratio of species to genera and families is exceptionally high. Indeed, when twenty floras around the world were compared the Cape had the highest ratio of species to genus (8.94) and the second highest ratio of species to family (57.8) (Fenner *et al.*, 1997).

However, the species are not evenly distributed throughout the genera but concentrated in a few large genera. The most remarkable of these genera is *Erica*; with 658 species present within the CFR it alone makes up 7.3% of the flora. Thirteen genera contain over 100 species each (Table 1.1), and together they comprise around 25% of the flora (Goldblatt & Manning, 2000). Even more significant are those genera that can also be shown to have had their origin in the Cape and then diversified there. Although it is not

possible to predict *a priori* where a genus originated, it can be assumed that the higher the proportion of species that are endemic to an area, the more likely that the area was the origin for diversification within the genus. Table 1.1 indicates that seven of the thirteen largest genera are also near endemic (>70% endemism).

While there are inherent problems in comparing higher taxa in assessments of floral diversity, the high proportion of species found in only a few near-endemic taxa is a remarkable feature of the CFR. As these taxa constitute such a large proportion of the flora, explaining their evolution will make a significant contribution to our understanding of the history of the flora as a whole. In this study, a detailed analysis of the taxonomy, phylogeny and biogeography of the genus *Cliffortia*, one of the largest near-endemic genera, is reconstructed.

Table 1.1. The thirteen genera with over 100 species present in the CFR. The number of species is given for the worldwide distribution of the genus, and the percent endemic highlights those large genera that are also predominantly Cape genera. Figures are taken from Goldblatt & Manning (2000).

Genus	Total no. of species	Endemic to CFR	Percent endemic
Aspalathus	278	257	92.4%
Agathosma	150	138	92.0%
Cliffortia	117	104	88.9%
Muraltia	115	100	87.0%
Phylica	150	126	84.0%
Lampranthus	155	118	76.1%
Erica	860	635	73.8%
Selago	190	79	41.6%
Moraea	195	79	40.5%
Gladiolus	250	86	34.4%
Pelargonium	250	79	31.6%
Oxalis	500	94	18.8%
Senecio	1200	58	4.8%

Cliffortia and the Cape Floristic Region:

The 117 currently accepted species of *Cliffortia* represent a diversity of growth forms that is only rarely matched by other genera within the CFR, e.g. *Pelargonium* (Bakker *et al.*, 1999b). Although the majority could be described as ericoid shrubs, some grow into small trees up to 5 m tall, while others form low sprawling semi-herbaceous ground-cover or impenetrable dense tangled thickets. Of the growth-forms present in fynbos only annual, succulent and geophytic life-styles are not represented by *Cliffortia*.

The species richness of *Cliffortia* is perhaps all the more surprising considering that all species are wind-pollinated and there is very little morphological variation in the

flowers. It is the most speciose wind-pollinated genus within the CFR, although the family Restionaceae has more endemic species. However, compared to Restionaceae, the inflorescence structure of *Cliffortia* is remarkably uniform. Usually flowers are solitary and scattered evenly over the plant, and only in a few species does this pattern vary and a more complex inflorescence can be described, e.g. in the serotinous species such as *C. conifera*.

Most species in fynbos have seeds that are either wind or ant-dispersed (Linder, 1985), although recently mouse-dispersal has also been demonstrated (Midgley, 2002). Only a few species have been shown to be bird dispersed (le Maitre & Midgley, 1992). Almost all *Cliffortia* species have hard dry seeds implying dispersal by ants or, in the species with larger seeds such as *C. dregeana*, mice. Therefore, it reflects the pattern found in most other fynbos genera, and differentiation of the species using fruit characteristics is predominantly on surface ornamentation. However, a few species have winged seeds adapted to wind dispersal, e.g. *C. alata* and *C. teretifolia*, such as is also found in some Restionaceae (Linder, 1991), and the serotinous species have flattened small light seeds that might also be wind dispersed, as is suspected for several *Leucadendron* species (Williams, 1972; Bond, 1988). Only *C. baccans* has slightly fleshy seeds, and it may be one of the few fynbos species that relies upon bird dispersal. Several species, e.g. *C. arcuata*, also have a membranous layer around the hard seed that swells upon wetting. However, the functional significance of this layer is not clear.

Therefore, species differentiation within *Cliffortia* is based primarily upon vegetative characters and fruit morphology, as opposed to pollination driven floral characters. This makes *Cliffortia* an excellent subject for examining growth-environment driven speciation within the Cape Flora (Johnson, 1996).

The CFR extends in a broad L-shape from the Bokkeveld Escarpment in the north to Port Elizabeth in the east. Five biomes are represented within this geographical area (Cowling, 1992), but it is dominated by the sclerophyllous heath-like vegetation known as fynbos (Cowling & Holmes, 1992b). Fynbos is almost entirely restricted to the CFR, although there are outliers in the hills towards Grahamstown in the east and on the high mountains of Namaqualand to the north. It is characterised by the presence of Restionaceae, as well as several other taxonomic groups, in particular Ericaceae and Proteaceae (Taylor, 1978; Campbell, 1985; Cowling & Holmes, 1992b).

Cliffortia species are found throughout the extent of the CFR. They grow almost exclusively in fynbos, although some also occur in the transition zones between fynbos

and the other biomes. Within the fynbos biome they are ubiquitous, from coastal sand plains to the peaks of the highest mountains (2325 m – Seweweekspoort Mt.). Certain species require their roots to be constantly surrounded by water while others exist in rock crevices of mountains on the arid edge of the Karoo. The majority of *Cliffortia* species is found in the acidic nutrient-poor sandstone-derived soils, but they are also found on alkaline limestone derived soils or the richer Bokkeveld shales. Outside of the CFR, *Cliffortia* species are almost entirely restricted to the afromontane heathlands from the southern Drakensberg to as far north as Mount Kenya, although they are occasional on the low-altitude sandstones of the Eastern Cape and Kwazulu-Natal or the dolerites of the Great Escarpment. Hence, *Cliffortia* is a useful taxonomic group for investigating the diversity of fynbos as it covers almost the complete range of available habitats and gives indications of the phytogeographical links to regions beyond the CFR.

Systematic position of *Cliffortia* within the Rosaceae:

The Rosaceae are a large family containing around 107 genera with 3100 species (Mabberley, 1987). At various times, it has been split into different numbers of subfamilies, containing varying groups of genera and there is no agreement upon which is the best one to use (Morgan *et al.*, 1994). Both Mabberley and Morgan *et al.* recognised four subfamilies within Rosaceae, Maloideae, Prunoideae, Rosoideae and Spiraeoideae. *Cliffortia* has always been placed within the Rosoideae and Morgan *et al.* (1994), using *rbc*L gene sequence data, showed that most of the taxa traditionally placed into that subfamily formed a well-supported monophyletic group sister to the rest of the family. The position of Rosoideae within the Rosaceae has been confirmed by further sampling of different chloroplast gene regions across the family (Potter *et al.*, 2002), while more comprehensive sampling of the genera within the Rosoideae using the nuclear ribosomal ITS gene region has supported the monophyly of this group (Eriksson *et al.*, 1998).

Within the subfamily Rosoideae, *Cliffortia* has consistently been placed in the group or tribe Sanguisorbeae (sometimes also referred to as Poterieae) (Jussieux, 1789; De Candolle, 1825; Harvey, 1862; Bentham & Hooker, 1865; Weimarck, 1934; Kalkman, 1988). Using the nuclear ribosomal ITS DNA region, Sanguisorbeae, represented by four taxa, was resolved by Eriksson *et al.* (1998) as polyphyletic in the Rosoideae. This was because the *Agrimonia* clade was placed within the *Alchemilla* clade and not sister to *Sanguisorba*. However, a tree only one step longer resolved a *Sanguisorba*-

Agrimonia clade, thereby supporting the recognition of the Sanguisorbeae. Eriksson et al preferred this tree, suggesting that the erroneous placement was due to taxon sampling. Twelve out of the 14 genera of the Sanguisorbeae were sampled by Helfgott *et al.* (2000) and the monophyly of the taxon was this time strongly supported.

The Sanguisorbeae were split by Weimarck (1934) into those still retaining their petals (*Sanguisorbeae petaliferae*) and those in which the petals have been lost (*Sanguisorbeae apetalae*). Both are shown to be monophyletic by Helfgott *et al.* (2000) based upon ITS nuclear ribosomal sequences. The position of *Cliffortia* within the *Sanguisorbeae apetalae* is more uncertain. *Sanguisorba* itself is shown to be paraphyletic and *Cliffortia* is placed as sister to two species of *Sanguisorba* (*S. canadensis* and *S. officinalis*). But Malin Hibbs (pers. comm.) has cast doubt upon this placement and her preliminary investigations into the position of *Acaena* and *Polylepis* have suggested that these genera (*Acaena* being paraphyletic to *Polylepis*) are the sister taxa to *Cliffortia*. Helfgott *et al.* also resolve *Cliffortia* as monophyletic, but there are only two species included in the analysis.

Overall, the position of *Cliffortia* within the Rosaceae has been relatively stable and this has been supported recently by molecular work. Only the immediate sister taxon to *Cliffortia* remains questionable. This is of interest because of the different distributions of the two possibilities: *Sanguisorba* has a Holarctic distribution, while *Acaena* is distinctly Gondwanan. Therefore, choice of one over the other would dramatically affect the interpretation of how the progenitor of *Cliffortia* arrived in South Africa. However, this will not be investigated in this study as work is already underway by Hibbs.

Previous studies on *Cliffortia*:

Weimarck was the last person to monograph the genus *Cliffortia* (Weimarck, 1934). He continued to describe new species (Weimarck, 1937; Weimarck, 1940a; Weimarck, 1940b; Weimarck, 1946)and then produced an updated taxonomical survey of the genus (Weimarck, 1948). After this he described a further six species (Weimarck, 1953; Weimarck, 1959) but carried out no more in depth analysis or discussion of the genus.

Weimarck (1934) gave a good overview of taxonomic work prior to his monograph. The first three *Cliffortia* species to be mentioned in literature were by Plukenet (1696), although the galled specimens led him to designate *C. strobilifera* as a cedar (*Cedrus*)! Linnaeus (1753) described four species in his Species Plantarum, and there was

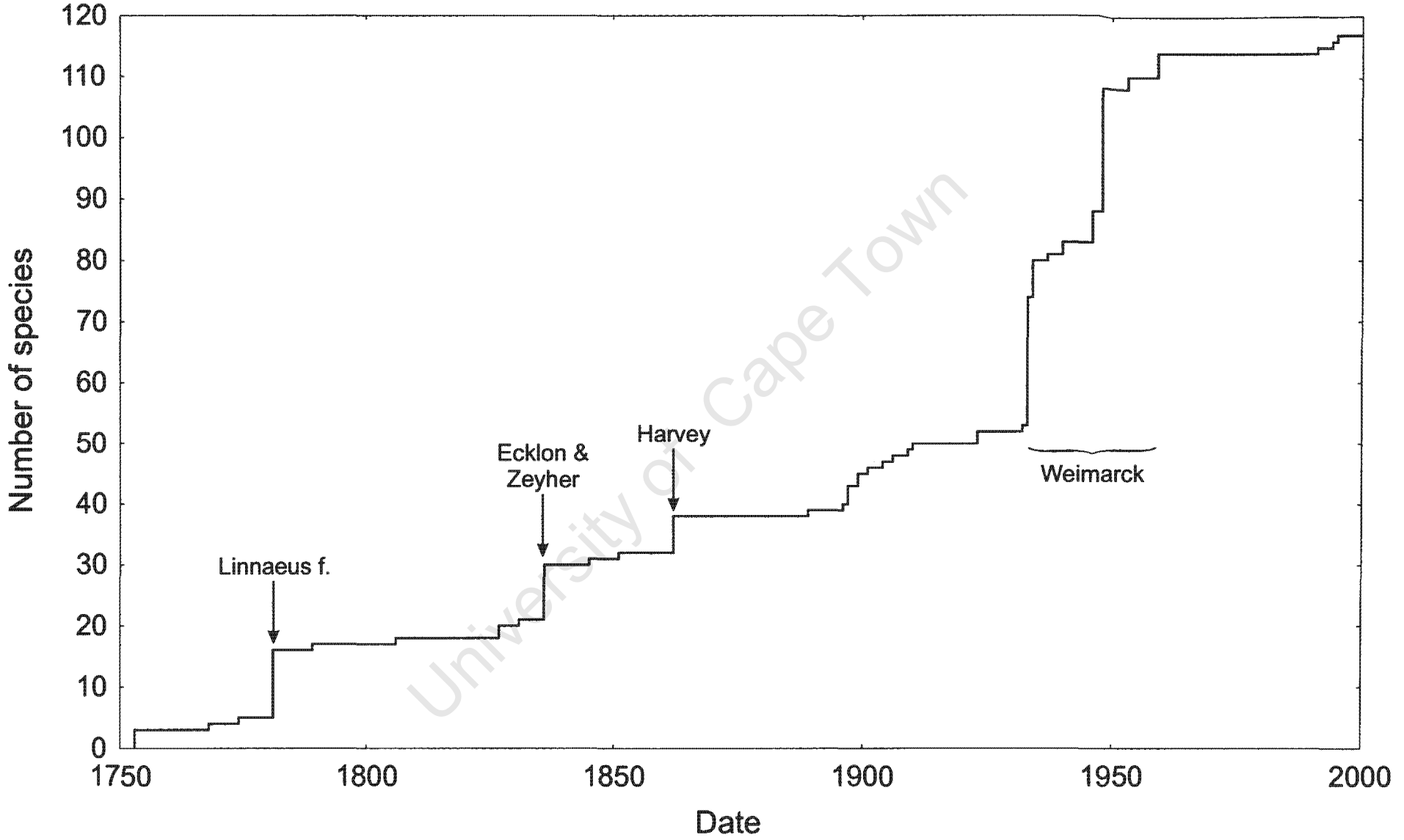


Fig. 1.1. Chart showing the increase in number of known species of *Cliffortia* through time. Based upon date of description for those species that are currently still recognised.

subsequently a steady rate of description of new species by various authors, with major contributions by the younger Linnaeus (1781), based on work by Thunberg, and by Ecklon and Zeyher (1836) (Fig. 1.1). Prior to Weimarck, the last major treatment of the genus in its entirety was by Harvey (1862) for the Flora Capensis. In total only 269 different names have been used for *Cliffortia*, which is comparatively conservative for a large genus with morphologically variable species.

It is worth noting that only two attempts were ever made to subdivide the genus into separate genera, *Morilandia* (Necker, 1808) and *Monographidium* (Presl, 1849), neither of which was ever taken up and used (Weimarck, 1934). In addition, the only species of *Cliffortia* that have been placed in the wrong genera upon description were as a result of serious misidentifications (i.e. also the wrong family, e.g. *Aspalathus*, *Anthospermum*) rather than uncertain delimitations. Hence, the cohesive nature of *Cliffortia* has never been questioned and the taxonomy has been stable.

Subsequent to Weimarck's thorough study, only Fellingham has worked on the genus to any extent. Her publications have predominantly been focussed on the description of new species and the clarification of species limits, including some nomenclatural work (Oliver & Fellingham, 1991; Fellingham, 1993a; Fellingham, 1993b; Oliver & Fellingham, 1994; Fellingham, 1994; Fellingham, 1995), although recently she undertook a study of the phenology and branching patterns of eight species of *Cliffortia* (Fellingham, 1999).

Although the insignificant flowers and difficulty in distinguishing the species may have reduced the interest in this large Cape taxon, especially compared to the charismatic ericas, geophytes and proteas, the paucity of recent taxonomic work on *Cliffortia* can also be attributed to the accuracy and comprehensiveness of Weimarck's study. Species were generally well-defined and the alpha taxonomy has withstood close scrutiny (e.g. Fellingham, 1993b; Fellingham, 1994). Consequently, *Cliffortia* has had a relatively uncomplicated taxonomic history, permitting this current work to be undertaken without a need to revise the underlying taxonomy first.

Weimarck's sectional classification is still currently used, as are his species definitions (Table 1.2), with the addition of those described by Fellingham (1991; 1994; 1995). The only people to have questioned his species delimitations are those working on species outside the Cape: *C. aequatorialis* was sunk into *C. nitidula* by Graham (1960), while Hilliard & Burtt (1987) doubted that *C. spathulata* could be maintained as distinct from *C. browniana*. Hence, in the most recent conspectus of the genus for the CFR all

Weimarck's species were retained (Fellingham, 2000). This makes a total of 111 species currently described and accepted within the CFR and a further six, including *C. aequatorialis* and *C. spathulata*, found exclusively outside the CFR.

Table 1.2. Tabulation of the sections recognised by Weimarck, with the currently recognised 117 species assigned to their appropriate sections.

Section	Species
Complanatae Weimarck	*C. complanata* E. Mey. ex Harv., *C. dentata* Willd., *C. filicauloides* Weim., *C. gracilis* Harv., *C. hantamensis* Diels, *C. propinqua* Eckl. & Zeyh.
Hermaphroditicae Weimarck	*C. hermaphroditica* Weim.
Petiolatae Weimarck	*C. arcuata* Weim., *C. drepanoides* Eckl. & Zeyh., *C. falcata* L.f., *C. lepida* Weim., *C. pedunculata* Schltr., *C. triloba* Weim.
Costatae Weimarck	*C. acocksii* Weim., *C. aequatorialis* R.E. Fr. & T.C.E. Fr., *C. amplexistipula* Schltr., *C. apiculata* Weim., *C. atrata* Weim., *C. browniana* Burtt Davy, *C. burchellii* Stapf, *C. carinata* Weim., *C. castanea* Weim., *C. concinna* Weim., *C. crassinervis* Weim., *C. curvifolia* Weim., *C. densa* Weim., *C. dispar* Weim., *C. dodecandra* Weim., *C. eriocephalina* Cham., *C. exilifolia* Weim., *C. filicaulis* Schldl., *C. filifolia* L.f., *C. geniculata* Weim., *C. glauca* Weim., *C. hirta* Burm.f., *C. incana* Weim., *C. juniperina* L.f., *C. linearifolia* Eckl. & Zeyh., *C. marginata* Eckl. & Zeyh., *C. montana* Weim., *C. nitidula* (Engl.) R.E. & T.C.E. Fr., *C. obcordata* L.f., *C. obovata* E. Mey. ex Harv., *C. paucistaminea* Weim., *C. polita* Weim., *C. pterocarpa* (Harv.) Weim., *C. ramosissima* Schltr., *C. rigida* Weim., *C. robusta* Weim., *C. serpyllifolia* Cham. & Schldl., *C. setifolia* Weim., *C. spathulata* Weim., *C. stricta* Weim., *C. subsetacea* (Eckl. & Zeyh.) Diels ex Bolus & Wolley Dod, *C. tenuis* Weim., *C. tuberculata* (Harv.) Weim.
Bacciformes Weimarck	*C. baccans* Harv., *C. micrantha* Weim.
Alatae Weimarck	*C. alata* N.E. Br., *C. burgersii* E.G.H. Oliv. & A.C. Fellingham, *C. semiteres* Weim., *C. teretifolia* L.f.
Inflexae Weimarck	*C. cristata* Weim., *C. hexandra* Weim., *C. lanata* Weim., *C. polygonifolia* L., *C. sericea* Eckl. & Zeyh., *C. subdura* Weim.
Arboreae Weimarck	*C. arborea* Marloth, *C. conifera* E.G.H. Oliv. & A.C. Fellingham
Bifoliolae DC.	*C. crenata* L.f., *C. crenulata* Weim., *C. mirabilis* Weim., *C. phyllanthoides* Schltr., *C. pulchella* L.f., *C. varians* Weim.
Multinerviae DC.	*C. aculeata* Weim., *C. cervicornu* Weim.*, *C. cymbifolia* Weim., *C. dregeana* Presl, *C. graminea* L.f., *C. grandifolia* Eckl. & Zeyh., *C. heterophylla* Weim., *C. ilicifolia* L., *C. integerrima* Weim., *C. intermedia* Eckl. & Zeyh., *C. lanceolata* Weim., *C. multiformis* Weim., *C. nivenioides* A.C. Fellingham, *C. ovalis* Weim., *C. ruscifolia* L., *C. strigosa* Weim., *C. theodori-friesii* Weim., *C. verrucosa* Weim., *C. virgata* Weim.
Simplices Weimarck	*C. acutifolia* Weim., *C. brevifolia* Weim., *C. cuneata* Aiton, *C. discolor* Weim., *C. erectisepala* Weim., *C. ericifolia* L.f., *C. esterhuyseniae* Weim., *C. ferruginea* L.f., *C. hirsuta* Eckl. & Zeyh., *C. longifolia* (Eckl. & Zeyh.) Weim., *C. monophylla* Weim., *C. neglecta* Schltr., *C. odorata* L.f., *C. phillipsii* Weim., *C. pilifera* Bolus, *C. pungens* Presl, *C. repens* Schltr., *C. reticulata* Eckl. & Zeyh., *C. strobilifera* Murr., *C. tricuspidata* Harv., *C. uncinata* Weim., *C. viridis* Weim.

*Weimarck (1959) suggested that *C. cervicornu* should belong to a section of its own but that there was insufficient material present at the time and implied that its greatest similarity was with sect. Multinerviae.

Objectives:

The genus *Cliffortia* represents one of the large Cape taxa that may have explosively radiated within the CFR. Two simplified scenarios for this radiation can be hypothesised. 1) The diversity observed can be assigned to just a single key innovation that permitted speciation to exploit a large number of new niches. 2) The diversity is a result of a multiplicity of different factors not related to any one attribute of the genus, each contributing something towards the diversification of the genus. The former scenario will highlight speciation processes that are peculiar to the genus, or might be found in other taxa with the same attributes, while the latter will reveal processes that are applicable across a wide range of taxa within the CFR.

This study aims to:

a) Establish a supra-specific classification based upon a phylogenetic hypothesis.

b) Search for a mechanism that might have generated the species richness within *Cliffortia*.

c) Evaluate the biogeographical patterns in the genus.

These objectives are achieved by reconstructing a phylogenetic hypothesis based upon molecular and morphological data. A protocol similar to that proposed by Bateman (1999) is employed. Four datasets are assembled: nuclear gene sequence data, plastid gene sequence data, morphological data, and extrinsic data in the form of habitat and distributional information. The first three are used to infer a phylogenetic hypothesis using parsimony analysis (Chapter 2). Three steps are used to analyse the collected data: 1) individual cladograms are generated for each of the datasets; 2) the topologies of the individual datasets are compared for congruence and the cause of any discrepancies are discussed; 3) the data is combined to produce the most parsimonious phylogeny. The application of the resultant phylogenetic hypothesis is discussed in Chapters 3–5.

Chapter 3 outlines the methods used in constructing a revised classification based upon phylogenetic principles. The current classification of the genus (Weimarck, 1934; Weimarck, 1948) is reinterpreted in the light of the phylogeny produced in Chapter 2. Possible classifications based on both traditional Linnaean nomenclature (Greuter *et al.*, 2000) and the new PhyloCode (Cantino & de Queiroz, 2000) are presented.

In Chapter 4, patterns within the morphology and extrinsic data are compared with the phylogeny. Possible processes that have influenced speciation events within *Cliffortia*

are suggested. Previous evolutionary hypotheses are also tested against the current phylogeny to examine if they are still valid.

In Chapter 5, distributional data is used to examine the biogeographical patterns within the CFR by identifying areas of high diversity and endemism within *Cliffortia*. Phytogeographical links between the Cape Flora and those to the north are also investigated.

Finally, Chapter 6 expands upon the significance of hybridization. The role of hybridization as a factor in the speciation and diversification of *Cliffortia* is discussed and consequently how this affects our understanding of the Cape flora and its conservation.

Molecular systematic work is already being conducted upon several other large CFR genera, e.g. *Agathosma*, *Aspalathus*, *Erica*, *Lampranthus*, *Moraea* (Goldblatt *et al.*, 2002), *Muraltia*, *Pelargonium* (Bakker *et al.*, 1998; Bakker *et al.*, 1999a), and *Phylica* (Richardson *et al.*, 2001a; Richardson *et al.*, 2001b), as well as important Cape taxa, e.g. Iridacae, Proteaceae and Restionaceae (Eldenas & Linder, 2000). When phylogenetic hypotheses have been obtained for many of these taxa, common patterns can be sought between them. These patterns can then be used to identify the major causes of species diversity within the CFR (Linder, 1996). The study presented here is an important addition to this ongoing research into the Cape Flora, both in understanding its origins and for helping to conserve its future.

2. Phylogenetic Reconstruction

Introduction:

The explosion in molecular systematics over the past 20 years has been remarkable (Crawford, 2000). Nowadays, few phylogenetic studies are conducted without resorting to some molecular techniques. The ease with which gene sequences can be rapidly produced has led to a wealth of reassessments of generic and familial boundaries (e.g. Morgan *et al.*, 1994; Graham *et al.*, 1998; Muasya *et al.*, 1998; Hu *et al.*, 2000; Bayer *et al.*, 2000; Eldenas & Linder, 2000; Baker *et al.*, 2000b; Richardson *et al.*, 2000; van der Bank *et al.*, 2002). However, species-level molecular phylogenies of plants have only recently become more common (e.g. Sang *et al.*, 1997; Fuertes Aguilar *et al.*, 1999; Jorgensen & Frydenberg, 1999; Caujapé-Castells *et al.*, 1999; Alice & Campbell, 1999; Schultheis & Baldwin, 1999; Baker *et al.*, 2000a; Helfgott *et al.*, 2000; Ohsako & Ohnishi, 2000; Meimberg *et al.*, 2001; Chandler *et al.*, 2001; Bellstedt *et al.*, 2001; Yoo *et al.*, 2002). The reason for this is three-fold. Firstly, the genetic markers used for higher-level phylogenies are too conserved at the species-level and there is difficulty in finding suitably variable DNA regions (Soltis & Soltis, 2001). Secondly, there is uncertainty as to what will adequately represent the chosen terminal taxa in a phylogeny. When generic boundaries are not being questioned in a family level phylogeny, one or two carefully chosen exemplars can be used to represent a genus in a phylogeny (e.g. the study of Rosaceae by Morgan et al. (1994)). However, the concept of species and thus their delimitation is still so much under debate that the boundary between systematics and population biology is constantly blurred (Baum & Shaw, 1995; Avise & Wollenberg, 1997). The inevitable presence of paraphyletic species (Rieseberg & Brouillet, 1994; Crisp & Chandler, 1996), as well as the increasing likelihood of detecting polyphyletic multiple-origin species (Soltis & Soltis, 1999; Grant, 2002) (e.g. Ashton & Abbott, 1992; Ayres & Strong, 2001; Hagen *et al.*, 2001), means that the true evolutionary history for the species may not always be revealed by a single representative.

Providing there are enough resources, sequencing more DNA regions or increasing the number of collections sampled can be relatively easily achieved. However, the inclusion of more data can also make producing a phylogeny even more intractable, for increasing evidence is being found in species-level phylogenies that different DNA regions may

track different phylogenetic histories. Many phylogenies, because of time and expense, employ just a single gene region and a single sample per species and this needs to be redressed (Rieseberg & Soltis, 1991). Multiple gene trees need to be assessed to determine where they agree and where conflict occurs. Although some of the gene tree discrepancies can be put down to other causes (Brower *et al.*, 1996; Lowinski & Page, 1999), increasing evidence is being found that reticulate evolution is playing a large role in the evolution of plant (Rieseberg & Carney, 1998; Bachmann, 2000), as well as animal (Dowling & Secor, 1997), species. Consequently, the possibility that a strictly dichotomously branching tree can be produced that will reflect an accurate phylogeny for all taxa is becoming increasingly remote (Doolittle, 1999). Reticulations have already been proposed in reconstructing the trees of many genera, e.g. *Actinidia* (Li *et al.*, 2002), *Aeonium* (Jorgensen & Frydenberg, 1999), *Amelanchier* (Campbell *et al.*, 1997), *Arygyranthemum* (Francisco-Ortega *et al.*, 1996), *Armeria* (Fuertes Aguilar *et al.*, 1999), *Dubautia* (Baldwin, 1997), *Geum* (Smedmark & Eriksson, 2002), *Gossypium* (Cronn *et al.*, 2002), *Helianthus* (Rieseberg & Morefield, 1995), *Machaeranthera* (Morgan, 1997), *Osmorhiza* (Yoo *et al.*, 2002), *Paeonia* (Sang *et al.*, 1997), *Penstemon* (Wolfe *et al.*, 1998), *Phlox* (Ferguson & Jansen, 2002), *Populus* (Smith & Sytsma, 1990), *Rhododendron* (Milne *et al.*, 1999), *Senecio* (Comes & Abbott, 2001), and even above the genus level, hybrid origin is being postulated for some families or subfamilies (e.g. Phipps *et al.*, 1991).

Molecular analyses:

The ease with which genetic sequences can be obtained makes the use of molecular markers much more amenable than morphological ones, but caution is necessary to avoid introducing certain errors. Firstly, the terminal taxa need to be correctly identified. At the generic level, exemplar species can be relatively easily chosen that unequivocally belong to the genus. However, at the species level, a thorough understanding of the taxonomy of the genus and a comprehension of the variation patterns within the species needs to be developed before selection of exemplar collections to represent a species can be done reliably. Ideally, more than one collection should be chosen to cover the range of morphological and geographic diversity found within the species (e.g. Ohsako & Ohnishi, 2000; Utelli *et al.*, 2000; Ferguson & Jansen, 2002). When the number of accessions needs to be limited due to the size of the study, a specimen should be chosen that lies within the middle of the range of variation. If the knowledge of the genus is only superficial, an arbitrary choice of a specimen can

result in one that lies at the boundary between two species or represents a case where introgression has occurred with a sympatric species.

A major advantage of molecular work over morphology is the number of characters that can be easily obtained and that delimitation of the character states is unambiguous (Hillis, 1987). However, care is still needed with molecular characters, as the problems are sometimes less evident than with morphological ones. Our lack of understanding of how the DNA evolves means that the characters might not be independent (Dixon & Hillis, 1993), e.g. secondary structure in the chloroplast genome to assist the splicing of introns from the RNA precursors (Clegg *et al.*, 1994). As a result of this hidden structure, such as stem-loops and inversions, character changes can often be linked but scored as two independent characters thereby giving a single evolutionary change double the weight it should receive (Wheeler & Honeycutt, 1988). Nevertheless, although mutational changes are not randomly distributed across the genome, the assumption that they are almost so is usually adequate for most studies (Dixon & Hillis, 1993). Similarly, indels provide an additional problem, for although base pair substitutions can be regarded as discontinuous, insertions and deletions of parts of the gene region can occur adjacent to one another or be superimposed. There is then the risk of multiple evolutionary steps being scored as a single one. Therefore, care is needed to score them in a way that reflects the different possible evolutionary steps that created them (Simmons & Ochoterena, 2000; Lee, 2001).

For plants, it is strongly recommended that evidence from both the plastid and nuclear genomes should be used in reconstructing the phylogeny (Rieseberg & Soltis, 1991; Cronn *et al.*, 2002), but problems exist with both sources. The plastid DNA, i.e. chloroplast and mitochondrial, is much more slowly evolving than the nuclear DNA. (The mitochondrial DNA of plants is even slower than that from the chloroplast, with few substitutional changes and frequent rearrangements, so is rarely used in botanical studies (Soltis & Soltis, 2001).) Rates of substitution are so slow that often multiple regions need to be combined to gain a phylogenetic signal for lower level studies (e.g. Sang *et al.*, 1997; Ohsako & Ohnishi, 2000; Udovicic & Ladiges, 2000; Chandler *et al.*, 2001; Cronn *et al.*, 2002). The other problem is that using solely cytoplasmic DNA can lead to erroneous phylogenetic conclusions, as only the maternal lineage is reconstructed (Rieseberg & Soltis, 1991; Cronn *et al.*, 2002). The gene regions employed, despite being single copy, are present in high numbers and hence are relatively easily amplified, especially compared to the nuclear genome. As a

consequence, many of the molecular phylogenies for plants published in the past few years have been based solely upon the chloroplast genome, especially using *rbcL* and *trn*L-*trn*F. Any conclusions drawn from these phylogenies have to be preliminary until they are compared with nuclear histories (Rieseberg & Soltis, 1991; Baldwin *et al.*, 1995; Cronn *et al.*, 2002).

Unfortunately, the nuclear genome has its own inherent problems. The DNA occurs in relatively low quantity, consequently the amplification of single-copy genes can be problematic. Hence, multiple copy gene regions, such as nuclear ribosomal DNA, are generally chosen, as these are easier to amplify (Long & Dawid, 1980; Hillis & Dixon, 1991). However, despite the effect of concerted evolution on the genome (Long & Dawid, 1980; Hillis *et al.*, 1991), there may be paralogous copies present of a particular region, which have evolved independently. It has been shown that which region gets amplified can vary depending upon the quality of the DNA and reaction conditions used (Mayol & Rossello, 2001). As a result, the apparent homology of the sequences retrieved has recently been called into question for these regions, especially the much used ITS (Baldwin *et al.*, 1995; Mayol & Rossello, 2001). This problem can be alleviated in part by cloning all the copies present, but this is an expensive and time-consuming technique, nor does it guarantee that all copies will be found (Baldwin *et al.*, 1995).

Gene regions employed:

For this study, information from both the nuclear and chloroplast genome was desired. It was also necessary to ensure that enough gene regions were sampled to give adequate variation, and hence characters, for a well-corroborated tree for each genome. Two nuclear regions, ITS and 5S NTS, and four chloroplast regions, *trn*L, *trn*K-*mat*K, *rps*16, and *psb*A-*trn*H, were screened for phylogenetic usefulness.

ITS (Internal Transcribed Spacer) is a spacer region found within the repeat unit of 18–26S nuclear ribosomal DNA (White *et al.*, 1990). It is split into two regions (ITS-1 and ITS-2) separated by the 5.8S gene. It has been used for a large number of inter- and infrageneric studies because of its ease of amplification across a wide range of groups, its fairly short length and yet variable enough to be used in lower-level studies (e.g. Sang *et al.*, 1995; Baldwin *et al.*, 1995; Campbell *et al.*, 1997; Eldenas *et al.*, 1998; Eriksson *et al.*, 1998; Baldwin & Sanderson, 1998; Bakker *et al.*, 1998; Fuertes Aguilar *et al.*, 1999; Jorgensen & Frydenberg, 1999; Helfgott *et al.*, 2000; Iwata *et al.*, 2000; Baker *et al.*, 2000b; Meerow & Snijman, 2001; Li *et al.*, 2002). Only the ITS-1 was

sequenced for screening as this region has been shown generally to have higher levels of variation than ITS-2 (Baldwin *et al.*, 1995).

5S rDNA non-transcribed spacer region (5S NTS) is found between the tandem arrays of the 5S ribosomal DNA gene (Cox *et al.*, 1992). The 5S gene is highly conserved amongst green plants and so the region is easily amplified. The spacer is short but highly variable and so useful for determining species-level relationships. Unfortunately, concerted evolution among the arrays is often incomplete and multiple different copies of the spacers often exist at different loci, requiring cloning of individual repeats to separate them out (e.g. Udovicic *et al.*, 1995; Cronn *et al.*, 1996; Kellogg *et al.*, 1996). Because of the expense and time involved in cloning, especially for this study where over 100 species were to be sequenced, it was hoped to be avoided. It was found that although examination of the sequence chromatograms gave evidence that the concerted evolution of the repeats was sometimes incomplete, a reading of the majority of the sequence could still be made. Even so, compared to the other gene regions employed there was a relatively high proportion of ambiguities in the dataset (0.3% of total bases, 0.5% of bases in informative characters).

*trn*L-*trn*F comprises of the *trn*L intron region, situated between the two *trn*L exons, and the spacer region between the *trn*L exon and *trn*F gene (Taberlet *et al.*, 1991). Easy amplification and the relatively small size of the region, coupled with quite high sequence divergence, makes it ideal for phylogenetic study. Consequently, it has been widely employed at the generic level and below (e.g. Bayer *et al.*, 2000; Eldenas & Linder, 2000; Bellstedt *et al.*, 2001).

*trn*K-*mat*K is a combination of part of the *trn*K intron and the *mat*K coding gene (Johnson & Soltis, 1995). The *mat*K gene, which is found inside the *trn*K intron, has frequently been used for generic-level studies but is too long to sequence with just two primers and amplification of the whole region requires a long PCR. The *trn*K intron has been shown to have a higher rate of base-pair substitution than *mat*K, although as it is shorter there are fewer substitutions in total. Furthermore, the 5' end of the *trn*K intron has been shown to contain more information than the 3' end because it is much longer and evolves only slightly slower (Johnson & Soltis, 1995; Meimberg *et al.*, 2001) and the 5' end of the *mat*K gene itself evolves more quickly than its 3' end (Hilu & Liang, 1997). For this study therefore, the whole of the 5' end of the intron was used, as well as part of the *mat*K coding region, following the example set by Chandler et al. (2001) and Ohsako & Ohnishi (2000).

*rps*16 intron is a medium sized region found between the two *rps*16 exons (Oxelman *et al.*, 1997). The region is easily amplified and has provided some resolution in a few species-level analyses (e.g. Oxelman *et al.*, 1997; Lidén *et al.*, 1997a; Baker *et al.*, 2000b).

*psb*A-*trn*H intergenic spacer is a short region found between two highly conserved genes (Sang *et al.*, 1997). It is therefore easily amplified. It contains an AT rich area which leads to a high occurrence of slipped-strand mispairing resulting in several duplications or deletions of previous duplications of short parts of the genome. These duplication events are generally found to be reliably informative at lower taxonomic levels. Stem-loop structures have also been shown to be present and care has been required when interpreting substitutions to ensure that they are not linked. The region as a whole shows quite high levels of mutation and so has been useful for lower level and phylogeographic studies (e.g. Sang *et al.*, 1997; Iwata *et al.*, 2000; Utelli *et al.*, 2000; Chandler *et al.*, 2001).

Morphological analyses:

The utility of morphological characters in phylogenetic analysis has been widely questioned, either because of procedural problems or biological attributes of the organisms (Sytsma, 1990). The independence of characters is almost impossible to establish and separating characters into discrete states is often difficult to justify (Stevens, 1991). This makes constructing a reliable matrix a difficult task and very often, even with detailed justification, the use of some characters can only be tenuously supported. Morphological characters also have a tendency to be more homoplasious than molecular ones as their evolution is driven by environmental factors that promote the selection of useful traits (Givnish & Sytsma, 1997). Nonetheless, many morphological phylogenies have been produced (e.g. Linder & Vlok, 1991; Goldblatt & Manning, 1996; Linder & Mann, 1998; Goldblatt & Manning, 1998) and when viewed in combination with molecular work they can help to independently support common lineages (Vane-Wright *et al.*, 1992). In addition, morphology can be used to complement the molecular datasets in parts of the tree where resolution would otherwise be poorly supported (Eldenas & Linder, 2000). This is especially so at the tips of the branches, where molecular techniques as yet do not reveal enough variation in the gene regions to resolve closely related species groups (Pennington, 1996).

There has been much debate about whether it is legitimate to combine all datasets, both molecular data from unlinked gene regions and morphology, in what is termed 'total

evidence' or to keep them separate (e.g. Miyamoto, 1985; Barrett *et al.*, 1991; Kluge & Wolf, 1993; de Queiroz, 1993; Miyamoto & Fitch, 1995; de Queiroz *et al.*, 1995; Page, 1996; Huelsenbeck *et al.*, 1996; Givnish & Sytsma, 1997). The advantage of using all the data available is that phylogenetic signal should assert itself over homoplasious characters that create noise in the individual datasets (Barrett *et al.*, 1991; de Queiroz *et al.*, 1995). The problem with 'total evidence' is that if the different datasets reflect different phylogenetic histories then this information will be lost (Smith & Sytsma, 1990; Givnish & Sytsma, 1997). An example of separate histories is when hybrids are present, where the nuclear genome contains evidence from both parents (or even just the paternal lineage), while the plastid genome, at least in most Angiosperms, tracks only the maternal history (Mogensen, 1996). Although there is now widespread support for using all the data in a 'total evidence', no matter what their origin, as long as they are heritable (Grandcolas *et al.*, 2001), the proviso is often added that the datasets should be checked first for combinability (Bull *et al.*, 1993; de Queiroz, 1993; de Queiroz *et al.*, 1995). While this approach is good in theory, the methods used to evaluate combinability are in themselves often contentious (e.g. Yoder *et al.*, 2001; Dowton & Austin, 2002).

Hybridization and its detection in phylogenetic analyses:

Determining the presence of hybridization in phylogenetic reconstruction is a difficult task (McDade, 1995). Grant (1981) lists six mechanisms by which hybrids can become stabilized and thus provide a platform for speciation: hybrid speciation with external barriers and recombinational speciation (together known as homoploid hybridization), allopolyploidy, apomictic speciation, permanent structural hybridity and numerical hybridity. The last three create microspecies, which span the range of variation between the parents, while the first three create sexual derivatives and hence the potential for new biological species (Rieseberg, 1997). Although hybridization has been widely suggested in monographs as an explanation for collections that appear intermediate (e.g. Weimarck, 1934; Williams, 1972; Grey-Wilson, 1980; Goldblatt, 1985; Bruyns & Linder, 1991; Whitehouse *et al.*, 2001), unambiguous evidence for the hybrid status of these plants is much harder to achieve. This is especially so for homoploid speciation as it usually occurs between genetically close relatives (Rieseberg, 1997; Wolfe *et al.*, 1998; e.g. Morrell & Rieseberg, 1998; Ungerer *et al.*, 1998; Rieseberg & Linder, 1999; Ferguson & Sang, 2001). Allopolyploidy on the other hand can occur between more disparate species and hence the incongruence within the genome or morphology caused

by its hybrid nature is easier to detect (e.g. Wendel *et al.*, 1995; Sang & Zhang, 1999; Widmer & Baltisberger, 1999; Alice *et al.*, 2001; Szalanski *et al.*, 2001; Raymond *et al.*, 2002; Smedmark & Eriksson, 2002; Li *et al.*, 2002).

However, when establishing phylogenetic hypotheses, it is vital to detect the presence of hybrids or else wrong conclusions can be drawn about the evolutionary history of the species. Several ways of assessing the data to identify the presence of hybrids have been *suggested* but none are entirely satisfactory and a combination of methods is usually needed (Funk, 1985). There are three common ways that the presence of hybrids can be detected in phylogenetic analysis (Sang & Zhong, 2000): (1) identification before the analyses; (2) detection according to their cladistic behaviour; (3) reconstruction by comparing discordant positions on independent datasets.

A priori identification of hybrids and omitting them from the analysis is the simplest to implement but has severe problems. Firstly, the hybrid nature of the specimen or taxon is often far from obvious. Secondly, determining which is the hybrid and which is the parent can sometimes be arbitrary and hence it is debatable as to which one is omitted. Thirdly, and most seriously, is that if whole lineages are suspected of having a hybrid origin then large portions of the dataset might need to be removed, which can severely reduce its informativeness and stability (de Queiroz *et al.*, 1995; Wiens, 1998).

The "total evidence" approach calls for combining all the evidence together and identifying the hybrids by their intermediate position and homoplasious character states only after the cladogram has been produced (Skála & Zrzavý, 1994). While simplifying preparation of data for the analysis by removing any *a priori* decisions as to which taxa should be removed, the subsequent detection of both the hybrids and the true phylogeny can be very problematic. Firstly where conflict occurs across disparate parts of the tree it can cause the consensus tree to collapse and the deeper nodes to be lost (Bremer & Wanntorp, 1979). Secondly, the behaviour of hybrids in a phylogenetic tree is not always predictable (Funk, 1985; Rieseberg & Ellstrand, 1993). Finally, the unequal contribution of data can mask a conflicting signal from an opposing dataset and phylogenetic information is lost (Doyle, 1992; Huelsenbeck *et al.*, 1996). Wiens (1998) showed, unsurprisingly, that in those parts of the tree where conflict occurred accuracy diminished by combining the datasets, while in those parts where the datasets were congruent, accuracy was improved. His suggestion was therefore to use a combination of the first two methods by combining the data only for the particular portions of the tree where there was no conflict.

The final approach is one that is becoming more possible with the rise in molecular techniques to produce independent datasets with differing evolutionary histories. Most useful is the basis that the chloroplast genome is almost always maternally inherited (Mogensen, 1996) and therefore will often conflict with the nuclear if it is of hybrid origin (Rieseberg *et al.*, 1996; Bachmann, 2000). Unfortunately, the hybrid origin of the species will only show around 50% of the time, as each nuclear gene will only conflict strongly with the chloroplast when concerted evolution in that region has been complete and unidirectional towards the paternal genome. This can be overcome by broader sampling of unlinked nuclear genes, thus increasing the chance of detecting conflict with the maternal genome. Where concerted evolution of the gene region has not had time to complete, then either cloning techniques can be used to separate out the two parental sequences (Sang & Zhang, 1999) or nuclear sequences can be examined for evidence of additivity or intermediacy (Sang *et al.*, 1995; Baldwin *et al.*, 1995; Campbell *et al.*, 1997). Even when separate gene phylogenies have been produced, reconstructing the species phylogeny can be difficult, especially if the number of taxa involved is large and reticulations have occurred at all levels between them (Rieseberg & Soltis, 1991). In addition, not all conflict between gene trees has necessarily been caused by hybridization. There are three reasons why a gene tree might differ from a species tree: gene duplication (paralogy), deep gene coalescence (lineage sorting) and horizontal gene transfer (Doyle, 1992; Lowinski & Page, 1999). Only the final one is equivalent to that caused by hybridization.

At present, statistical tests and computer programs to analyse hybridization hypotheses nearly all rely upon the identification of which species are the hybrids, i.e. implementing Methods 1 or 2 (e.g. Rieseberg & Morefield, 1995; Sang & Zhong, 2000; Xu, 2000). No computational method has yet been identified that detects the presence of hybrids either directly from the original datasets or from the resulting phylogenetic trees. The closest attempt has been GeneTree (Lowinski & Page, 1999), which is used for reconciling gene and species trees, but it only implements resolving gene duplication and deep gene coalescence stating that "optimization procedures for lateral transfer are much more difficult to implement". The reason for the difficulties of creating a program to construct these reticulate phylogenies are clear in that current algorithms of cladistic analysis are designed to produce a single hierarchic dichotomously branching tree. Hybridization events will always be inconsistent with this aim (Funk, 1985). Although algorithms designed to accept reticulations should be designed (Rieseberg & Soltis, 1991), implementation is hampered by the lack of goodness-of-fit criteria (Legendre,

2000) and the unpredictable nature of hybrid characters (Rieseberg & Ellstrand, 1993; Wendel *et al.*, 1995). One way around this is to use one of the many network approaches that were designed for population genetics but these are primarily distance rather than parsimony based methods (Posada & Crandall, 2001).

To add to this problem, just as the number of trees in a phylogenetic analyses increases exponentially with increased number of taxa, so also does the number of possible *reticulations* and the analysis soon becomes almost intractable. For example, Nelson (1973) outlined a procedure for analysing cladograms for the presence of reticulations by inserting reticulations that decrease homoplasy. Rieseberg & Morefield (1995) implemented this procedure in their RETICLAD program, although it could only deal with terminal reticulations (not between internal nodes) and *a priori* entering of all possible hybrid combinations. But as Funk (1985) points out, the number of possibilities soon becomes intractably large and the methodology unworkable with multiple reticulations and large datasets.

Most of the analytical advances that have been made in testing hybridization hypotheses have been highly theoretical and based upon closely related species. Sang & Zhong (2000) explain how their model can be extended to cover any size of tree and the species included in it, but again *a priori* prediction of the hybrids is needed. (Their model is really designed to distinguish between hypotheses of hybridization against lineage sorting or paralogy rather than to identify hybrids in incongruent trees and even its ability to do that has been questioned by Holder et al. (2001).) Xu (2000) also suggests that his method can be used for reticulations deep within a tree and between distantly related species, but it also relies upon using gene frequency data rather than sequence data and knowing the model under which the taxa have diverged. McDade (1995) points out that most analysis of hybrids is generally carried out on 'primary hybrids' and that ancient 'derived hybrids' are even harder to spot as they accumulate autapomorphies or themselves go on to diverge into new species. Indeed, she practically dismisses 'derived hybrids' as not having "much more impact on phylogenies than primary hybrids" as they are mostly between close relatives. She does point out that there are "worst case scenarios" between non-sister taxa or where all lineages are still extant and this can cause "considerable disruption of the relationships of the non-hybrid taxa". Certainly it appears that where these scenarios exist no cladistic analysis has yet been worked out to deal with them adequately and manual inspection is often the only means of disentangling the various threads.

Methods:

Plant material and extraction of DNA:

DNA was extracted from 107 of the 117 currently accepted species (Table 1.1, Appendix 4). The species excluded were either taxonomically dubious, and hence were not extensively searched for, especially if the type locality was in inaccessible areas, or thorough searching of the type locality failed to reveal the species. A further 43 samples were added from putative new species or collections of uncertain identity. For nine widespread species (i.e. *C. erectisepala*, *C. eriocephalina*, *C. linearifolia*, *C. montana*, *C. odorata*, *C. pilosa*, *C. ramosissima*, *C. serpyllifolia* and *C. teretifolia*) more than one representative was included to represent the geographic range of the species.

The outgroup species were selected based upon Helfgott et al. (2000). From their phylogeny, a single representative from each of the two closest clades to *Cliffortia* was chosen, *Sanguisorba officinalis* and *Acaena latebrosa*, as well as *Sanguisorba minor* which was sister to a clade comprising all three groups. For *trn*L-F two additional sequences were donated by Malin Hibbs (University of Maryland) from the *Acaena-Polylepis* clade: *Acaena cylindristachya* and *Polylepis tarapacana*.

The leaf material for DNA extraction was collected in the field in silica gel. Freshly collected material was used for *C. tuberculata* (C. Whitehouse 48) but as this proved harder to grind and extract, giving slightly poorer although still adequate results, material was preferentially collected and dried in silica gel. For species not found in the field, herbarium material was used when available. Recent collections for *Cliffortia*, especially the rarer species, were sparse. Hence, species not found in the field were only represented in the herbaria by a few collections, often collected over 50 years ago. The efficiency of the extraction was then very dependent upon the quality of the original pressing and subsequent storage, and the success rate was low (Table 2.1).

The protocol for extraction of the DNA was very similar to Doyle & Doyle (1987), with slight modification. Material was initially ground in a mortar and pestle with liquid nitrogen, but it was subsequently found that less material was needed and more consistent results obtained by grinding with a plastic pestle directly in a 1.5 ml Eppendorf tube in a liquid nitrogen bath. CTAB was used with 1% PVP-40 (1g of PVP dissolved in 100 ml of CTAB). In addition, a second ethanol precipitation was found to be unnecessary for the quality of DNA required. When the DNA was clearly present but failed to amplify, it was cleaned on QIAquick™ PCR Purification columns.

Table 2.1. List of herbarium specimens from which DNA extraction was attempted, indicating for which gene regions subsequent amplification was successful.

Species	Collection	Year	Successful
C. acocksii	Esterhuysen 16001	1949	-
C. brevifolia	Esterhuysen 26433	1956	-
C. sp. cf. arborea	Viviers & Vlok 211	1987	5S NTS, *psb*A
C. ericifolia	Esterhuysen 34433	1976	-
C. gracilis	Esterhuysen 12813	1946	-
C. lanata	Meyer 741	1994	5S NTS, *trn*L, *psb*A
C. linearifolia	Linder 3976	1986	5S NTS, *psb*A
C. marginata	Parker 3475	1940	5S NTS, *psb*A
C. mirabilis	Bean, Vlok & Viviers 1951	1987	*psb*A
C. nitidula	Schelpe 600	1955	-
C. serpyllifolia	Balkwill, Manning & Cadman 1243	1984	5S NTS
C. sp. cf. viridis	Esterhuysen 25071	1955	-

DNA sequence analysis:

Selected gene regions were amplified using the polymerase chain reaction (PCR). A standard protocol with slight variation was used for all reactions. Each 25 µl reaction was prepared as follows: 16.85 µl of sterilised water, 2.5 µl of 10× Taq reaction buffer, 2.5 µl of 50mM MgCl solution, 0.75 µl of each primer at 10 mM strength, 1 µl of dNTPs at 10 mM, and 0.15 µl of Biotaq DNA Polymerase. Template DNA (0.5 µl) was added at varying dilutions for optimum PCR product. Products were checked on a 1% agarose gel stained with ethidium bromide and viewed with a UV light source. With 5S NTS products more than one band was usually seen. The larger bands were regarded as polymers of the main smallest band, since the larger bands tended to be even multiples of the smallest. Initially the smaller band was cut out and reamplified using reduced levels of magnesium (0.5 µl) and primers (0.25 µl) to get a single bright band, but this extra step was soon deemed unnecessary as products could be sequenced adequately from the original amplification. Occasionally double bands that were obviously not polymers were seen upon visualisation and in these cases cutting out and reamplification was regarded as necessary to produce a single product. Although ideally both bands would have been amplified, to help reduce costs only the band closest to the predominating bands found in other reactions was cut out. Baker et al. (2000a) and Udovicic et al. (1995) have shown that even when different copies are present in a species they still resolve as monophyletic groups, so the choice of band was not regarded as too critical. PCR reactions were carried out on a Hybaid PCR Sprint Thermal Cycler with conditions as in Table 2.2.

Table 2.2. PCR conditions employed for the various gene regions sampled. References for the primers of each gene region are cited in the introduction.

	ITS	5S NTS	*trn*L	*trn*K	*rps*16	*psb*A
Initial denaturation	95°C 2'	95°C 2'	95°C 2'	95°C 2'	95°C 2'	95°C 2'
Denaturation	93°C 35"	94°C 1'	94°C 1'	94°C 1'	94°C 1'	94°C 1'
Annealing	49°C 35"	55°C 45"	52°C 45"	52°C 45"	62°C 45"	52°C 45"
Extension	72°C 2'	72°C 1'	72°C 1'30"	72°C 1'30"	72°C 1'30"	72°C 1'
Cycles	35×	30–35×	30–35×	30–35×	30×	30×
Final extension	72°C 7'	72°C 8'	72°C 8'	72°C 8'	72°C 8'	72°C 8'
Forward primer*	ITS5	PIII	c	3914F (dicot)	rpsF	psbAF
Reverse primer*	ITS2c	PIV	f	1168R	rpsR2	trnHR

*See introduction for references to primers.

PCR products were cleaned using the QIAquick™ PCR Purification Kit and then cycle-sequenced using an ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit with 5× Sequencing Buffer. The Ready Reaction mix was used at quarter strength, except for 5S NTS and *psb*A when only eighth strength was used. The subsequent reactions were submitted to the Core DNA Sequencing Facility, Stellenbosch University for sequencing on an ABI3100 Genetic Analyzer from Applied Biosystems. The sequences thus obtained were aligned manually using GeneDoc – Multiple Sequence Alignment Editor and Shading Utility (Nicholas & Nicholas, 1997). Gaps and poly-AT regions with variable lengths were coded using the 'simple indel coding' method, scoring each different sized gap with a unique character (Simmons & Ochoterena, 2000). Parts of the sequence with gaps were retained for the analysis and reductive coding was used by interpreting the inapplicable data as missing (Strong & Lipscomb, 1999).

Morphology:

The species were studied from live field-collected material wherever possible. Measurements were made under a Zeiss binocular microscope using a graticule. Multiple collections were examined to capture the range of variation present in species

with wide distributions, that were morphologically variable or had critical limits between species.

For each morphometric character, the average and range of at least five measurements were made per specimen, unless there was insufficient material of a particular organ in a fresh and mature state. Measurements were made to the nearest 0.1 mm using a graticule; for those characters too large to be measured under the microscope a ruler was *used* and measured to the closest 0.5 mm. Discrete characters were scored as appropriate for each specimen. A list of characters assessed is given in Appendix 3. Most discrete characters were coded directly from observation rather than measurement, even if they were in essence quantitative (Stevens, 1991). However, when enough evidence was present for state delimitation from measurement data, the justification is given in the character description. This was rare as most measurement data showed no discontinuities.

Herbarium material from the Bolus Herbarium (BOL, University of Cape Town), Selmar Schonland Herbarium (GRA, Albany Museum, Grahamstown), Compton Herbarium (NBG, National Botanical Institute, Kirstenbsoch), Bews Herbarium (NU, University of Natal, Pietermaritzburg) and National Herbarium (PRE, National Botanical Institute, Pretoria) was examined to check for polymorphisms in multistate characters. It was also used for additional measurements when particular vegetative or sexual organs were not found in the field and to cover the range of variation found in leaf morphology. The three outgroup taxa were also scored from herbarium material. However, many of the characters were inapplicable to them, as their homology to *Cliffortia* was difficult to establish.

A DELTA database (Dallwitz, 1980; Dallwitz *et al.*, 1993) was constructed by extracting the total ranges for each species from a spreadsheet, while the multistate characters were entered directly. A morphological matrix for use in the phylogenetic analysis was retrieved by creating a Nexus file directly from the DELTA database.

To compile a combined dataset, the morphological data for a species was directly matched to the single molecular accession. In the cases where multiple accessions for a species had been included in the molecular analysis, an additional entry was needed for the morphological dataset. The additional entry was scored just from the specimen for which the DNA for the extra accession had been extracted, hence any polymorphisms for the species were ignored for that accession. The additional entry was constructed for the specimen for which the fewest number of DNA regions had been sampled or

arbitrarily if equal. Molecular accessions that had not yet been attributed to a particular species or were believed to be of hybrid origin, were also given a separate entry for the morphological dataset, based on the same collections from which the molecular data were derived. This ensured that where species limits were doubtful, the molecular data would correspond directly to morphological observations for that specimen in the combined dataset.

Phylogenetic analysis:

Cladistic analysis was mostly carried out using Paup* 4.0b10 (Swofford, 2001). For the Parsimony Ratchet searches WinClada (Nixon, 1999b) was used to invoke Nona (Goloboff, 1993) to carry out the analysis and then the resulting trees transferred back into Paup* for further analysis. Unless stated otherwise, all Paup* searches were carried out using tree-bisection-reconnection (TBR) branch swapping, MULTREES set to on and steepest descent off. A variety of search methods were used as outlined below:

Search 1. An initial search was carried out on the datasets using the Parsimony Ratchet method (Nixon, 1999a). An initial subset of the shortest trees was gathered from across a wide sample of tree space by carrying out 20,000 replicates changing 10% of the informative characters and constraining 10% of the nodes. To check that there was not a shorter tree amongst the islands that were found, the resulting subset of the shortest trees was used in a more complete heuristic search. To limit the time taken a maximum of 20,000 trees was retained and these then swapped to completion.

Search 2. Successive weighting was conducted on the trees from Search 1 (Farris, 1969). This was used to reduce the effect of homoplasious characters in the dataset. The trees recovered were also compared to the unweighted trees to see if the topology changed significantly. The characters were reweighted by the rescaled consistency index using a base weight of 1,000 and 100 random addition sequence replicates were then done, saving five trees for each replicate. The resulting shortest trees were then used to carry out a heuristic search until 10,000 trees were found. The process was repeated until the tree topology stabilised, at which point the final trees were allowed to swap to completion. For analyses that included the morphology dataset a base weight of only ten was used, as otherwise the high degree of homoplasy in that dataset meant that minute changes occurred at each reweighting and the topology did not stabilise without numerous successive weightings; 500 random addition sequences, only saving a single tree for each replicate, were also used to ensure that the shortest tree was found on each successive weighting.

Search 3. A jackknife analysis (Farris *et al.*, 1996) was carried out to determine support for nodes resolved in Searches 1 and 2. Jackknifing gives very similar results to bootstrapping so only the one type of support value was calculated (Mort *et al.*, 2000). Each jackknife replicate consisted of using the shortest trees from three random addition sequence replicates, saving three trees for each replicate, with 36.79% of characters deleted. Groups that appeared in at least 50% of the trees produced by 2,000 replicates were retained. The search method employed for each jackknife replicate was used as a compromise between the fast jackknife and more thorough branch-swapping jackknife analyses tested by Mort et al. (2000).

Analyses were carried out upon each dataset individually. As the chloroplast regions are all maternally inherited as single copies without any recombination, the three most complete datasets of the chloroplast genome (*trn*L, *trn*K and *psb*A) can therefore be treated as having identical evolutionary histories and combined into a single dataset for analysis.

Testing for congruence of datasets:

The three datasets used in the phylogenetic analysis, nuclear, chloroplast and morphology, were compared to investigate if they were congruent with each other. The simplest method is just to compare visually the topology and respective supports for the datasets (Mason-Gamer & Kellogg, 1996). For example, Eldenas & Linder (2000) suggest that if incongruence exists between the datasets, but these are not hard conflicts (i.e. with strong support values), then the discrepancy can be put down to sampling or other errors in the datasets. A 'robustly supported' node was defined by the minimum level of support required so that there was no conflict between the datasets. As the support level required for a 'robustly supported' node was relatively low (bootstrap 60%, Bremer 2) they justified their combining of datasets without carrying out any incongruence tests.

To confirm congruence statistically a partition-homogeneity (= ILD, incongruence length differential) test was carried out between the three separate datasets (Farris *et al.*, 1994). For each test 999 heuristic searches were conducted, each search using three random addition sequence replicates and saving a maximum of three trees per replicate.

In addition to the ILD test a more complex method was used as outlined by Graham et al. (1998) for testing congruence of datasets using the Templeton or Kishino-Hasegawa

tests on transformed trees. The shortest trees for a given dataset were compared to the shortest trees obtained when the same dataset was constrained by an opposing dataset's topology. To prevent poorly supported branches in one dataset causing conflict and hence incongruence, the constraining trees were derived from Jackknife analyses at various cut-off percentages. This was also useful to determine the level at which conflict between the datasets disappeared.

To ensure unbiased comparison, only those taxa with all datasets complete were included in the analysis. To obtain the constraint trees, 10,000 jackknife replicates were carried out with 36.79% deletion of characters using the Faststep search option. This search option was used in preference to a more thorough heuristic search, as it is a more conservative estimate of incongruence. Being a less thorough search strategy, each node in the jackknife tree would be more poorly supported and hence there would be fewer branches in the constraint trees to cause conflict. Constraint trees at 5% intervals from 50–100% were saved for use and the number of nodes in each constraint tree recorded.

Each constraint tree was then applied to the opposing datasets in turn and the shortest trees were then found by carrying out 100 random addition sequence replicates, saving a maximum of three trees at each replicate. A tree from this constrained search was chosen arbitrarily and compared with one from an unconstrained search, the increase in length recorded and a Kishino-Hasegawa Test carried out to test if they were significantly different or not. The Templeton test was not implemented as the results were previously found to be very similar to those of the Kishino-Hasegawa Test (Graham *et al.*, 1998). Only a single tree from each of the constrained and the unconstrained searches were compared, except when levels of significance were close to the boundary of the 5% level. However, further pairwise testing of individual trees showed that the degree of probability did not vary much and never affected whether the results were significant or not.

Detecting long-branches:

Some incongruence within the dataset might be due to error in phylogenetic reconstruction due to 'long-branch attraction' (Felsenstein, 1978). Often the solution suggested is that additional taxa should be added to 'break up the long branches' (Graybeal, 1998). However, this is not always possible because the taxa with characters that are able to 'break up the branches' might not exist, especially if taxon sampling is already quite complete. Indeed, it is possible that adding further taxa can even make the matter worse (Poe & Swofford, 1999), especially if they also have a high rate of

character change (Kim, 1996). Therefore, some other means of testing whether a placement is 'true' or due to the high degree of homoplasy naturally present in 'long-branches'.

To examine this problem, the taxa involved in the putative long-branch can be pruned from the tree. Each taxon or clade involved can then be replaced independently to examine if they occupy the same position without the presence of the other taxa involved. If they remain in the same position on the tree then the effect of long-branch attraction upon their placement can be regarded as negligible.

Reconstructing the total evidence trees:

Whether or not incongruence was proved, all the data were combined into a single analysis to produce a 'total evidence' tree. However, if the diverse datasets track different phylogenetic histories then that analysis might reflect an inaccurate species phylogeny (Lowinski & Page, 1999). In cases of strongly conflicting datasets, three options are available to present the data: as individual trees, as a single reticulating tree, or as a non-reticulating tree representing the dominant pattern (de Queiroz *et al.*, 1995). The first option is possible by examining the trees produced by the individual datasets, while a means to produce a reticulating tree is presented below. Here a method to construct a tree that represents the dominant pattern in the three datasets is proposed.

Two different approaches to producing a non-reticulating tree from conflicting datasets are possible: to identify conflicting taxa or data and removing them prior to combining the datasets, or to identify the points of congruence and preferentially select them. Most datasets do not have many points of conflict and therefore excision of the taxa is the easiest and most widely used solution (e.g. de Queiroz *et al.*, 1995; Kellogg *et al.*, 1996; Mort *et al.*, 2002). However, this method requires *a priori* knowledge or presumptions, and becomes increasingly harder the larger and more reticulate the dataset (de Queiroz *et al.*, 1995). Therefore, in cases where reticulation is believed to be common, the third option, identification of the points of congruence to retrieve the dominant pattern is a more practical approach.

One way to do this is to produce reduced cladistic consensus trees (Wilkinson, 1994) using the computer program RadCon (Thorley & Page, 2000). This method should be able to identify the subtrees that are congruent within the separate datasets and exclude those taxa that conflict without any *a priori* assumptions. One can then go back to option two, and remove the conflicting taxa before reanalysing the data using total

evidence. However, this program failed to cope effectively with the large number of trees produced when processing the separate chloroplast and nuclear datasets. The number of taxa needed to be pruned down to around 90 before it was able to process the trees. This rather negated the purpose of using the program in the first place as then an *a priori* decision was still required upon which taxa to include and which to exclude.

The advantage of using total evidence against separate analyses is that there are more characters available to support real nodes and to mask homoplasy in the datasets (de Queiroz, 1993; Page, 1996). The disadvantage is that when diverse datasets strongly conflict, one dataset will override the other or if their contribution is almost equal that resolution will be lost (Hillis, 1987; Doyle, 1992; de Queiroz *et al.*, 1995). A method was therefore devised that identified firstly the points of agreement in the datasets, and secondly the points that were strongly supported by one or more datasets and not conflicted in the others.

The separate analyses showed that the morphology data were highly homoplasious. Hence, conflict between the morphology and molecular datasets could just as likely be because of homoplasy as differing evolutionary histories (Givnish & Sytsma, 1997). It was important therefore to identify the points of agreement in the less homoplasious molecular datasets before including the morphology for further support.

To ensure that missing data in any dataset did not influence the result disproportionately, the number of accessions used was reduced to include those for which the 5S NTS dataset and at least two chloroplast datasets were present. Two sets of trees were produced by pruning the taxa from the trees of Search 2 that did not have at least the 5S NTS and two chloroplast datasets from the separate analyses. A semi-strict consensus was calculated to identify nodes that were unconflicting in all the trees. These nodes formed the basis for reconstructing monophyly and backbone constraint trees. Further nodes were added to these constraint trees as points of agreement between the two datasets were identified. A node could only be added to a constraint tree if it met the criterion that when the original unweighted dataset, for either the chloroplast or nuclear, was constrained by that tree it was no longer than when unconstrained. This would ensure that the trees found by searching with the constraint trees enforced would always be a subset of the unconstrained trees.

For the monophyly constraint tree, addition of nodes was relatively easy and unambiguous, as there was just a single optimal tree possible with the maximum number of nodes constrained. However, for the backbone constraint tree more than one

tree could be produced. Addition of certain nodes was possible only when others were removed, including some from the original nodes that had been identified. Hence an optimality criterion needed to be used. As the monophyly constraint will generally identify nodes towards the tips of the branches, nodes for the backbone constraint were preferentially chosen if they held together branches deeper within the tree. Furthermore, the two constraint trees were intended to be complementary. As a monophyly constraint tree is more forceful than a backbone constraint tree, if a node was present in the monophyly constraint tree it was not deemed necessary in the backbone one, especially if this permitted additional nodes to be added. By doing this, one of the constraint trees is not necessarily congruent with the other. This situation will occur whenever a complete lineage is well supported (i.e. present in the monophyly constraint tree), but its placement within the context of the overall tree is conflicting (i.e. not present in the backbone constraint tree). However they should still be complementary and applicable in tandem to the datasets while maintaining the above criterion.

Unfortunately, Paup* only allows a single constraint tree to be in effect at a time. To circumvent this problem with two non-combinable but not incompatible constraint trees, the search can be conducted using one constraint tree and then the set of trees found can be filtered for those that fit the other. In a large dataset and with innumerable tree arrangements, thousands of trees may be needed before one is found that fits both constraints. Therefore, a matrix representation of the monophyly constraint tree was constructed and the characters added to the dataset. These characters were then upweighted to 1,000 (or higher) so that the nodes would be forced together and the dataset then searched with the backbone constraint enforced. The trees produced as a result of this method should still correspond to the shortest trees of the unconstrained dataset, if the two constraint trees are indeed compatible with each other.

With as many as possible of the congruent nodes between the two molecular datasets having now been identified and included in the constraint trees, all the datasets can be combined. However, there is still the danger that strongly conflicting nodes will result in erroneous reconstruction. Hence, a presumption was made that where nodes strongly conflict they will not receive strong support in a jackknife analysis of the combined data.

Therefore, a jackknife tree was produced (1,000 replicates with 36.79% character deletion and a heuristic search strategy of three replicates saving a single tree with each replicate) from the total evidence with the two constraint trees enforced. Nodes with less

than 50% support were collapsed. The resulting tree could either be used as a very conservative hypothesis based upon the total evidence, or for a more resolved hypothesis, applied as a constraint tree to any of the separate datasets depending upon what was being investigated. However, it should be noted that applying this constraint tree to any single dataset would result in suboptimal trees.

Reconstructing the reticulate trees:

The most accurate reflection of the evolutionary history of the species if reticulations are suspected to be common is to analyse each gene separately (Huelsenbeck *et al.*, 1996), and subsequently compare the position of taxa on the resulting trees. In many smaller studies of hybridization, reticulations can be placed to show the origins of each hybrid on a single tree. As the number of taxa increases, and hence potential reticulations, this becomes a much more difficult task, especially if the reticulations occur deep within the tree.

Using the successively weighted datasets, the two gene trees, combined chloroplast and 5S NTS, were compared by eye, and branches and taxa placed so as to minimise the number of reticulations needed to accurately portray the two gene histories on a single tree. Because of the difficulty of dealing with such a large number of apparently incongruent taxa, the trees were subdivided into smaller groups of between 4 and 22 related taxa based upon nodes recovered by the selective total evidence tree. In each group at least two taxa were regarded as being of 'non-hybrid' origin in order to fix the branching. As many potential 'non-hybrid' taxa as possible were identified within the groups, with polytomies that were resolved in the opposing dataset regarded as resolved to reduce the number of possible reticulations. In cases of equal-sized different sets of 'non-hybrid' taxa, the set was chosen that minimised the number of reticulations required for placement of the remaining taxa. By keeping the groups small this is relatively easy to do, although sometimes the choice of 'non-hybrid' taxa sets was still arbitrary. However, as the group size increases there is less and less guarantee that the optimum number of reticulations can be found.

To place all the groups together an overall framework tree is needed. From each group a taxon that had no reticulations within the group was chosen to represent the placement of the group in the framework tree. The method used was similar, but so many taxa needed to be included that for simplicity in the reconstruction only those branches supported at the 50% jackknife level were used.

Adding support for the reticulate trees:

Incongruence is expressed as alternative positions for a taxon on different gene trees. However, detecting the cause of each individual incongruence, whether due to erroneous tree construction or a real expression of the history of the taxon, can be difficult. Some measure is needed as to how justifiable it is to invoke a reticulation for any particular incongruence observed.

A method was devised to estimate the degree of incongruence for each reticulation on the tree, using the jackknife values already obtained for the separate analyses. Each of these estimates has two elements: the greatest support for a taxon within a different clade to that where it is placed on the opposing gene tree, and the greatest support for its placement outside the clade where it is placed on the opposing gene tree. For example, in Fig. 2.1 the placement of taxon B in the nuclear tree is supported by 70% that it occurs within a different clade to where it is placed on the chloroplast tree and 80% that it does not occur in the same clade as where it is placed on the chloroplast tree. Likewise for taxon B in the chloroplast tree the values are 70% and 60% respectively. For each estimate the greater of the two elements represent its contribution towards the incongruence, but the strength of the incongruence will be represented by the lower of the two estimates. Therefore in the example given here, the incongruence is expressed as 80% in the nuclear and 70% in the chloroplast, but the latter lower value will be indicative of the strength of the incongruence.

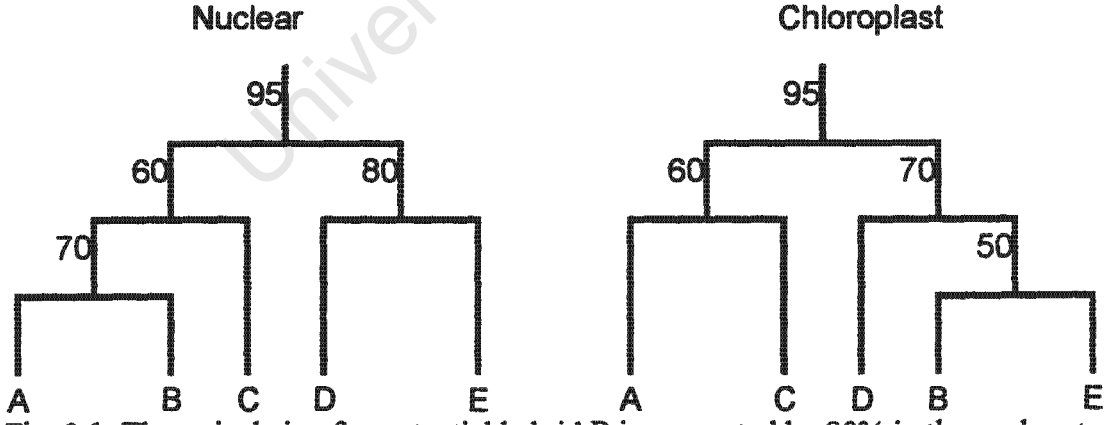


Fig. 2.1. The reticulation for potential hybrid B is supported by 80% in the nuclear tree and 70% in the chloroplast tree, and the overall value for the incongruence is 70%.

While the above example is simple to implement, when more than one reticulation occurs within the clades in question it is often only possible to establish the first element, i.e. that the taxon belongs within a different clade. This is because the composition of the opposing clade has changed by more than just the taxon in question and different circumscriptions of that clade are then possible. Hence, in this study only

the first element was calculated for each potential reticulation, but this can be regarded as a conservative estimate, as determining the second element as well can only increase the values obtained.

The difficulty with only using the first element occurs when one of the parents is missing or has become extinct and no closer relative exists along one lineage of the tree. Naturally, if the two parents were originally sister species then this makes no difference. Indeed the hybrid and its lone parent will then actually resolve as two sister species (McDade, 1995). If they are not sister species then a value can only be calculated from the gene tree with the parent and the other value will be missing (see Fig. 2.2). It cannot be regarded either as 0% or 100% as both these can give erroneous presumptions about the evidence for its hybrid status. Therefore, in these cases the second element where possible was also calculated to provide support for the placement of a reticulation at that point and where not possible the value was left blank and the support for the placement regarded as ambiguous.

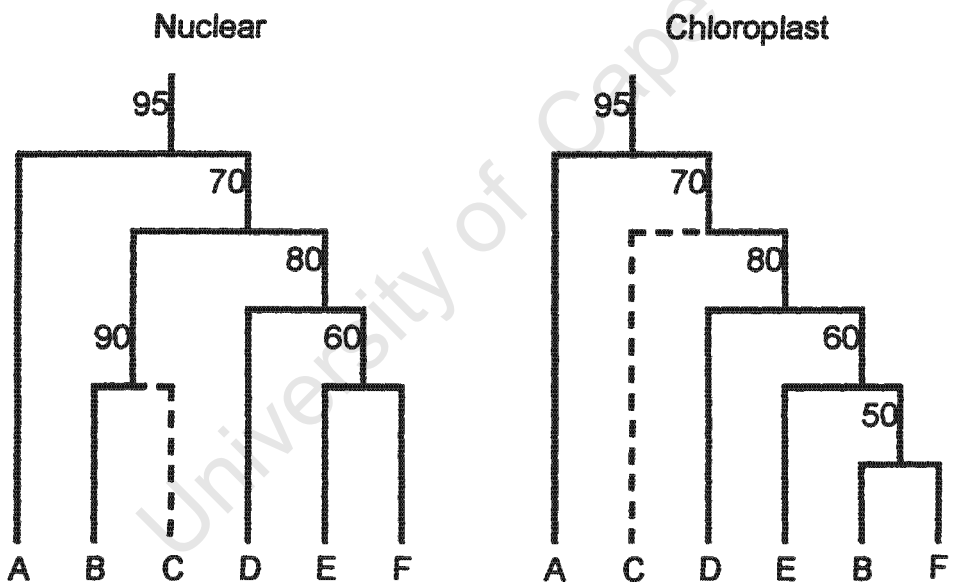


Fig. 2.2. When taxon C is included, the reticulation of hybrid B is supported by 90% in the nuclear tree and 80% in the chloroplast and thus shows strong incongruence, but if taxon C is excluded the reticulation only shows 60% support in the chloroplast tree and the value for the nuclear tree must be calculated from the second element, i.e. 80%.

Additivity of sequence data:

For putative recent hybrids, for which both parents are still believed to be extant, the original chromatograms for nuclear sequence data can be examined for evidence of additivity. Where equal double peaks in the chromatograms corresponded to differing base pair changes in the putative parents, this was regarded as evidence for additivity of sequences (for example see Fig. 2.3).

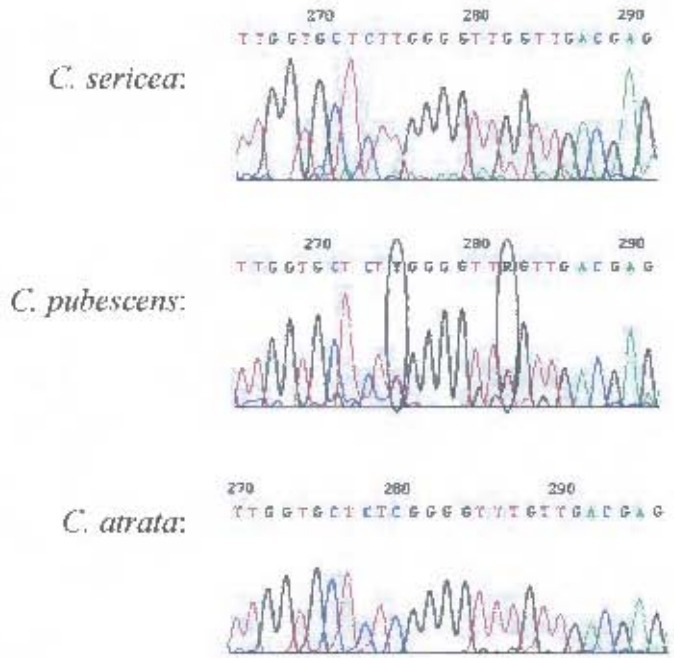


Fig. 2.3. Example sections of chromatograms showing evidence of additivity (circled) of sequences between *C. sericea* and *C. atrata* in the putative hybrid, *C. pubescens* MS.

Results:*

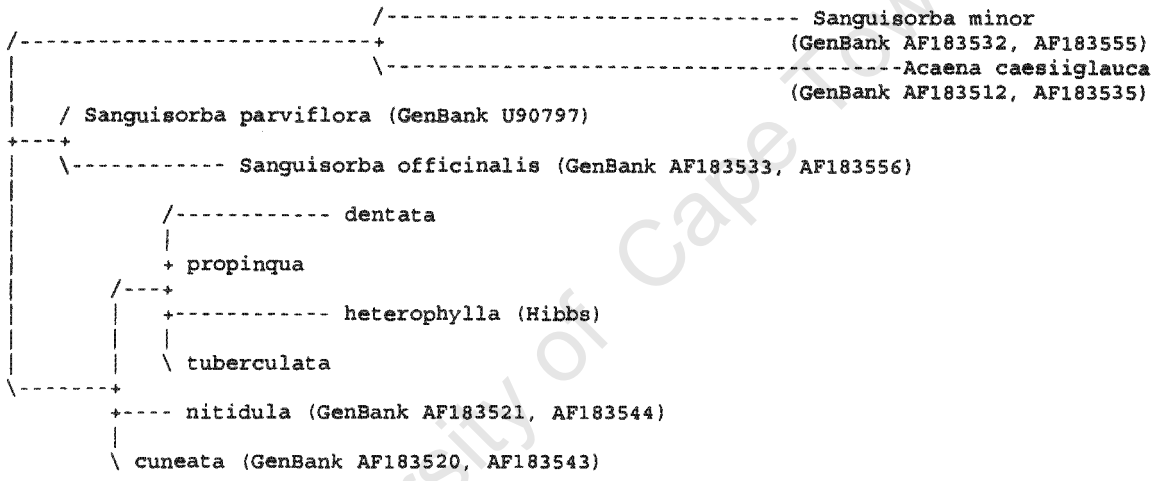
Table 2.3. Comparison of the number of taxa sampled and characters found for the respective gene regions screened.

	ITS-1	5S NTS	*trn*L	RPS16	*trn*K	*psb*A
Total sequences	10	150	157	9	102	143
Currently accepted *Cliffortia* species sampled	6	106	105	9	90	103
Outgroup sampled	4	3	5	0	2	3
Unaligned length of *Cliffortia* sequences	255	371–668	949–1122	832–861	1053–1095	262–376
Aligned length of sequences	256	1209	1348	877	1208	722
Variable sites	31	461	239	39	268	120
Percent variable sites	12.1%	38.1%	17.8%	4.4%	22.2%	16.6%
Indels & inversions	1	24	26	2	7	26
Parsimony informative characters	13	287	138	10	104	90
Percent informative characters	5.1%	23.7%	10.2%	1.1%	8.6%	12.5%
Base pair proportions:						
A	0.173	0.251	0.353	0.341	0.324	0.356
C	0.334	0.176	0.162	0.134	0.145	0.101
G	0.302	0.229	0.156	0.193	0.183	0.157
T	0.191	0.344	0.329	0.332	0.349	0.386
Transition/transversion ratio	1.907	0.956	0.423	0.616	1.342	0.379

* Classification follows the revised Linnaean classification as presented in Chapter 3 (Table 3.2 and Appendix 1). Unpublished species names are given the suffix 'MS' (manuscript).

ITS:

ITS-1 was screened for six species: three were sequenced in this study (*C. dentata*, *C. propinqua* and *C. tuberculata*), two sequences were already present in GenBank and another one donated by Malin Hibbs. Sequences of *Acaena caesiiglauca* and of three species of *Sanguisorba* were also obtained from GenBank to be used as outgroups. Only eight base pair (bp) substitutions were found in total for ITS-1 between the six species of *Cliffortia*. *C. tuberculata* and *C. propinqua* have identical sequences, and just a single substitution was parsimony informative. Compared to the closest member of the outgroup, only a further three base pairs difference was found (Fig. 2.4). For ITS-2 four base pair substitutions were found for the three species of *Cliffortia* for which that part was present, showing that it too had barely enough information to be phylogenetically informative at the level required.



---- = 1 change

Fig. 2.4. One of four equally parsimonious trees from the ITS-1 dataset indicating low levels of nucleotide variation among ingroup taxa.

5S NTS:

The 5S non-transcribed spacer proved to be more informative than ITS, showing variability even between closely related species. It was sequenced for 150 collections, including the three outgroup taxa and 106 of the currently recognised species. The unaligned length of the sequences varied between 371 for *C. aculeata* and 668 for *C. ericifolia*. The length of the aligned sequences including gaps was 1209. Of the characters present, 748 were constant, another 198 were unique autapomorphies, and the remaining 263 were phylogenetically informative. A further 24 characters were scored for the presence or absence of informative indels.

Phylogenetic reconstruction

The ratchet analysis of Search 1 produced 13,537 equally parsimonious trees of 1033 steps in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.4860, RI = 0.7220). The analysis of the reweighted dataset was terminated at 10,000 trees of length 303,461 (CI = 0.7072, RI = 0.8855). The trees from the reweighted dataset were a single step longer in length than the trees from the unweighted dataset when the weighting was removed. The strict consensus and jackknife support for the reweighted trees is shown in Fig. 2.5.

Topology of major clades

The monophyly of *Cliffortia* is well supported (jackknife = 99%). At the base of the tree, the three subgenera Graminea, Arborea, and Eriocephalina, as well as *C. curvifolia*, form a grade to the rest of *Cliffortia*. The latter three share a large insertion (189–226 bp) and all four are missing a medium-sized insertion (47 bp), which is a synapomorphy for all the other species of *Cliffortia*. In addition, the series Subsetacea is placed as sister to subgenus Eriocephalina. However this may be due to long-branch attraction as both taxa have very divergent sequences from the remainder of *Cliffortia*. The two species of series Subsetacea also lack the large insertion, but have the medium-sized one that would normally place them in a more derived position. The 'long branch attraction' test failed to corroborate this hypothesis as individually both clades remained attached to the basal node of *Cliffortia*. However, this may be the result of the highly divergent nature of the sequences and therefore each was independently being 'attracted' to the outgroup. Hence, the outgroup species were also removed and the tree rooted with subgenus Arborea. Subsequently, the 'long branch attraction' test did corroborate the suspicion that long branch attraction was the cause: subgenus Eriocephalina formed a polytomy with subgenus Graminea, while series Subsetacea was placed in an intermediate position between the basal grade and the remainder of *Cliffortia*.

The weakly supported clade that can be circumscribed with *C. falcata* and *C. dentata* is sister to a well-supported clade containing the rest of the *Cliffortia* species. That clade is itself divided up into ten smaller clades on an unresolved polytomy.

*trn*L:

The *trn*L intron and *trn*L-F intergenic spacer were easy to amplify and sequence using just two primers. Sequences were generated for 155 accessions, including three outgroup taxa and 105 of the currently recognised species; a further two outgroup taxa

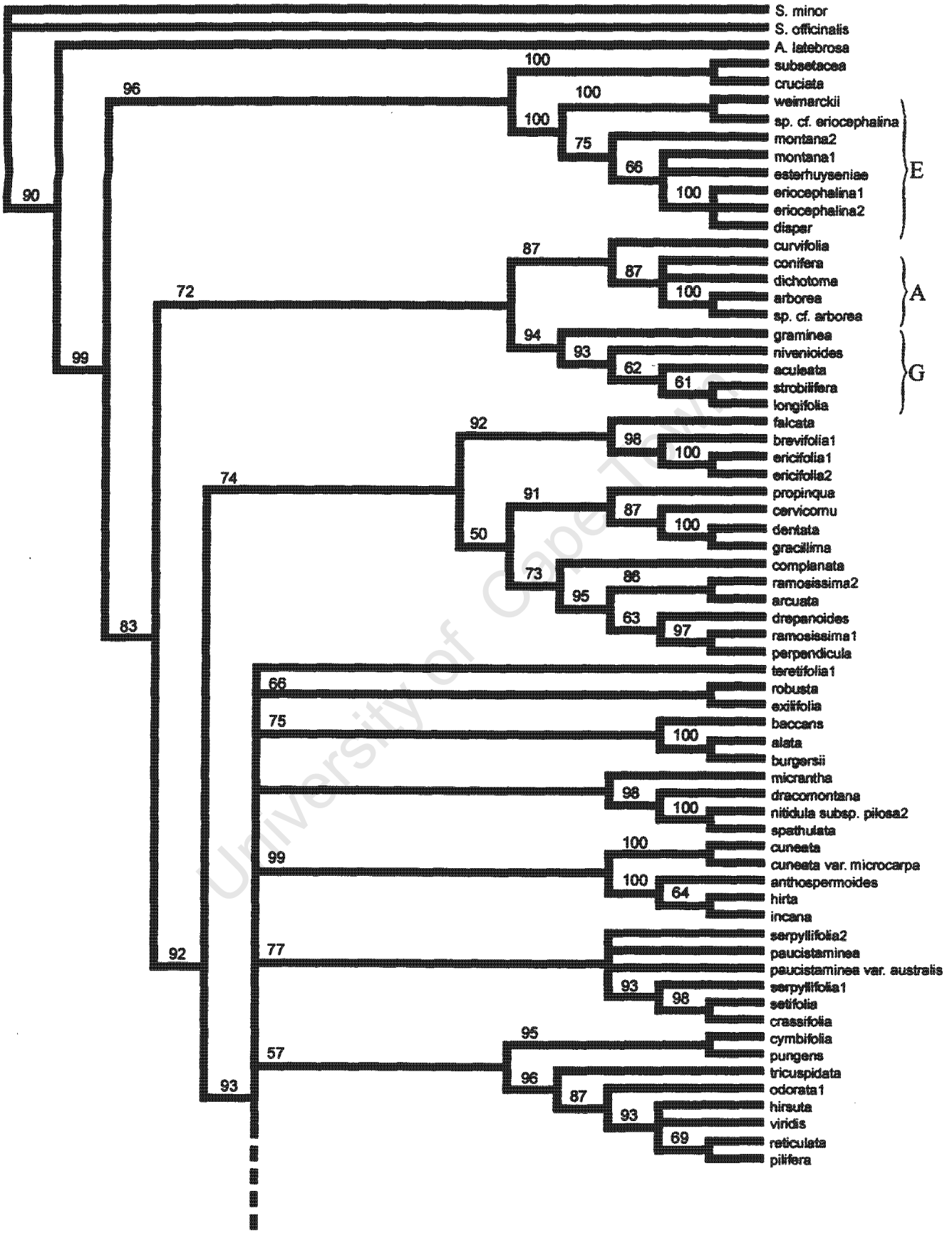


Fig. 2.5. Strict consensus tree of the successively reweighted nuclear 5S dataset. Figures above the line are jackknife support values greater than 50%. Subgenera Arborea (A), Eriocephalina (E) and Graminea (G) are labelled, all the remaining ingroup taxa belong to subgenus Cliffortia.

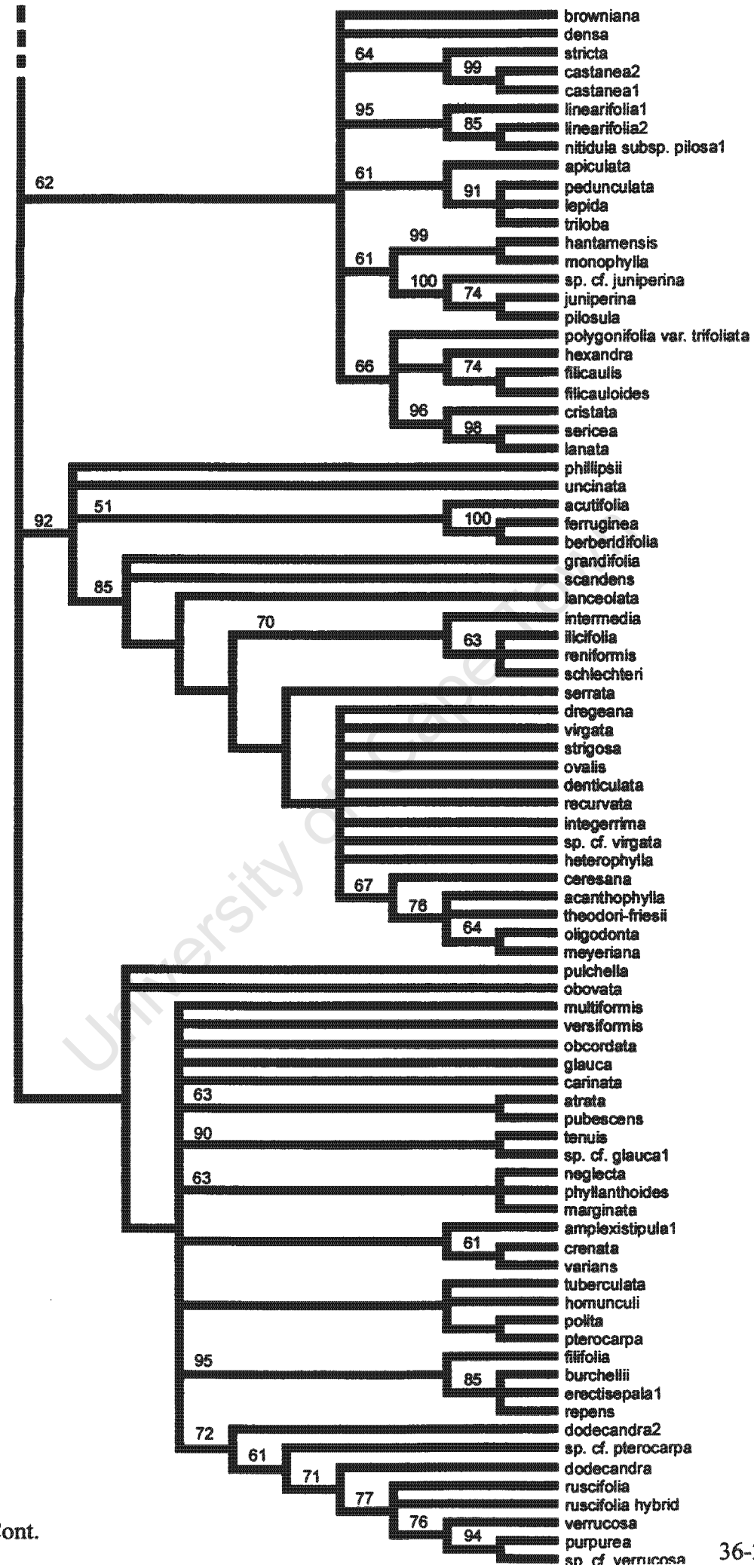

browniana
densa
stricta
castanea2
castanea1
linearifolia1
linearifolia2
nitidula subsp. pilosa1
apiculata
pedunculata
lepida
triloba
hantamensis
monophylla
sp. cf. juniperina
juniperina
pilosula
polygonifolia var. trifoliata
hexandra
filicaulis
filicauloides
cristata
sericea
lanata
phillipsii
uncinata
acutifolia
ferruginea
berberidifolia
grandifolia
scandens
lanceolata
intermedia
ilicifolia
reniformis
schlechteri
serrata
dregeana
virgata
strigosa
ovalis
denticulata
recurvata
integerrima
sp. cf. virgata
heterophylla
ceresana
acanthophylla
theodori-friesii
oligodonta
meyeriana
pulchella
obovata
multiformis
versiformis
obcordata
glauca
carinata
atrata
pubescens
tenuis
sp. cf. glauca1
neglecta
phyllanthoides
marginata
amplexistipula1
crenata
varians
tuberculata
homunculi
polita
pterocarpa
filifolia
burchellii
erectisepala1
repens
dodecandra2
sp. cf. pterocarpa
dodecandra
ruscifolia
ruscifolia hybrid
verrucosa
purpurea
sp. cf. verrucosa

Fig. 2.5. Cont.

were donated by Malin Hibbs. Excluding the outgroups, the average unaligned length of the sequences was 987 bp, varying between 949 bp for *C. densa* and 1122 bp for *C. polita*. The latter species had a unique large insertion of length 104 bp, the next highest length was only 1027 bp for a variety of *C. cuneata*. With the outgroups present the length of the aligned sequences including gaps was 1348 bp. Of the characters present, 1109 were constant, another 127 were unique autapomorphies and the remaining 112 characters were phylogenetically informative. A further 26 characters were scored for the presence or absence of informative indels. The relative compositions of characters for the different parts of the gene region for *Cliffortia* alone are shown in table 2.3.

Table 2.3. Statistics for *trn*L gene region from the 152 *Cliffortia* species sampled.

	trnL intron	trnL exon	trnL-F spacer	Total
Aligned length	799	50	479	1328
Constant	680	49	366	1095
Autapomorphic	59	1	43	103
Parsimony informative	50	0	55	105
Informative indels	10	0	15	25
Total informative characters	60	0	70	130

Phylogenetic reconstruction

The ratchet analysis of Search 1 produced 9,159 equally parsimonious trees of 301 steps in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.6047, RI = 0.8379). The analysis of the reweighted dataset was terminated at 10,000 trees of length 150,782 steps (CI = 0.8281, RI = 0.9437). The trees from the reweighted dataset were identical in length to the trees from the unweighted dataset when the weighting was removed. However, successive weighting improved the resolution found in the strict consensus tree, hence the trees found were a subset of the unweighted ones.

Topology of major clades

The *trn*L dataset has the largest number of outgroups present and the monophyly of *Cliffortia* is well supported. Several species groups are supported but there is little resolution among these. The only character that supports resolution among the groups at a deeper level within the tree is a six base pair repeat unit at position 1017–1022. It is scored as a single indel so has a very weak influence upon tree structure in the unweighted dataset. However, the group defined by the absence of the repeat unit does not conflict with the strict consensus and the same group is present upon successive weighting of the dataset. The relationships supported by the indel might be corroborated

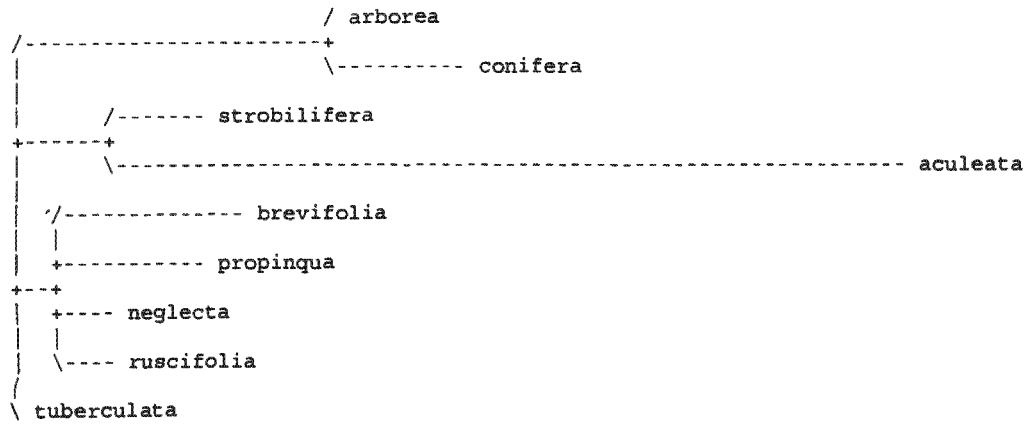
by further data, but in this dataset there is no additional support to determine how homoplasious it is. The dataset as a whole is most informative near the tips of the tree, but as it contains almost no informative characters at deeper levels it needs to be combined with other datasets for more complete resolution.

*rps*16:

The *rps*16 intron was sequenced for nine species representing six sections in *Cliffortia*, as well as both representatives of two species pairs, to establish the usefulness of the region at various levels within *Cliffortia*. A poly-T region close to the beginning of the forward primer meant that some of the sequences were difficult to read from the 5' end.

Phylogenetic analysis of the data (using Branch and Bound, possible because of the small sample size) produced five equally parsimonious trees. The strict consensus of these collapsed all nodes except those supporting the two species pairs, *C. arborea* & *C. conifera* and *C. strobilifera* & *C. aculeata*. By excluding a potentially ambiguous character in the middle of a poly-G region, a single tree was produced (Fig. 2.6). This tree showed the same topology as those produced by other chloroplast regions.

The species pairs were well-supported, indicating that the gene might be informative at confirming relationships at the tips of the tree. However, there was just a single character, an extra T at position 686, that gave any indication of the presence of synapomorphies to support nodes deeper within the tree. As the *trn*L dataset, which was already quite complete, already supported the species groups quite strongly, the addition of further characters to support them was deemed unnecessary. Therefore, as only a single character in the *rps*16 intron showed any potential for identifying nodes deeper within the tree it was abandoned.



-- = 1 change

Fig. 2.6. One of five equally parsimonious tree from the *rps*16 dataset, identical in topology to the single tree produced when the ambiguous character in the poly-G region is removed. Despite its small size there is no conflict between this dataset and the other chloroplast ones.

*trn*K:

The 5' end of the *trn*K intron, including part of the *mat*K gene, proved difficult to amplify for certain species and only 102 accessions were sequenced, including 90 of the currently recognised species and two of the outgroup taxa. Two species, *C. burchellii* and *C. repens*, contained an identical inversion, which was reversed, and the presence of the inversion scored in the same way as an indel.

The average unaligned length of the sequences was 1069 bp, ranging between 1053 bp for *C. filicaulis* and 1095 bp for *C. filifolia*. The length of the aligned sequences including gaps was 1208. Of these characters, 978 were constant, another 171 were unique autapomorphies, and the remaining 97 characters were phylogenetically informative. A further six characters were scored for the presence or absence of informative indels, plus the single character for the inversion. The relative compositions of the different parts of the gene region for *Cliffortia* alone are shown in table 2.4.

Table 2.4: Statistics for *trn*K gene region from the 100 *Cliffortia* species sampled.

	5' end of trnK intron	part of MatK gene	Total
Aligned length	846	356	1202
Constant	683	297	980
Autapomorphic	97	35	132
Parsimony informative	66	24	90
Indels & inversions	7	0	7
Total informative characters	73	24	97

Phylogenetic reconstruction

The ratchet analysis of Search 1 produced 4,389 equally parsimonious trees of 187 steps in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.6047, RI = 0.8379). The reweighted dataset yielded 10,000 trees of length 92984 steps (CI = 0.8859, RI = 0.9548). The trees from the reweighted dataset were identical in length to the trees from the unweighted dataset when the weighting was removed. However, successive weighting improved the resolution found in the strict consensus tree, hence the trees found were a subset of the unweighted ones.

Topology of major clades

The monophyly of *Cliffortia* was once again supported (jackknife = 78%). As with the 5S NTS, the same four basal lineages, subgenera Arborea, Eriocephalina and Graminea, plus *C. curvifolia*, resolve along a polytomy to the rest of *Cliffortia*. Also present on the same basal polytomy are a small clade containing the section Tuberculatae and *C. cruciata* MS, and nine other species: *C. crassinervis* and the remaining members of section Heteromorphae. Once again the majority of *Cliffortia* species form a large polytomy directly beneath the basal polytomy divided up into many smaller supported clades.

Of note is that section Tuberculatae along with *C. cruciata* MS form a clade that is found in a transitional position between the basal polytomy and the large derived polytomy in 74% of the unweighted trees. Indeed, if *C. crassinervis* is omitted from the analysis then the clade is found in that position in the strict consensus too.

*psb*A:

The *psb*A-*trn*H intergenic spacer was easily amplified, and consequently 143 accessions were sequenced, including three outgroup taxa and 103 of the currently recognised species. Excluding the outgroups, the average unaligned length of the sequences was 321 bp, varying between 262 bp for *C. exilifolia* and 376 bp for *C. geniculata*, this includes 51 bp of the *psb*A gene at the 5' end. The length of the aligned sequences was 722. A highly polymorphic region between 640–692, which proved difficult to align between species groups because it contained several varying repeat units, was excluded from the analysis. Within species groups this area might be very useful, especially for phylogeographic studies, but would probably have influenced the complete dataset too strongly if left in. Other variable regions were scored for the presence of matching repeat units but otherwise excluded; in total 398 characters were excluded. Of the

included characters, 204 were constant, another 56 were unique autapomorphies, and the remaining 64 characters were phylogenetically informative. A few inversions were also noted, including a large and frequently occurring one between positions 222–283; these were returned to the same direction as the rest of the species and scored as present or absent. In total, a further 26 characters were scored for the presence or absence of informative indels and inversions.

Phylogenetic reconstruction

The ratchet analysis of Search 1 produced 12,592 equally parsimonious trees of 292 steps in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.5205, RI = 0.8194). The reweighted dataset yielded 10,000 trees of length 110,272 (CI = 0.7589, RI = 0.9322). The trees from the reweighted dataset were four steps longer in length than the trees from the unweighted dataset when the weighting was removed.

Topology of major clades

This dataset was the only one where the monophyly of *Cliffortia* was not fully supported as *Acaena latebrosa* formed a polytomy with the basal lineages of *Cliffortia* and *C. crassinervis* was placed as sister to this in the reweighted tree. This can be attributed to a few mutations in *C. crassinervis* in common with the outgroup taxa in a less conserved part of the gene.

In the unweighted dataset the pattern was very similar to that found in the *trn*L analysis, but with successive weighting much more resolution was revealed with the four basal lineages again coming to the fore along with section Heteromorphae and *C. cruciata* MS (but not section Tuberculatae). There was also a higher degree of resolution within the large derived polytomy with two large clades being resolved, although not well-supported (jackknife = 53% and <50%)

Combined chloroplast genes:

Sequences for all three chloroplast genes were available for 100 accessions, a further 40 were complete for two of the genes (usually *trn*L and *psb*A) and 21 accessions only had the sequence for a single gene. Whether the dataset was restricted to taxa with all sequences present or taxa with at least one DNA region were included, did not alter the general topology of the tree much, hence all 162 accessions were included in the final analysis. This is supported by Wiens (1998) who found that adding characters with missing data usually improved the accuracy of the resulting phylogeny even up to 50%

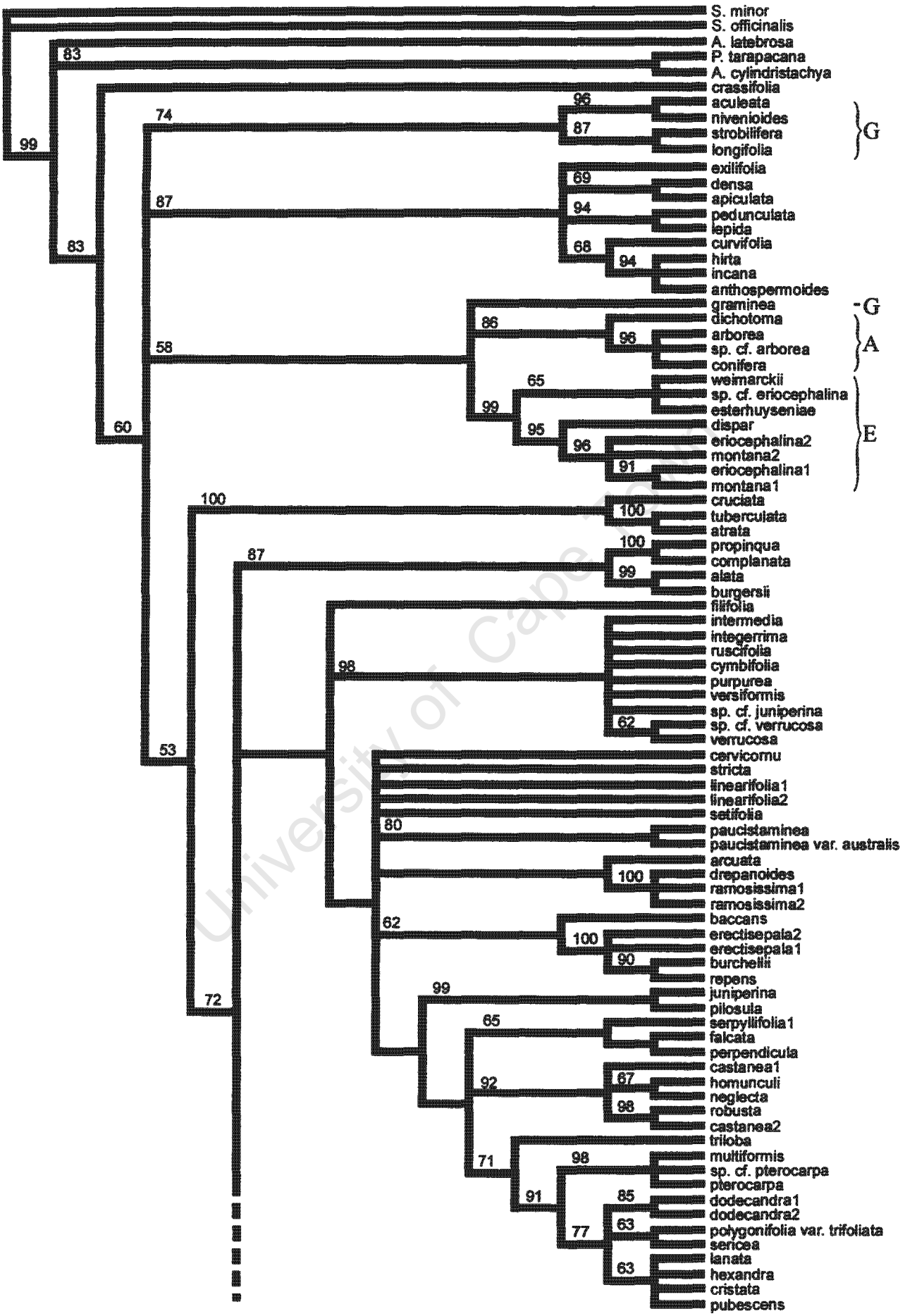


Fig. 2.7. Strict consensus tree of the successively reweighted combined chloroplast datasets. Figures above the line are jackknife support values greater than 50%. Subgenera are labelled as in Fig. 2.5.

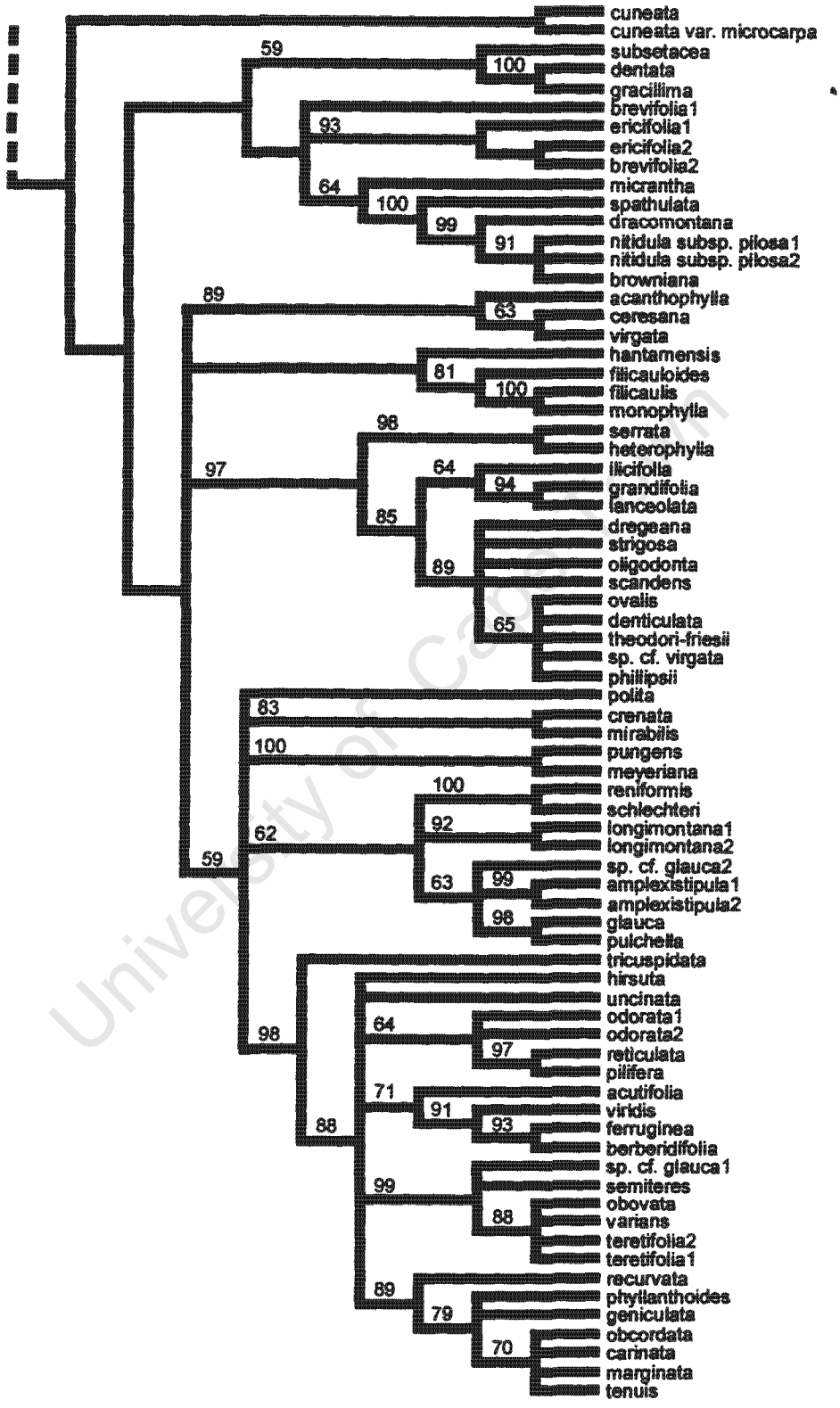

cuneata
cuneata var. microcarpa
subsetacea
dentata
gracillima
brevifolia1
ericifolia1
ericifolia2
brevifolia2
micrantha
spathulata
dracomontana
nitidula subsp. pilosa1
nitidula subsp. pilosa2
browniana
acanthophylla
ceresana
virgata
hantamensis
filicauloides
filicaulis
monophylla
serrata
heterophylla
ilicifolia
grandifolia
lanceolata
dregeana
strigosa
oligodonta
scandens
ovalis
denticulata
theodori-friesii
sp. cf. virgata
phillipsii
polita
crenata
mirabilis
pungens
meyeriana
reniformis
schlechteri
longimontana1
longimontana2
sp. cf. glauca2
amplexistipula1
amplexistipula2
glauca
pulchella
tricuspidata
hirsuta
uncinata
odorata1
odorata2
reticulata
pilifera
acutifolia
viridis
ferruginea
berberidifolia
sp. cf. glauca1
semiteres
obovata
varians
teretifolia2
teretifolia1
recurvata
phyllanthoides
geniculata
obcordata
carinata
marginata
tenuis

Fig. 2.7. Cont.

level of missing data. This dataset has only 19.5% cells of missing data (as the result of missing sequences rather than gaps in sequences). There were a total of 332 informative characters in the final dataset, which is an average of 2.05 characters per accession.

Phylogenetic reconstruction

The ratchet analysis of Search 1 produced 5039 equally parsimonious trees of 818 in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.5660, RI = 0.8055). The reweighted dataset yielded 10,000 trees of length 339,631 (CI = 0.8189, RI = 0.9366). With weights removed, the trees had length of 825. The strict consensus and jackknife support for the reweighted trees is shown in Fig. 2.7.

Topology of major clades

The monophyly of *Cliffortia* was supported, although only receiving 63% jackknife support. This was probably due to *C. crassinervis*, which was placed as sister to the rest of *Cliffortia*, but as already mentioned this is probably an artefact of the *psb*A dataset. There is not a single character to support this placement in either of the other two genes. The large basal polytomy includes the well-defined clades that were usually supported in at least two of the datasets, namely subgenera Arborea, Eriocephalina, Graminea and section Heteromorphae. *C. graminea* itself is well supported within this polytomy, but as it was either sister to subgenus Arborea or Graminea, its placement is still unclear and it drops to the base of the two. Sister to the remainder of *Cliffortia* is a small clade containing section Tuberculatae and *C. cruciata* MS. Two large clades splitting the rest of *Cliffortia* are present in the successively weighted combined analysis. These are based on two small indel events, one in the *trn*L dataset, which has already been mentioned, and another in the *psb*A, the latter being a slightly smaller subset of the former. However, they are not completely in agreement and hence there is still low support for these nodes, and the 50% jackknife tree reveals the same large polytomy containing the majority of *Cliffortia* in relatively small well-defined clades.

Morphology:

A total of 81 parsimony informative characters were scored for the morphological matrix (see Appendix 3). All the currently accepted taxa for which herbarium material was available were scored as well as any putative new taxa. Including the five outgroup species, 145 taxa were included in the analysis.

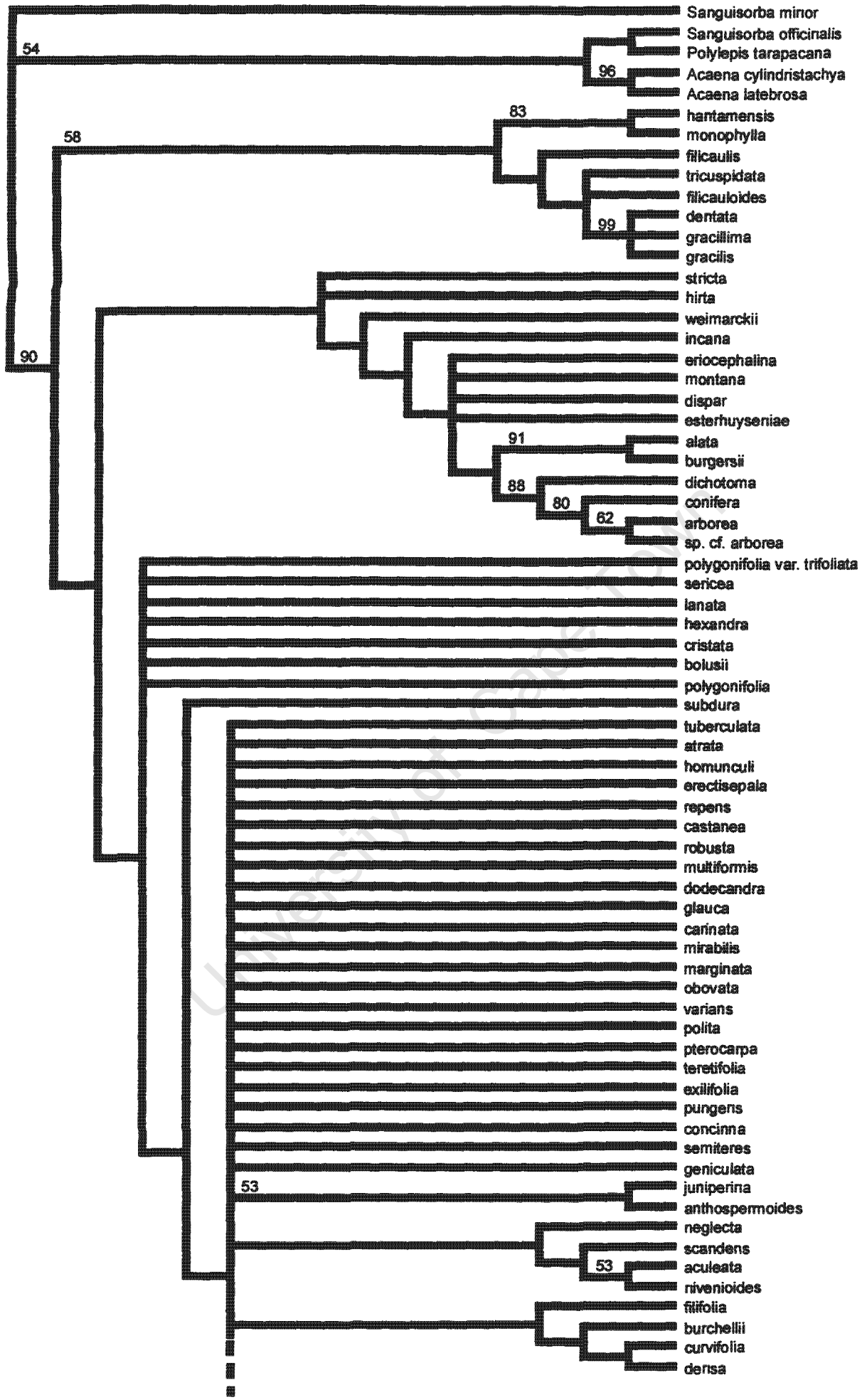


Fig. 2.8. Strict consensus tree of the successively reweighted morphology dataset. Figures above the line are jackknife support values greater than 50%.

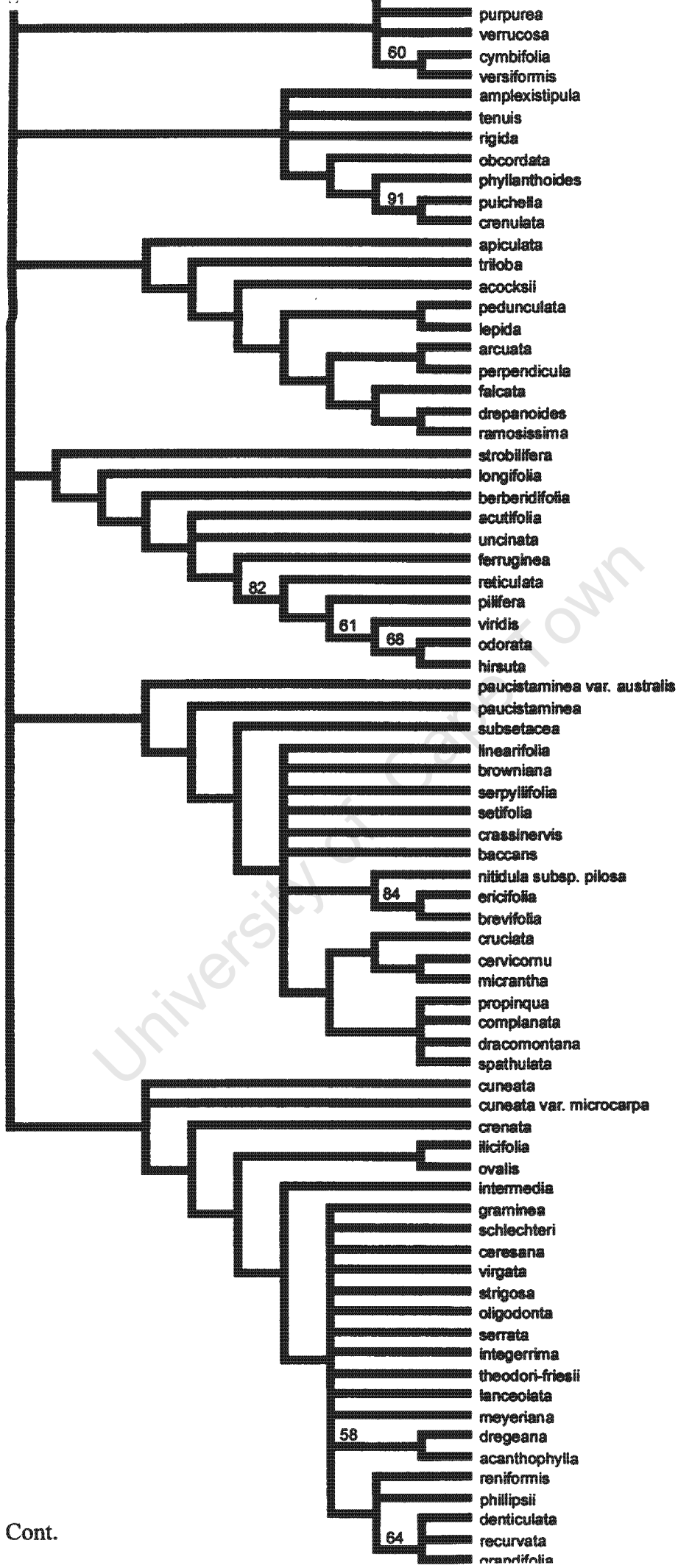

purpurea
verrucosa
60
cymbifolia
versiformis
amplexistipula
tenuis
rigida
obcordata
phyllanthoides
91
puichella
crenulata
apiculata
triloba
acocksii
pedunculata
lepida
arcuata
perpendicula
falcata
drepanoides
ramosissima
strobilifera
longifolia
berberidifolia
acutifolia
uncinata
ferruginea
82
reticulata
pilifera
61
viridis
68
odorata
hirsuta
paucistaminea var. australis
paucistaminea
subsetacea
linearifolia
browniana
serpyllifolia
setifolia
crassinervis
baccans
nitidula subsp. pilosa
84
ericifolia
brevifolia
cruciata
cervicornu
micrantha
propinqua
complanata
dracomontana
spathulata
cuneata
cuneata var. microcarpa
crenata
ilicifolia
ovalis
intermedia
graminea
schlechteri
ceresana
virgata
strigosa
oligodonta
serrata
integerrima
theodori-friesii
lanceolata
meyeriana
58
dregeana
acanthophylla
reniformis
phillipsii
denticulata
64
recurvata
grandifolia

Fig. 2.8. Cont.

Phylogenetic reconstruction

The ratchet analysis of Search 1 produced 3136 equally parsimonious trees of 629 in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.1828, RI = 0.7091). The reweighted dataset yielded 10,000 trees of length 761 (CI = 0.3495, RI = 0.7952). The trees from the reweighted dataset were between 38–43 *steps* (6–7%) longer in length than the trees from the unweighted dataset when the weighting was removed. This reflects the low CIs, and shows that the dataset is highly homoplasious. The strict consensus and jackknife support for the reweighted trees is shown in Fig. 2.8.

Topology of major clades

The monophyly of *Cliffortia* was supported (>50% jackknife support), which was noteworthy considering that only 22 nodes were supported within *Cliffortia* even in the reweighted analysis and the outgroup taxa had numerous missing characters. Within *Cliffortia* there was little significantly supported structure, with only a few very closely related species pairs being held together. However, although lacking significant support, the strict consensus of the reweighted dataset did have a high degree of resolution.

Total evidence:

When all 163 taxa were included in the dataset there were a total of 700 informative characters. The ratchet analysis of Search 1 produced 7149 equally parsimonious trees of 2988 in length. No shorter tree was found upon complete swapping of 20,000 trees (CI = 0.3614, RI = 0.6613). The reweighted dataset yielded 10,000 trees of length 6278 (CI = 0.6722, RI = 0.8516). The strict consensus and jackknife support for the reweighted trees is shown in Fig. 2.9.

Selective total evidence tree:

For the 'selective total evidence' tree 133 accessions were used which had at least two chloroplast datasets present and as a result only 4.6% of cells in the matrix had missing data (not including gaps). Most of the morphological range of diversity found within *Cliffortia* was covered. Only *C. integerrima*, *C. marginata*, *C. mirabilis* and *C. varians* were excluded out of the currently accepted species for which there was some molecular data. Hence, most of the accessions excluded were duplicates of species or undetermined collections.

Only 16 nodes were located in the semi-strict consensus of the two sets of trees.

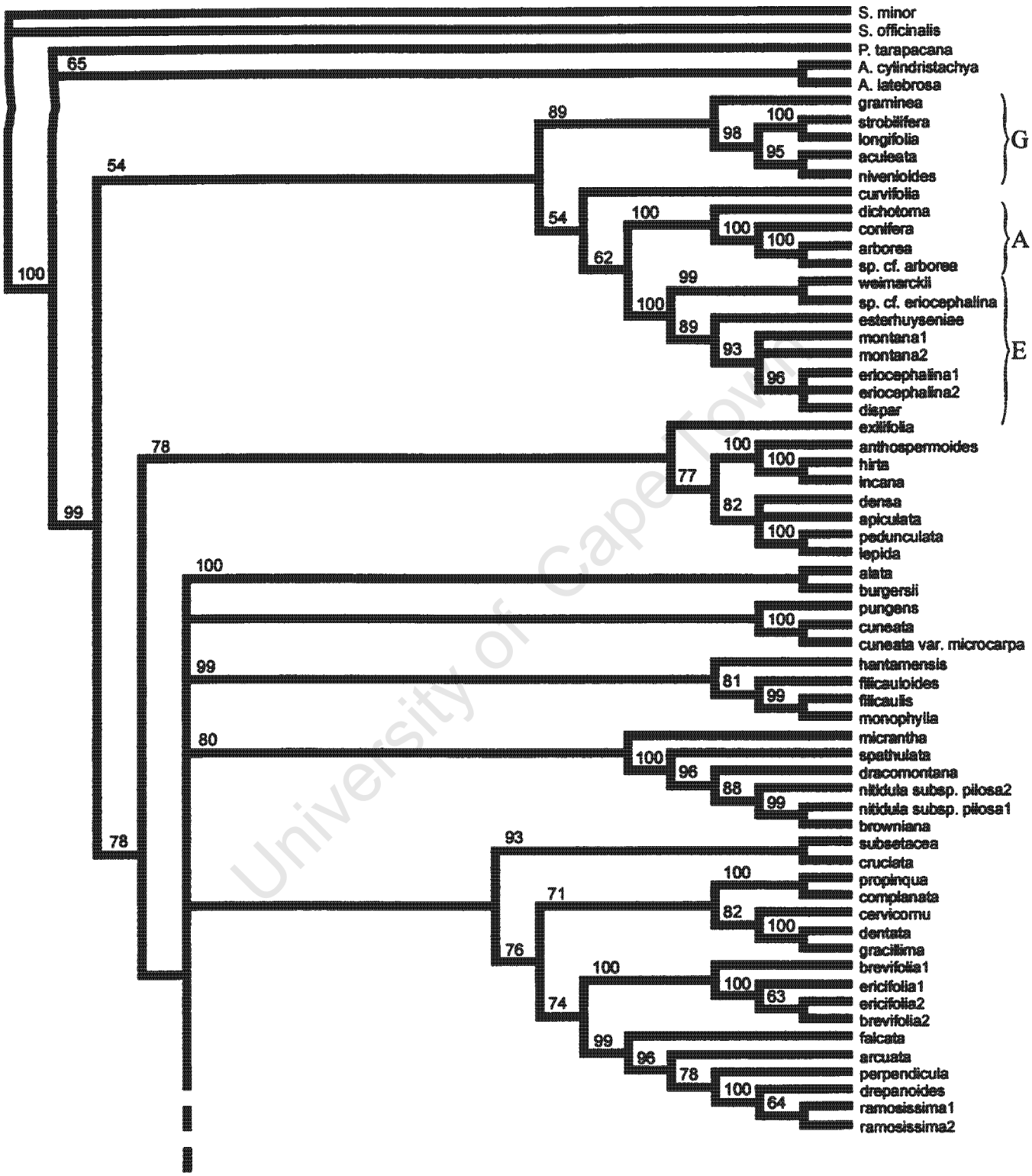


Fig. 2.9. Strict consensus tree of the successively reweighted 'total evidence'. Figures above the line are jackknife support values greater than 50%. Subgenera are labelled as in Fig. 2.5.

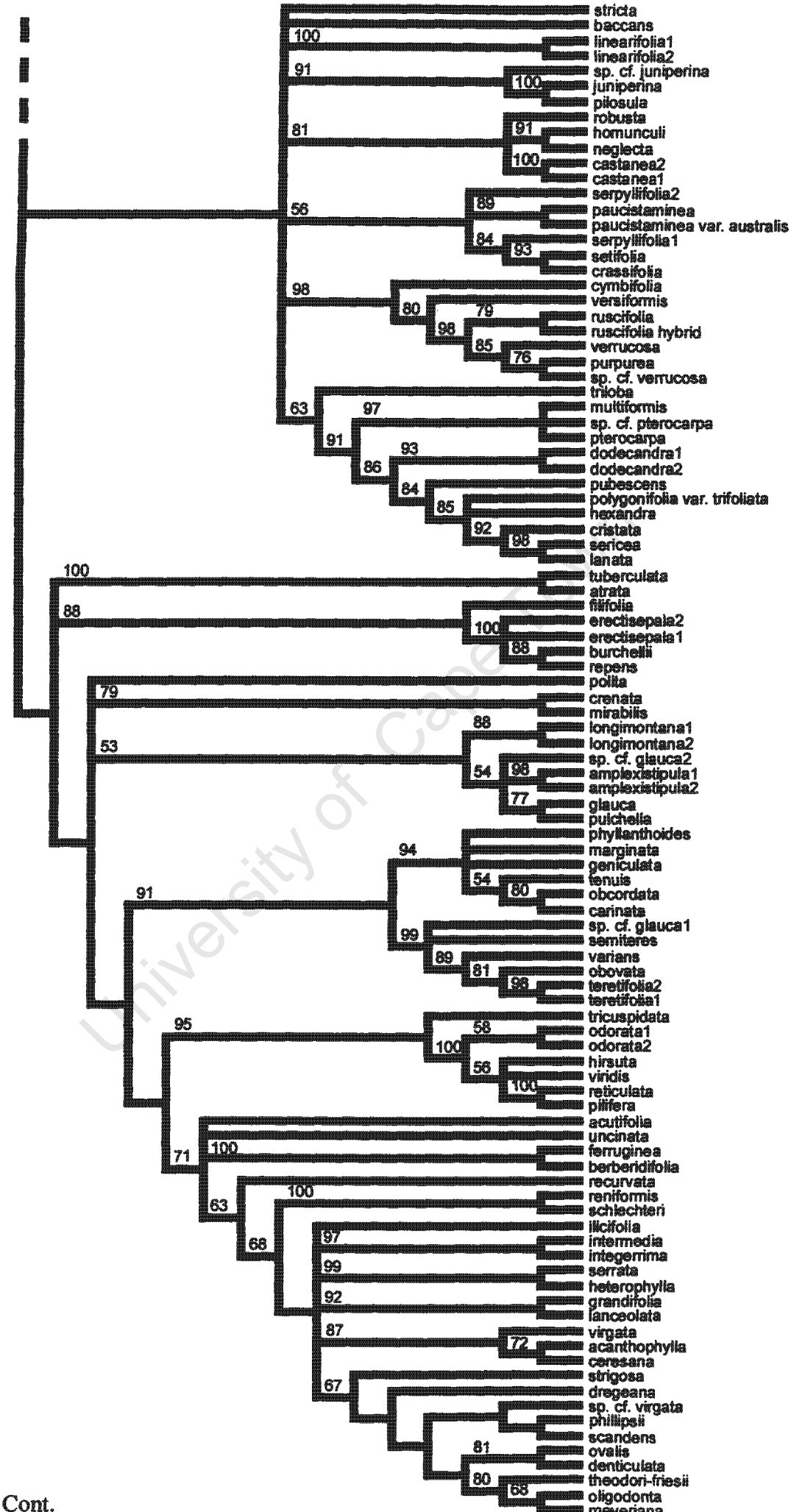

stricta
baccans
linearifolia1
linearifolia2
sp. cf. juniperina
juniperina
pilosula
robusta
homunculi
neglecta
castanea2
castanea1
serpyllifolia2
paucistaminea
paucistaminea var. australis
serpyllifolia1
setifolia
crassifolia
cymbifolia
versiformis
ruscifolia
ruscifolia hybrid
verrucosa
purpurea
sp. cf. verrucosa
triloba
multiformis
sp. cf. pterocarpa
pterocarpa
dodecandra1
dodecandra2
pubescens
polygonifolia var. trifoliata
hexandra
cristata
sericea
lanata
tuberculata
atrata
filifolia
erectisepala2
erectisepala1
burchellii
repens
polita
crenata
mirabilis
longimontana1
longimontana2
sp. cf. glauca2
amplexistipula1
amplexistipula2
glauca
pulchella
phyllanthoides
marginata
geniculata
tenuis
obcordata
carinata
sp. cf. glauca1
semiteres
varians
obovata
teretifolia2
teretifolia1
tricuspidata
odorata1
odorata2
hirsuta
viridis
reticulata
pilifera
acutifolia
uncinata
ferruginea
berberidifolia
recurvata
reniformis
schlechteri
ilicifolia
intermedia
integerrima
serrata
heterophylla
grandifolia
lanceolata
virgata
acanthophylla
ceresana
strigosa
dregeana
sp. cf. virgata
phillipsii
scandens
ovalis
denticulata
theodori-friesii
oligodonta
meyeriana

Fig. 2.9. Cont.

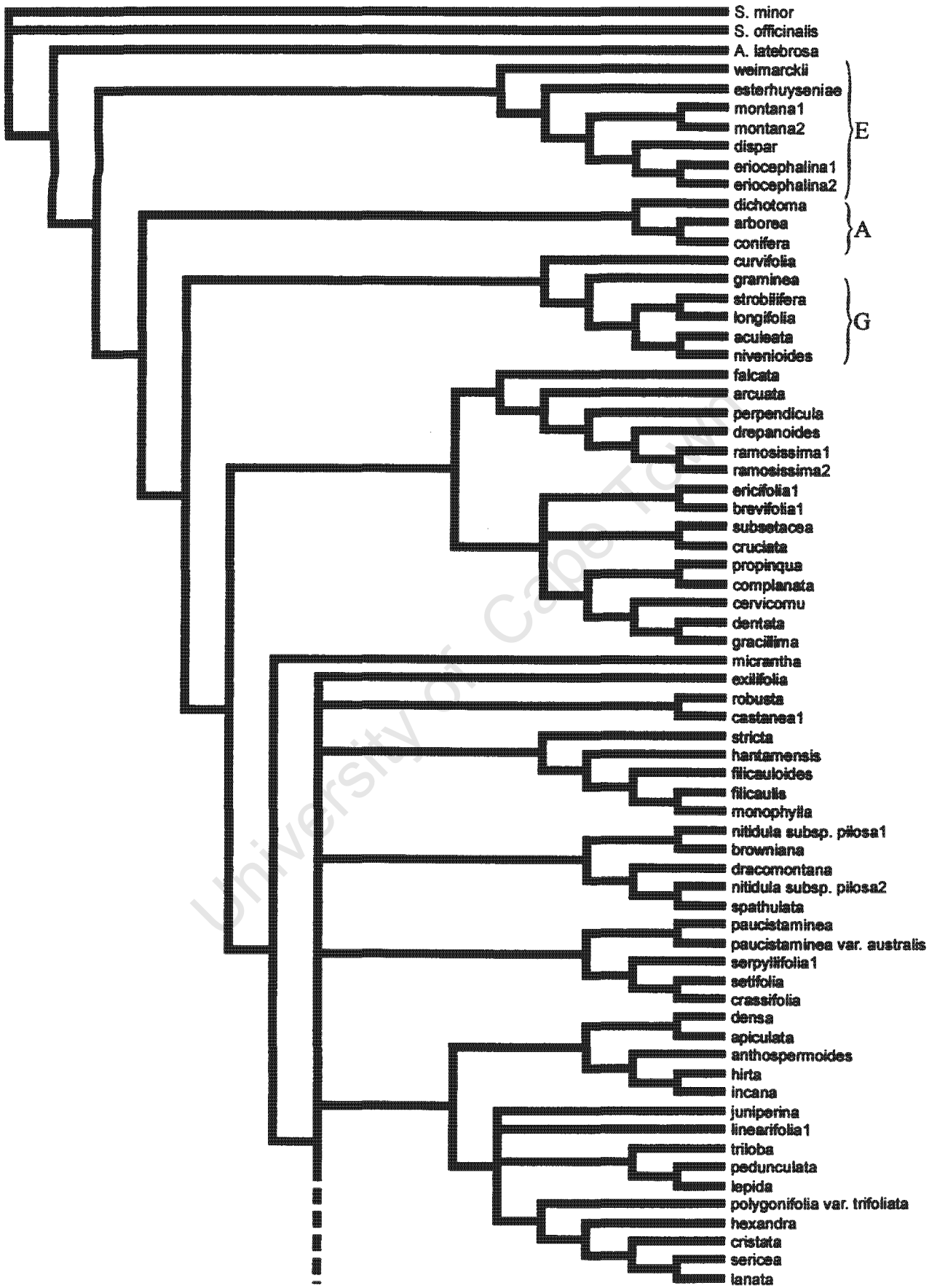


Fig. 2.10. Strict consensus tree of the 5S data when constrained by the methods described for creating the 'selective total evidence' tree. Subgenera are labelled as in Fig. 2.5.

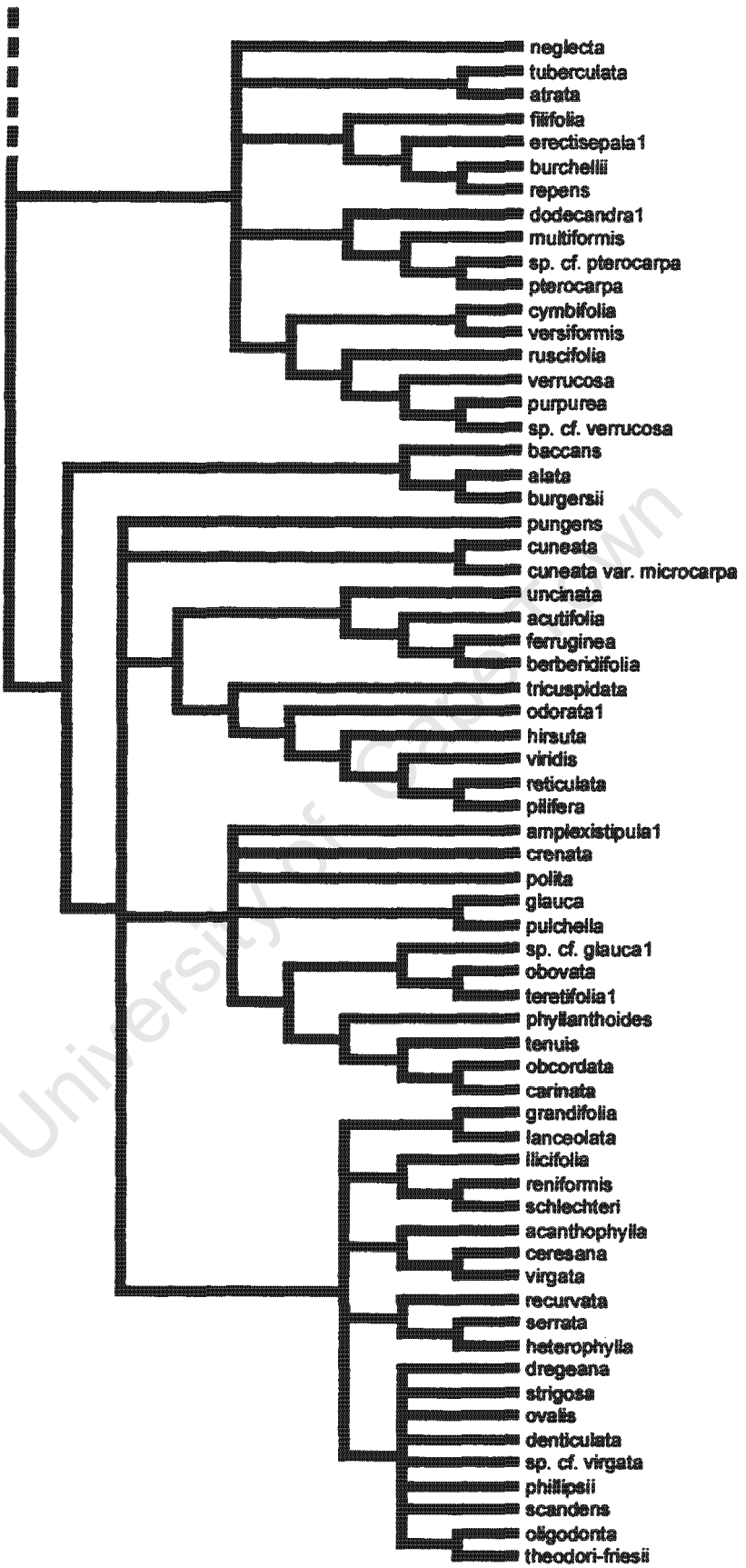

neglecta
tuberculata
atrata
filifolia
erectisepala1
burchellii
repens
dodecandra1
multiformis
sp. cf. pterocarpa
pterocarpa
cymbifolia
versiformis
ruscifolia
verrucosa
purpurea
sp. cf. verrucosa
baccans
alata
burgersii
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata1
hirsuta
viridis
reticulata
pilifera
amplexistipula1
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia1
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii

Fig. 2.10. Cont.

The monophyly constraint tree that was constructed had 23 nodes, which included all the 16 nodes present in the semi-strict consensus of the two datasets. For the backbone constraint, however, it was found to be better to remove some of the original 16 nodes as they caused conflict with some of the deeper nodes within the tree. A total of 79 taxa and 34 nodes were included in the backbone constraint tree.

No tree that fitted both constraint trees was found in over 50,000 trees when searching using only one constraint tree enforced. The combined backbone constraint and monophyly constraint (using the upweighted characters from the matrix representation tree) produced trees that were equal to the shortest trees on the unconstrained datasets showing that both constraint trees were compatible and combinable.

The final constraint tree from the jackknife analysis of combined data had 91 nodes. When applied to the different datasets the length of the shortest trees produced are shown in table 2.5. For comparative purposes, an unconstrained total evidence search was carried out on the same taxa and the different datasets independently mapped on to those trees. An example for the 5S NTS dataset is given in Fig. 2.10.

Table 2.5. Comparison of the length of the trees and increase in steps against the unconstrained trees when the different datasets are constrained by the selective total evidence jackknife tree and then searched, or when the characters are mapped over the total evidence tree.

	Nuclear	Chloroplast	Morphology	Nuclear + morphology	Total evidence
Informative characters	266	310	81	347	657
Unconstrained tree length	985	767	600	1754	2784
Constrained tree length	1084	888	739	1855	2789
Increase	99	121	139	101	5
Mapped on to total evidence	1111–1121	910–920	751–755	1864–1874	2784
Increase	126–136	143–153	151–155	110–120	0

Comparison and congruence of chloroplast, nuclear and morphology datasets:

Visual comparison of the two molecular datasets showed strong conflict between them as there were some nodes with 99% jackknife support in each consensus tree that conflicted. The morphology dataset had so few nodes supported that conflict is rarer, but it can be seen that there is a greater degree of conflict against the chloroplast dataset than the nuclear (table 2.6). All results from the pair-wise ILD tests gave P values < 0.001 (table 2.7).

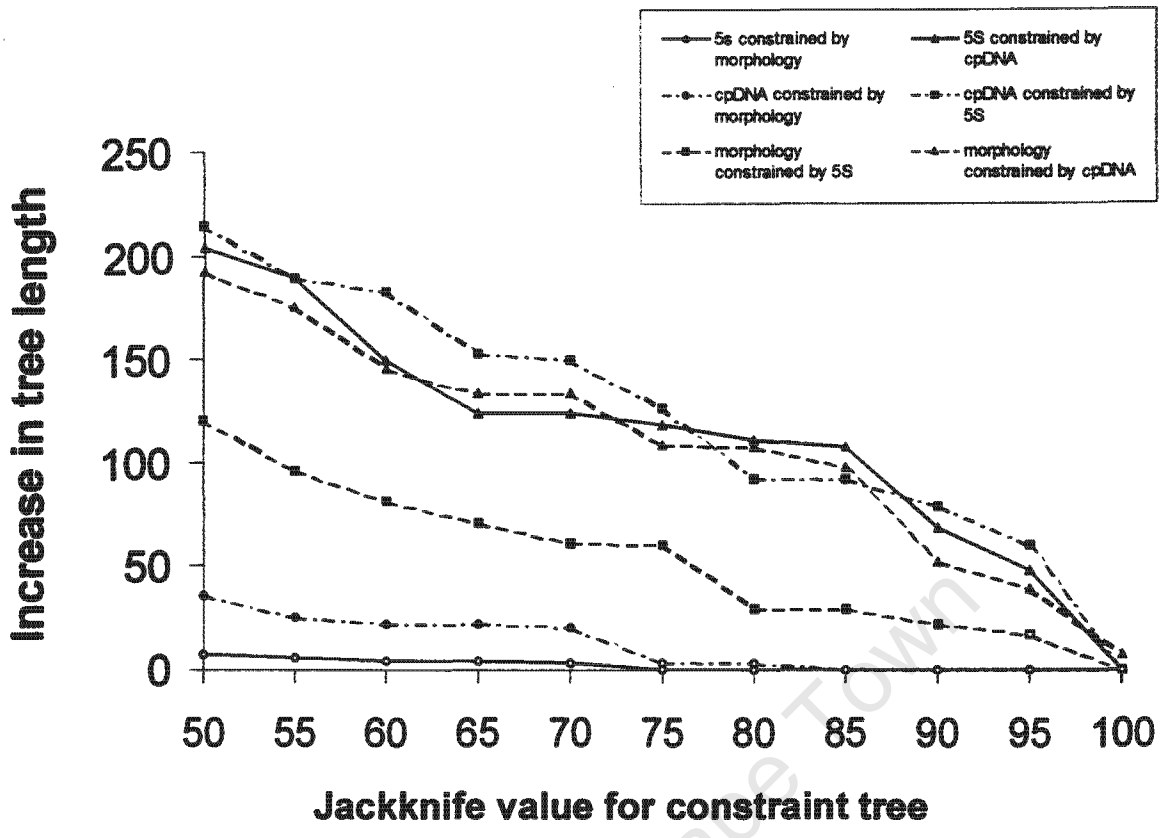


Fig. 2.11. Graph to show the effect of constraining the different datasets by the jackknife trees at varying levels of support for an opposing dataset. Empty symbols indicate tree length increases that were not significantly different from the unconstrained analyses.

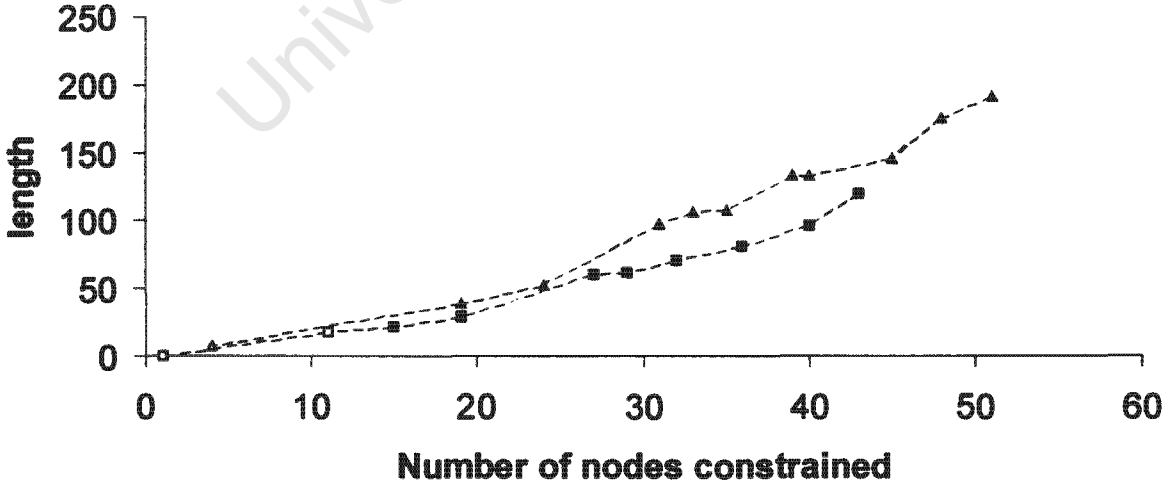


Fig. 2.12. Graph to show the effect of the number of nodes present in the chloroplast and nuclear jackknife trees when they constrain the morphology dataset. Legend is the same as for Fig. 2.11.

Table 2.6. Jackknife value above which no conflicting nodes are found between datasets (based upon non-weighted datasets).

	cpDNA	nrDNA
morphology	78	65
nrDNA	99	

Table 2.7: *P* value from partition homogeneity tests between different matrices to show incongruence of datasets. Only the 100 taxa for which datasets were complete for all three were included. The figures in brackets are the sum of tree lengths for the original partition and the range of lengths for subsequent partitions.

	cpDNA	nrDNA
morphology	0.001 (1206; 1313–1364)	0.001 (1374; 1439–1487)
nrDNA	0.001 (1519; 1643–1694)	

The Kishino-Hasegawa tests on the transformed trees always revealed a high degree of incongruence between the chloroplast and nuclear datasets and were significantly different from each other except with the 100% jackknife constraint trees (Fig. 2.11). When the morphology was used as the constraining dataset the most congruence was found with the nuclear 5S NTS dataset and even at the 50% level there was no incongruence (table 2.8).

Table 2.8: Comparison of the effect of constraining each dataset by the jackknife trees from the opposing datasets.

			Test dataset								
			5S NTS (844 steps)			cpDNA (674 steps)			morphology (520 steps)		
Constraining dataset	Jackknife percentage		50	75	95	50	75	95	50	75	95
	5S NTS	constrained nodes				43	27	11	43	27	11
		length increase				214	126	60	120	60	18
		SD of difference				21.75	14.05	8.42	21.01	13.22	8.96
		P				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0616*
	cpDNA	constrained nodes	51	35	19				51	35	19
		length increase	204	118	48				192	108	39
		SD of difference	23.40	14.39	8.41				25.01	18.78	12.23
		P	<0.0001	<0.0001	<0.0001				<0.0001	<0.0001	0.0021
	morphology	constrained nodes	13	3	0	13	3	0			
		length increase	7	0	n/a	36	3	n/a			
		SD of difference	5.38	n/a	n/a	7.57	3.32	n/a			
		P	0.1943*	1.0*	1.0*	<0.0001	0.3667*	1.0*			

* comparison of two arbitrary trees using Kishino-Hasegawa test shows that there is not a significant difference between them.

Reticulate trees:

The reconstruction of the reticulating trees produced 18 definable small groups, although the Ilicifolia and Glauca groups were rather large and the reticulation pattern may not be optimal, as well as an overall framework tree (Fig. 2.13). Table 2.9 lists the 55 taxa with strong incongruence (both alternative topological positions with more than 80% jackknife support) incongruence or ambiguous incongruence (with only one of the

alternative positions with more than 80% jackknife support). A further 35 showed weak incongruence (between 50–80% support in either topological position), making a total of 90 out of the 146 *Cliffortia* accessions (61.6%) that show incongruence between the nuclear and chloroplast genomes.

Table 2.9. List of putative hybrids based upon the incongruence support values of the reticulation. Only those with strong (>80%) support are listed, including ambiguously incongruent ones.

Taxon	Nuclear	Chloroplast
acanthophylla	92	-
acutifolia	92	98
alata	93	87
anthospermoides	99	87
apiculata	93	87
arcuata	95	-
atrata	93	-
berberidifolia	92	98
burgersii	93	87
castanea2	99	98
ceresana	92	-
cervicornu	87	-
crassinervis	98	-
cruciata	100	100
cymbifolia	95	98
densa	93	87
dispar	100	-
dodecandra1	81	91
dodecandra2	81	91
exilifolia	93	87
ferruginea	92	98
hirsuta	93	-
hirta	99	87
incana	99	87
integerrima	92	98
intermedia	92	98
lepida	93	87
meyeriana	92	100
monophylla	99	100
montana1	-	91
multiformis	81	91
neglecta	81	92
obovata	-	99
pedunculata	93	87
phillipsii	-	89
nitidula subsp. pilosa	95	100
pterocarpa	81	91
pubescens	81	91
pulchella	-	98
purpurea	94	-
recurvata	92	98
ruscifolia	81	-
serpyllifolia	93	-
sp. cf. glauca1	90	99
sp. cf. juniperina	100	98
sp. cf. pterocarpa	81	91
sp. cf. verrucosa	81	-
spathulata	100	-
teretifolia1	-	98
tuberculata	93	-
uncinata	92	98
verrucosa	81	-
versiformis	-	98
virgata	92	-
viridis	96	91

Additivity of sequence data:

A list of putative recent hybrids and whether there was evidence of additivity and between which putative parents is given in Table 2.10. Only eight of the 27 putative hybrids for which extant parents could be postulated showed signs of additivity in the nuclear sequences. This suggests that concerted evolution has been relatively complete in many of the hybrids. Hybrids that still show signs of additivity are possibly more recent ones, where concerted evolution has not had time to complete, and all are sympatric with at least one parent.

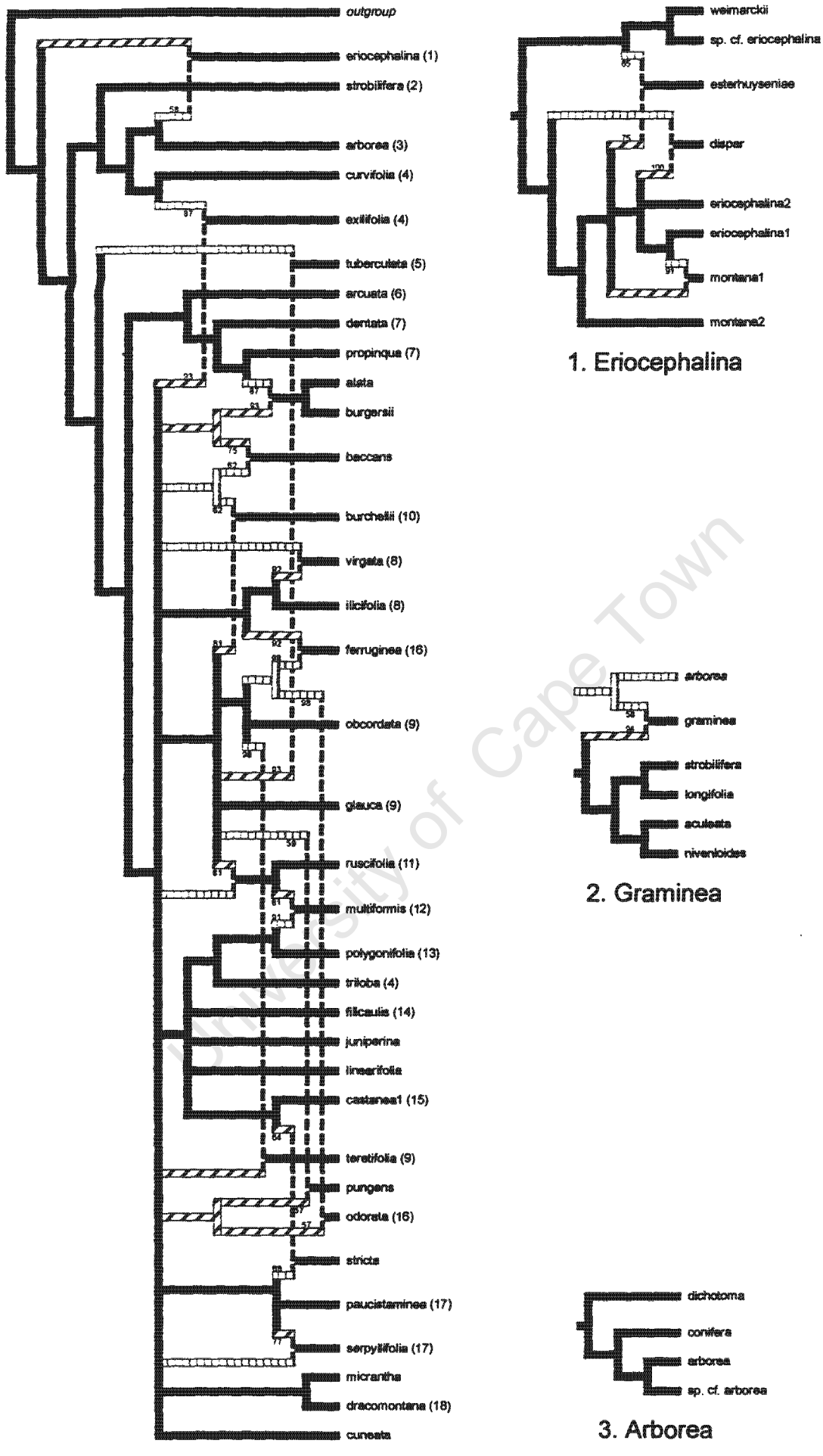
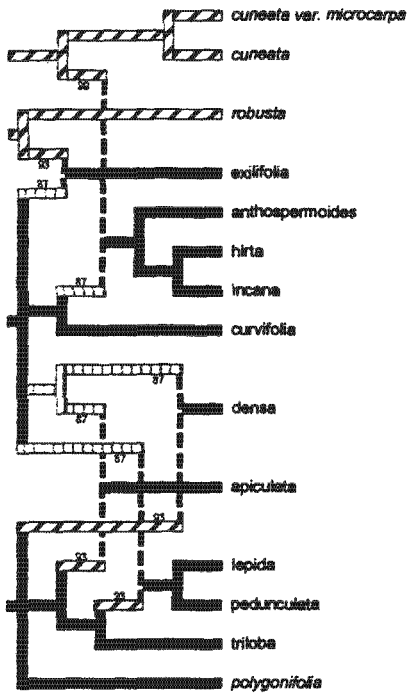
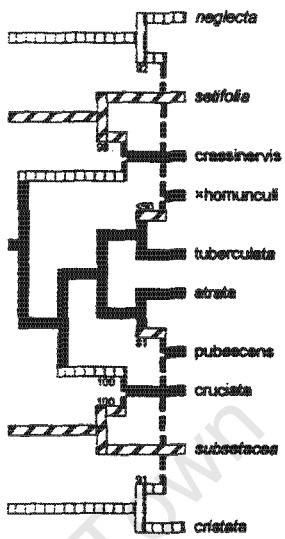


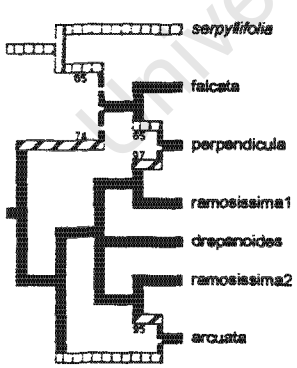
Fig. 2.13. Framework reticulate tree and associated subtrees. Diagonal hatching indicates that information for a branch comes from nuclear genome alone, vertical hatching from chloroplast. Dashed lines indicate putative hybrid reticulations. Numbers after taxa in framework tree give the subtree that the taxon can be found in. Numbers associated with branches indicate the incongruence support values for the reticulations.



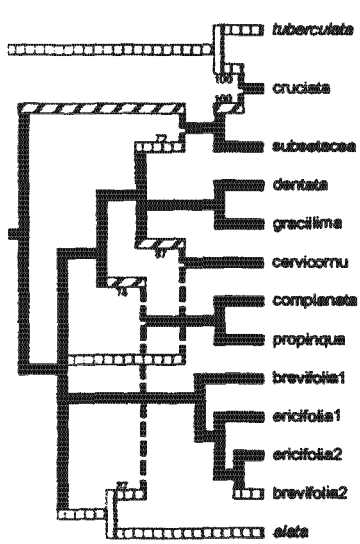
4. Curvifolia



5. Tuberculata

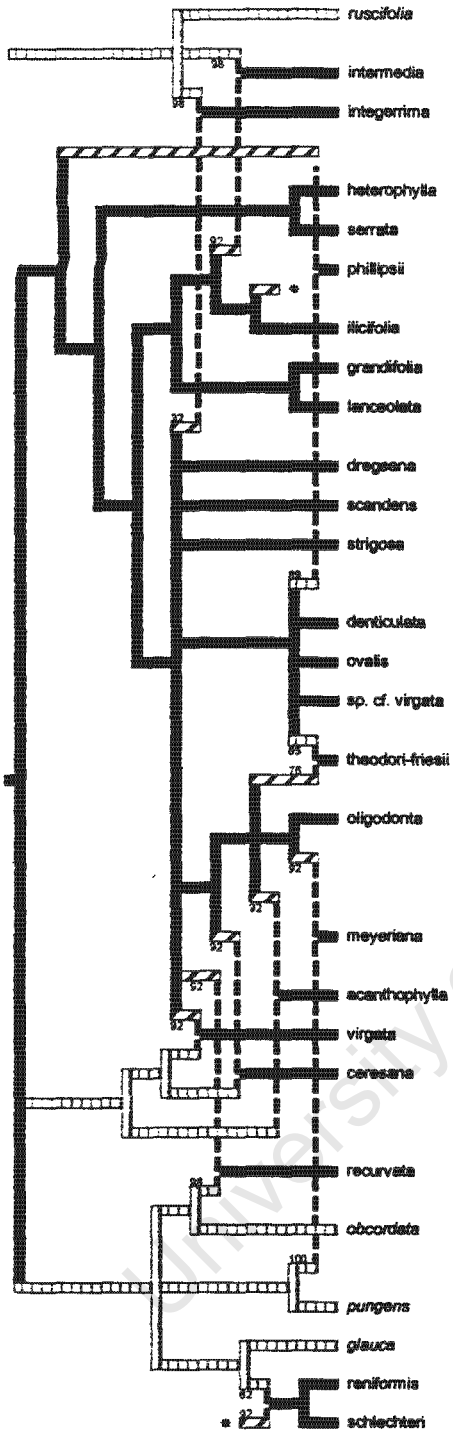


6. Falcata

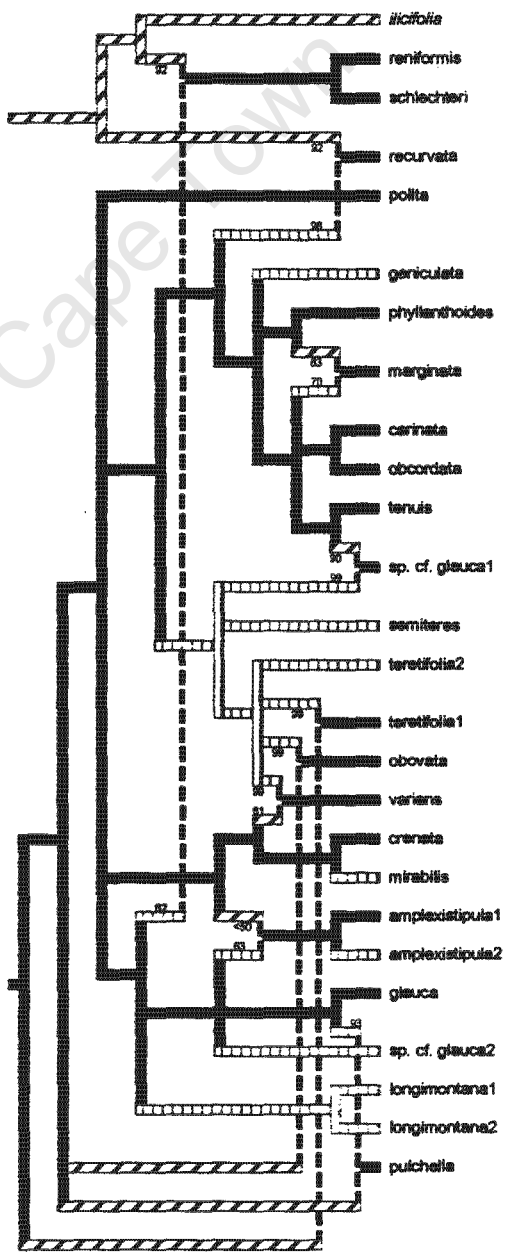


7. Dentata

Fig. 2.13 cont.

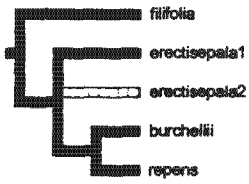


8. Ilicifolia

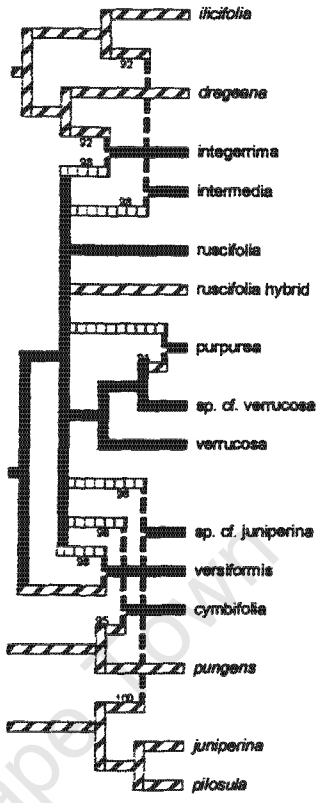


9. Glauca

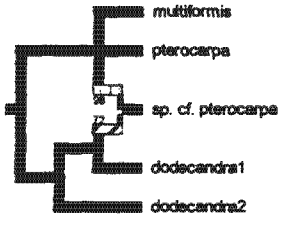
Fig. 2.13 cont.



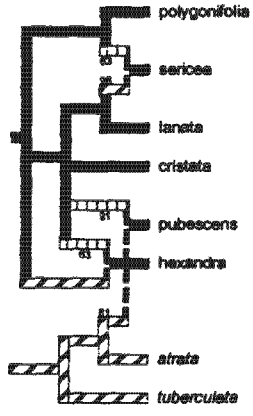
10. Burchellii



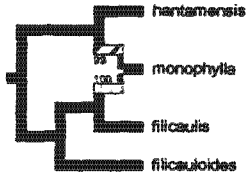
11. Ruscifolia



12. Multiformis

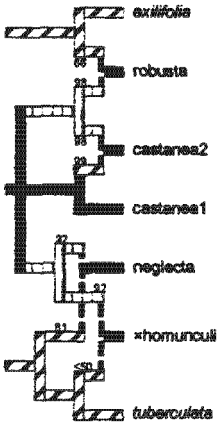


13. Polygonifolia

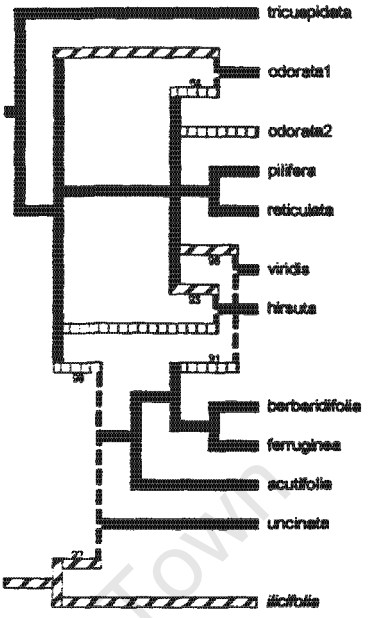


14. Filicaulis

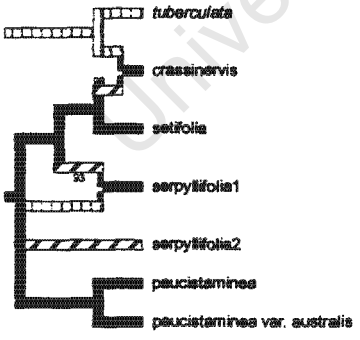
Fig. 2.13 cont.



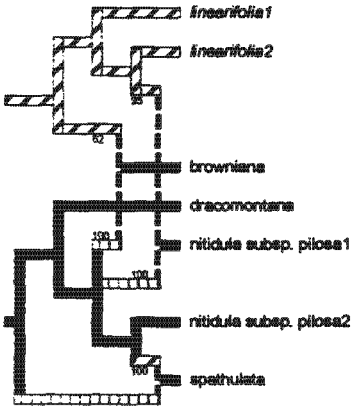
15. castanea1



16. Odorata



17. Paucistaminea



18. Dracomontana

Fig. 2.13 cont.

Table 2.10. List of putative hybrids and their suspected parents. Where true identity of the parent is uncertain, but belongs to the same lineage as the suspected parent, a question mark is placed before the taxon. Sympatry implies that the suspected parent has been found in at least one of the same grid squares as the putative hybrid.

Taxon	male parent	sympatric	female parent	sympatric	evidence of additivity
arcuata	ramosissima	✓	falcata	×	✓
browniana	?linearifolia	✓	nitidula	✓	✓
ceresana	virgata	✓	acanthophylla	×	✓
crassinervis	setifolia	✓	tuberculata	✓	×
cruciata	?subsetacea	×	atrata	✓	×
cymbifolia	pungens	×	ruscifolia	✓	×
×homunculi	tuberculata	✓	neglecta	✓	×
integerrima	?dregeana	✓	ruscifolia	✓	✓
intermedia	ilicifolia	✓	ruscifolia	✓	×
marginata	?phyllanthoides	×	tenuis	✓	×
meyeriana	?theodori-friesii	✓	pungens	✓	×
monophylla	hantamensis	×	filicaulis	✓	×
nitidula subsp. pilosa	linearifolia	✓	nitidula	✓	✓
perpendicula	ramosissima	✓	falcata	✓	✓
pubescens	atrata	✓	sericea	✓	✓
reniformis	ilicifolia	✓	?glauca	✓	×
reticulata	odorata	✓	pilifera	×	×
schlechteri	ilicifolia	×	?glauca	×	×
sericea	lanata	✓	polygonifolia	✓	×
sp. cf. eriocephalina	eriocephalina	×	weimarckii	✓	×
sp. cf. glauca1	tenuis	✓	obovata	✓	×
sp. cf. juniperina	juniperina	✓	ruscifolia	✓	×
sp. cf. verrucosa	purpurea	×	verrucosa	✓	×
triloba	pedunculata	×	?polygonifolia	✓	×
varians	crenata	✓	obovata	✓	✓
versiformis	obovata	✓	ruscifolia	✓	×
viridis	hirsuta	✓	ferruginea	✓	×

Discussion

Taxon sampling:

The sampling of taxa within *Cliffortia* has been thorough, with 91.5% of the currently accepted species being sequenced for at least one gene. Those that are missing are generally morphologically similar and found within the distribution of a commoner widespread species and their approximate taxonomic placement within *Cliffortia* is easily determined from morphology alone. The only exception to this is the undescribed *C. bolusii* Diels MS (=*C. sp.* 1 of Weimarck), which although poorly known, as neither flowers nor fruit have been recorded, is morphologically and geographically isolated (Weimarck, 1934).

The thoroughness of the sampling of *Cliffortia* can be demonstrated by pruning the trees created by using all the taxa to just the 100 taxa that had had all the four genes sequenced. By doing this to both the nuclear and combined chloroplast separately it

could be shown that there were still some trees remaining that were optimally short. This implies that those taxa that were pruned do not affect the overall topology of the trees. From this it can be postulated that adding any of the missing taxa, which were usually morphological variants of species already included, would also be unlikely to have affected the topology much. It can be noted that this was not so with the 'total evidence' trees, which failed to leave a single tree that was optimally short upon reduction, implying that the topology with the total evidence is more unstable, and influenced by the taxa included.

The comparative completeness of taxon sampling means that although long-branch attraction may well be the cause of misplacement of taxa within the trees, and possible incongruence, further addition of taxa to break up the long-branches is not possible. On the other hand, several analyses demonstrate that adding taxa can also have detrimental effects such as reducing support values (Sanderson & Wojciechowski, 2000), increasing computational times without increasing, and sometimes even reducing, accuracy (Kim, 1996; Poe & Swofford, 1999), and reducing congruence by adding missing data (Johnson, 2001). Clearly in every study there is a trade-off at some point between adding further taxa and adding more characters (Graybeal, 1998). In this study, because additional taxa are unlikely to affect the overall topology, further research should be based upon adding additional characters from new gene regions, especially nuclear, along with filling in the missing data (Wiens, 1998).

Usefulness of gene regions:

Of the regions screened the 5S NTS and *psb*A regions stood out as the most informative, providing resolution at all levels of the tree. The 5S NTS region was easy to align, despite the presence of some large indels, and the only problem is the possible presence of paralogous copies (see below). The *psb*A region, however, was difficult to align and some of the alignment was ambiguous. Thorough sampling of the taxa was needed to ensure that the alignment of the difficult portions of the gene was homologous. Although several methods have been suggested for dealing with alignment of these areas (Lee, 2001), they all increase computation times, which were already lengthy. Instead it was deemed better to omit them, as they were generally in highly homoplasious regions and hence would not provide support to deeper nodes, or to score them as simple multistate characters. The addition of more conserved chloroplast genes, such as *trn*L and *trn*K, was also vital to override the homoplasious nature of those regions and others. Indeed the large inversion that frequently occurred was shown to be

highly homoplasious, only two other single chloroplast characters when mapped on to the combined chloroplast trees required as many steps. This finding matches that of Sang et al.'s (1997) about the homoplasious nature of the inversions in this spacer region.

The ease of amplification of the *psb*A-*trn*H intergenic spacer was particularly useful, as sequences were obtained even for DNA extractions that had proved difficult for the other gene regions (e.g. *C. marginata* and *C. mirabilis* that were extracted from herbarium material). This is probably due to its short length, meaning that the entire length was often preserved even in degraded DNA, and high copy number. However, for two species, *C. lepida* and *C. nivenioides*, repeated attempts at different DNA dilutions failed to form a product. There were three possible reasons for this: the region was absent in these two species; the region contained a large insertion that meant that extension time was not long enough to complete the sequence at each cycle; or a mutation had occurred in one or other of the primer sites meaning that they could not bind properly.

The *trn*L and *trn*K datasets were easily and unambiguously alignable, except for a few poly-AT regions, and showed similar degrees of variation and resolution. They were vital for providing strong support for phylogenetic relationships towards the tips of the tree, thereby overriding any homoplasy in the *psb*A dataset. Often only one or two characters would support a node in either dataset but when combined they produced quite strong support. The *trn*K dataset was particularly useful at resolving nodes deeper within the chloroplast tree as it had three separate more or less unconflicted characters that supported the 'core' of *Cliffortia*.

Incongruence of datasets:

The most striking result from the above analyses of the three separate datasets is the high degree of incongruence between chloroplast and nuclear partitions. Over 38% of accessions show some sort of strong conflict, and as both incomplete heuristic searching (Mort *et al.*, 2000) and increased number of included taxa (Sanderson & Wojciechowski, 2000) reduce support values, the levels of incongruence shown will be lower than would be expected with fewer taxa and more thorough searching. Although the morphology dataset is also incongruent to the separate molecular datasets, the levels of homoplasy are so much higher in this dataset that the conflict is not as strong. The reasons for the incongruence need to be investigated to determine if it is a reflection of true organismal history or a result of erroneous laboratory or analysis methods. Several

putative reasons for the incongruence are suggested below, along with the evidence for and against each.

The incongruence is not real but due to too few characters in the individual datasets.

Three different methods were employed in examining the data for levels of incongruence between the three datasets and all showed significantly high levels of incongruence. However, it has been demonstrated that these tests indicate incongruence not only when there are different evolutionary histories, but also when there is a high proportion of homoplasy within the datasets themselves (Yoder *et al.*, 2001). Hence, a major argument for using total evidence is that it increases the number of characters available for phylogenetic reconstruction and thereby reduces the effect of homoplasious characters on the analyses. Here, a total evidence analysis was conducted and good resolution was obtained (Fig. 2.9). However, there was no increase in the number of well-supported nodes obtained. Indeed, it showed a slight decrease in number of supported nodes from the chloroplast dataset, which having the largest number of characters for an individual dataset is also the most robustly supported (Table 2.11.1). This is in marked contrast to the individual chloroplast gene regions, which always showed a significant increase in number of supported nodes when combined with one another (Table 2.11.2). This implies that although using total evidence more or less doubles the number of characters in the analysis, the levels of support on each node do not increase as would be expected with extra characters.

Table 2.11.1. Comparison of number of nodes with various levels of support when individual datasets are kept separate or combined. Based on fast jackknife search (nreps = 10000, 36.79% deletion, search = faststep) using only taxa with sequences for all gene regions (n = 100).

	No. of supported nodes		
Dataset	>50%	>75%	>95%
nuclear	43	27	11
chloroplast	51	35	19
morphology	13	3	0
nuclear + chloroplast	50	30	18
nuclear + morphology	40	27	11
chloroplast + morphology	45	27	16
total evidence	45	31	20

Table 2.11.2. Comparison of combination of chloroplast regions. Conditions same as for Table 2.11.1.

	No. of supported nodes		
Dataset	>50%	>75%	>95%
trnL	29	15	7
trnK	30	15	7
psbA	22	13	4
trnL + trnK	39	27	19
trnL + psbA	41	26	13
trnK + psbA	38	24	14
all chloroplast	51	35	19

The incongruence is because of incorrect phylogenetic reconstruction.

While this is possible, if it is true then the basis of cladistic analysis must be open to serious question. Although exhaustive searching for the shortest trees was not possible due to the large size of the dataset, the heuristic methods used were as comprehensive as they could be within the time limits imposed. The initial searches using a ratchet analysis ensures that a wide sample of tree space is found for input to any further searches. As these searches consistently found numerous trees of the same length, and no further trees were ever found upon subsequent swapping, the possibility that shorter trees exist appears to be remote. Additional characters will always help to resolve a phylogeny better, but from the evidence that has been gathered in this study one has to assent that the analysis of the data has been as thorough as it could be.

However, a well-known fault with the theory of parsimony analysis is that long-branches in the dataset can produce incorrect estimates of the phylogeny (Felsenstein, 1978). The recommended way to alleviate this problem is to add taxa to 'break up the branches'. As has already been shown, taxon sampling has been high for the study and that the missing taxa were unlikely to change the topology much. While, some of the incongruence can probably be ascribed to 'long-branch attraction' (e.g. series Subsetacea, see results for 5S NTS), to attribute all of the incongruence to this phenomenon would relegate parsimony analysis to a rather poor means of phylogenetic reconstruction.

The incongruence is because one of the datasets does not belong to Cliffortia.

For each gene region, a few of the sequences were submitted for BLAST searches (Altschul *et al.*, 1997). All revealed the sequences to belong to Angiosperms and hence the possibility of the phylogeny tracking an algal or fungal symbiont was unlikely.

The incongruence was because of incorrect alignment of the sequences.

In highly variable parts of the genome it is possible that there is more than one possible alignment. All alignments were done by eye and so there is the chance that the 'correct' alignment was not achieved. However, for the gene regions used in this study of *Cliffortia*, the problem was more in finding variation between the sequences than in the difficulty in aligning them to each other.

For the chloroplast dataset, both the *trn*L and *trn*K sequences were easily and unambiguously alignable. Only in a few poly-AT regions was there any ambiguity in the sequences and then these were very conservatively aligned so that the minimum number of possible informative characters would be included. However, the *psb*A was difficult to align in parts and some of the alignment was ambiguous. Hence, areas that were too difficult to align were either conservatively scored or omitted and because the *psb*A was used in combination with the *trn*L and *trn*K datasets, the effect of any homoplasy caused by an incorrect alignment should have been overridden.

The nuclear dataset however only included a single region, the 5S NTS. Despite its high degree of variability, it was easy to align. Although several indels were present, including some large ones, the regions on either side were generally conserved and so reliable placement of indels was easy and they were rarely overlapping. There were also very few repeat regions within the sequences and there was no evidence for any inversions. Generally, therefore, the 5S NTS sequences were also easy to align reliably.

The incongruence is because of lineage sorting in the genome.

The possibility of lineage sorting in the chloroplast and nuclear genomes has been postulated for some of the high degrees of incongruence seen in Mediterranean *Senecio* species (Comes & Abbott, 2001). If this was to be true for *Cliffortia* too, then it would require that an exceptionally high degree of genetic polymorphism to have been present in the ancestral species. Indeed, the fact that incongruence occurs between highly divergent branches of the tree would imply that the ancestral species had polymorphisms that covered the full range of genetic diversity now found within the genome and this would need to be followed by random sorting through more than one speciation event in a highly predictable manner! Therefore, as Fuertes Aguilar et al. (1999) suggest in a similar situation with *Armeria*, lineage sorting could theoretically account for the patterns seen in the nuclear tree but it is not very likely.

The incongruence is because of paralogy in the nuclear genome.

This problem requires serious consideration because of the frequent examples that have appeared in the literature recently, where nuclear genes have paralogous copies (e.g. Cronn *et al.*, 1996; Kellogg *et al.*, 1996; Baker *et al.*, 2000a; Mayol & Rossello, 2001). To overcome this, cloning of the PCR products is recommended to identify the different copies involved (although even this does not guarantee that all paralogous copies will be found). This was not carried out here and so the possibility remains that more than one set of divergent genes was amplified for the 5S NTS region, and hence the recorded conflict with the chloroplast. To test this two lines of evidence can be examined. Firstly, the sequences themselves and their reliability. Secondly, whether the 5S NTS phylogeny itself differs strongly from what would be predicted from other evidence.

If multiple and highly divergent copies of the spacer region were present, one would expect the sequences from direct PCR to be unreadable for most of the sequence. In this study this was not the case. A poly-T region near the 5' end sometimes meant that reading the sequence after that point was difficult or impossible, but this could have been due to incorrect reading of the *Taq* polymerase and the addition or deletion of extra Ts rather than multiple copies. As this was not uncommon in the single-copy trnL gene region also, one cannot necessarily attribute this difficulty in reading to multiple copies. Indeed, in all cases where the sequence was unreadable after the poly-T region, it was easily readable from the other direction and as the gene region was short, this was enough to cover the whole sequence.

However, occasionally there was evidence of multiple copies. Sometimes this was evident as the presence of equally strong peaks for a base pair (Fig. 2.3) or rarely by the deletion or insertion of a few base pairs that caused a stutter in the chromatogram. But in all these cases the ambiguities were few, especially in comparison to the variation found within the gene region as a whole. Hence, the presence or absence of these ambiguities would not have changed the position of the taxon within the tree. Indeed these ambiguities were useful additional support for the evidence that some of the incongruence may be due to hybridization (Table 2.10).

Sometimes multiple bands were present on the gel and one of the bands was cut out. When this was necessary, the resulting sequence was still readily alignable to the remainder of the sequences and its subsequent placement in the phylogenetic tree fitted with that predicted from morphology alone. For example, although the PCR product of *C. alata* showed signs of a double band and one was cut out and reamplified, it still

came out as sister to *C. burgersii*, and this positioning is strongly supported in both of the other two datasets.

However, having said all this, three sequences possibly showed some sign that they may actually be paralogous copies, namely *C. complanata*, *C. ericifolia*(1) and *C. ericifolia*(2). All three contained a large insertion (205, 245 & 245 bp) with an associated area of deletion, which was missing in their sister species (*C. propinqua* and *C. brevifolia* respectively as confirmed by both morphology and chloroplast data). These large insertions were alignable to the large insertion present in subgenera Arborea and Eriocephalina, but were placed within a different part of the spacer region. However, even if these were alternative copies to what was usually sequenced, the three sequences still placed the species within the predicted part of the tree close to their sister species. This fits with what has been found by other studies using 5S NTS, that even when multiple divergent copies of the spacer have been found within the same array, they still usually form monophyletic groups for the species (e.g. Udovicic *et al.*, 1995; Cronn *et al.*, 1996; Kellogg *et al.*, 1996; Baker *et al.*, 2000a). This is in contrast to when different arrays are present, for in those cases there has been little concerted evolution between the arrays and intraspecific variation between arrays is greater than interspecific variation within an array to such an extent that they are usually found to be of markedly different size (Kellogg *et al.*, 1996) or in separate branches of the tree (Cronn *et al.*, 1996). However, this appeared not to be the case in *Cliffortia*, as there was no evidence of such high divergence when there were signs of more than one copy being present. Hence, although paralogous copies may be present, they are contained within a single array and concerted evolution between them has been relatively complete within *Cliffortia*.

The other line of reasoning is to compare the phylogeny of the 5S NTS with what would have been expected from other evidence. If paralogy is a problem then the nuclear dataset will conflict strongly with the opposing datasets. The chloroplast has already been shown to conflict strongly, but what about the morphology dataset?

Results from the pair-wise ILD tests gave highly significant *P* values for all three datasets, thus suggesting strong incongruence all round. However, this test is profoundly insensitive (Yoder *et al.*, 2001) and unable to differentiate any variation in the degree of incongruence between the various combinations of datasets. Meerow & Snijman (2001) in a study of Amaryllidaceae based upon ITS sequences and morphology found substantial incongruence using the ILD test. However, they

attributed the incongruence to weak resolution in the morphology dataset and so pressed ahead with combining the morphology and molecular matrices. Yoder et al. (2001) actually showed an increase in bootstrap support and accuracy when congruence according to an ILD test decreased. They also point out that this insensitivity of the ILD test is in a large part due to high degrees of homoplasy present in one or other of the datasets. Dowton & Austin (2002) also demonstrate that it is highly affected by widely different sizes of datasets. Hence, while the significant incongruence reported by the ILD test might be a true reflection of what is happening in the chloroplast and nuclear datasets (which both have relatively high CIs), but between the nuclear and morphology datasets it might be a result of the high degree of homoplasy present in the latter coupled with the markedly smaller size.

Therefore, the Kishino-Hasegawa Tests were used to show variation in the degree of incongruence between the different datasets. The nuclear and chloroplast partitions were once again highly incongruent, but the morphology was less so. Indeed, when the nuclear dataset was constrained by the morphology dataset, it did not cause a significant increase in tree length for any level of jackknife constraint tree. This lack of incongruence can be put down partly to the low degree of support caused by the high amounts of homoplasy in the morphology dataset, and hence the fewer number of constraining nodes (only 13 in 50% jackknife tree). However, these nodes still had enough conflict to cause significant incongruence against the chloroplast dataset. In the reverse direction the pattern was the same. At all the levels of nuclear constraint trees, the morphology dataset had a smaller increase in tree length than the corresponding chloroplast one (Fig. 2.11). However, only at the 95% level was there a difference in the level of significance given by the Kishino-Hasegawa test. It is possible that this is due to the number of nodes constrained in the corresponding datasets. Tree length will always increase as the number of constrained nodes present increases (Mason-Gamer & Kellogg, 1996). The chloroplast constraint trees consistently had more nodes constrained for the same level of the nuclear constraint trees. However, comparison of the number of nodes constrained against increased tree length still shows the nuclear dataset as causing less distortion of the morphology dataset than did the chloroplast dataset (Fig. 2.12).

Hence, the nuclear dataset appears to track the morphology of the genus closer than the chloroplast dataset. If paralogy was a problem one would expect highly divergent nuclear genomes, which were more or less randomly distributed over the morphology.

However, the chloroplast genome has been demonstrated to have greater conflict with morphology than the nuclear. This matches findings by Ferguson & Jansen (2002) in *Phlox*, where they attribute the incongruence with morphology to introgression of the chloroplast genome and regard the nuclear-based tree as the "correct" reflection of the species phylogeny. Similarly, King & Ferris (2000) show that the chloroplast data for two *Alnus* species tracks geographic distributions while nuclear loci reflects the taxonomic. Comes and Abbott (1999) also regard the nuclear data as providing more accurate information on species relationships in *Senecio*, but warn that confusion can still occur due to partial nuclear capture and homogenization of sequences.

The incongruence is because of reticulation between the species.

As demonstrated above, the chloroplast dataset is relatively robust, having been derived from three separate single copy regions. Many of the nodes that cause the conflict with the nuclear phylogeny are supported by more than one of the individual genes that make up the chloroplast dataset. Although error in phylogenetic reconstruction is still possible, especially due to long-branch attraction, arguments against the chloroplast dataset are few when support for a particular branch is strong.

Incongruence in the nuclear dataset, on the other hand could be the result of paralogy, recombination or lineage sorting, as well as erroneous phylogenetic reconstruction and these could all be given as reasons for its conflict with the chloroplast phylogeny. However, the phylogeny from the nuclear dataset corresponds more closely to the morphology phylogeny than the chloroplast one and thereby any argument to consider discarding the 5S NTS region on the basis of paralogy is weaker than if it had conflicted strongly with the morphology as well as the chloroplast.

Therefore, if the two separate phylogenies from the nuclear and chloroplast datasets are both to be regarded as reliable, the only explanation left is that the incongruence seen is an accurate reflection of phylogenetic history. If this is the case then the most plausible cause for the differing phylogenetic histories is that it is a result of past hybridization events. This should not be regarded as surprising, as firstly Rieseberg et al. (1996) state that it is the "most common source of phylogenetic incongruence in plants" and secondly because of the very high incidence of hybridization within the family Rosaceae that has been inferred or demonstrated (e.g. Campbell *et al.*, 1997; Alice *et al.*, 2001; Smedmark & Eriksson, 2002).

3. Classification

Introduction:

Weimarck subdivided *Cliffortia* into two subgenera and 11 sections based solely upon morphology (Weimarck, 1934; Weimarck, 1948). His intention was to create a classification delimiting 'natural groups'. Although he was not explicit about what he regarded as 'natural', when discussing phytogeographical aspects of the genus he did allude to the importance of associating species with their systematic positions by considering the sections in which they belonged (Weimarck, 1934). Hence, he intended his sections of *Cliffortia* to be phylogenetically based and used discrete morphological characters that he regarded as having evolutionary significance. In particular, the characters pertaining to the number of carpels, presence of a petiole and number of leaflets, were chosen because they showed ancestral and derived states within *Cliffortia* relative to the sister taxa of the *Sanguisorbeae apetalae* (Weimarck, 1934). His classification resulted in easily identifiable groups with only occasional overlap in diagnostic characters (e.g. *C. filifolia*, despite having a short petiole, was not placed in sect. Petiolatae). This study provides additional characters, from both morphology and molecular data, for reassessing his classification. Furthermore, the cladistic analyses conducted in Chapter 2 provide a basis upon which to postulate a classification that reflects a more accurate phylogenetic history of the genus.

Creating a workable classification for a genus can be split into two steps: firstly, to recognise the species (or whatever are to be used as terminal taxa, e.g. Mishler, 1999) and to define their limits; and secondly, to erect the supra-specific structure for the genus.

Choosing a species concept:

A consistent delimitation of species boundaries in a complex genus like *Cliffortia*, requires an explicit species concept. The desirable qualities of a species concept are that it is theoretically significant, easily applied (operational) and encompasses natural biodiversity (Mayden, 1997; Hull, 1997). This aim has resulted in numerous species concepts being proposed over the last 50 years (summarised in Mayden, 1997). However, as yet, there is still no agreement upon a single species concept that can be universally applied (Mayden, 1997; Hull, 1997). This is because attempts to improve

one quality, e.g. more operational, always result in the lessening of another, e.g. theoretically less significant (Hull, 1997).

Hull (1997) notes that good test cases for the applicability of a species concept include how they deal with asexual species and species of hybrid origin. As these two modes of speciation are both suspected to be present within *Cliffortia*, only a species concept that is general enough to account for the presence of these modes will encompass the diversity found. In addition, certain characteristics, such as specific mate recognition, are not yet known for *Cliffortia*. Therefore, a species concepts that relies upon such biological information will not be operational here, e.g. Reproductive Competition Concept (Ghiselin, 1974) and Recognition Species Concept (Paterson, 1993).

Mayden recognised primary and secondary species concepts, the former being the theoretical goals that one is attempting to achieve, the latter the operational means by which one gets there. Of all the species concepts he only admitted the Evolutionary Species Concept of Wiley (1978) as a primary one, but pointed out that it was not operational. Hence, for *Cliffortia*, one or more of his secondary concepts, which are operational and will account for the observed diversity in a biologically meaningful way, are needed to identify species in practice.

Of the many secondary concepts cited by Mayden, several can be excluded, as they will fail to account for the processes that have created the diversity within *Cliffortia*. The Biological Species Concept (Mayr, 1942; Mayr, 1992) and Genealogical Species Concept (Baum & Shaw, 1995) among others can be excluded because they insist upon reproductive isolation. Hybrids are the result of breakdown in reproductive isolation, and therefore if the species involved are to be maintained the hybrids by necessity must be sterile or at least have reduced fitness compared to the parents so that they will not persist (Mayr, 1963). However hybrids are not always less fit (Arnold & Hodges, 1995; Rieseberg *et al.*, 1999; Arnold *et al.*, 2001) and several species within *Cliffortia* are presumed to be of hybrid origin (Chapter 2). Therefore, reproductive isolation cannot be used as a defining character for species delimitation. Furthermore, the criterion of reproductive isolation implicitly means that it can only be applied to sexually reproducing organisms. There are no criteria for recognising asexual lineages (Mayr, 1992), again suspected to be present within *Cliffortia*.

Similar reasons can be given for excluding any concept that insists upon monophyly to recognise species, e.g. Cladistic Species Concept (Ridley, 1989) or some versions of the Phylogenetic Species Concept (= Autapomorphic Species Concept) (de Queiroz &

Donoghue, 1988; de Queiroz & Donoghue, 1990). Hybridization between non-sister taxa creates para- or polyphyletic clades (Hedberg, 1995; Sosef, 1997). Therefore, hybrid species cannot be recognised if the parents are still extant. Furthermore, paraphyly is a common occurrence among plant species (Rieseberg & Brouillet, 1994; Crisp & Chandler, 1996). Sympatric speciation can easily occur in plants due to polyploidy and asexual reproduction, both suspected in *Cliffortia*, leaving the parental population still able to breed with other populations but not its progeny. To counter this the terms 'metaspecies' (de Queiroz & Donoghue, 1988) or 'ferespecies' (Graybeal, 1995) have been employed by some advocates of the monophyletic PSC for those species that were demonstrably paraphyletic ancestral species. However, this method of separation is not universally agreed upon and is deemed an unhelpful distinction to most taxonomists (Wheeler & Nixon, 1990; Crisp & Chandler, 1996). If monophyly was used to define species in *Cliffortia*, the result would be excessive reduction in the number of species to accommodate the reticulations within a monophyletic clade. While this has been done for some groups of plants such as agamospermous grasses (Kellogg, 1990), even there it was pointed out that such a step calls into question the purpose of a classification. For *Cliffortia*, such reduction of species would not adequately reflect the morphological and biological diversity found within the genus.

Therefore, a species concept is required that is broad enough to capture the morphological diversity within *Cliffortia* and the biological processes that created it, while remaining operational. There are two possible concepts, which are widely employed, that are both operational, i.e. do not require any information that is unavailable at present, and will be able to adequately reflect the diversity within the genus: Morphological Species Concept (MSC, du Rietz, 1930; Davis & Heywood, 1963; Blackwelder, 1967; Cronquist, 1978) and the diagnosable versions of the Phylogenetic Species Concept (PSC, Eldredge & Cracraft, 1980; Nelson & Platnick, 1981; Cracraft, 1983; Nixon & Wheeler, 1990).

The MSC identifies species primarily on morphological differences between entities as these are the easiest to use and most accessible to everyone. However, in principle non-morphological characters, such as chemical, ecological, geographical and molecular data, could also be incorporated to assist in the delineation of species (in which case it is frequently referred to as the Taxonomic Species Concept, Davis & Heywood, 1963; Blackwelder, 1967; Mayden, 1997; Gornall, 1997). Therefore, the MSC is highly operational. It is able to cope with both hybrid species and morphologically distinct

asexual lineages and is widely employed by taxonomists, especially those working on museum collections. However, it will fail to detect cryptic species unless non-morphological characters are also included.

The problem with the MSC is that it has no basis in biological principles. It defines species as classes, circumscribing them on the basis of particular morphological attributes. Two problems are inherent with this stance. Firstly, an arbitrary level of morphological divergence is employed to distinguish between species. Usually, it is taken to be at least two correlated characters (Gornall, 1997), but there is no fundamental biological reason why this should be so and a single evolutionarily significant character, such as flowering time, might be more 'important' than five minor phenotypically plastic ones. Secondly, there is no consideration of species as reproducing lineages through time. Theoretically the MSC could recognise the male and female of a species as two distinct species, or the larval form as distinct from the adult (Nelson & Platnick, 1981). Therefore, some element of descent or recognition of the life-cycle is usually assumed by the taxonomists in species definition (du Rietz, 1930). However, once this is incorporated then the MSC could be regarded as a version of the diagnosable PSC (Mayden, 1997).

The diagnosable PSC identifies "the smallest diagnosable cluster of organisms within which there is a parental pattern of ancestry and descent" (Cracraft, 1983). It therefore follows that a species that can be diagnosed using the MSC should also be diagnosable with the PSC, providing that direct ancestry can be proved. It is preferable to the monophyletic versions of the PSC for two reasons: (1) definition of species can be done prior to phylogenetic analysis (Nixon & Wheeler, 1990); (2) paraphyletic species can be diagnosed (Crisp & Chandler, 1996; Mayden, 1997). As with the MSC, problems arise in defining what constitutes a "diagnosable cluster" (Nixon & Wheeler, 1990; Avise & Wollenberg, 1997). Nixon & Wheeler (1990) resolve this problem by saying that they must have "a unique combination of character states in comparable individuals".

While the diagnosable PSC can deal easily with asexually reproducing lineages, the presence of reticulations can cause some problems (Mayden, 1997). The PSC relies upon recognising the boundary between tokogenetic and phylogenetic relationships, and this is not explicitly defined (Avise & Wollenberg, 1997; Mayden, 1997) nor is there necessarily a distinct point where this happens (Mishler, 1999). This is a problem as the difference between tokogenetic systems and those that create hybrid species is not clear. Therefore, although the PSC can be employed in principle for defining species of

Cliffortia, an arbitrary decision must be taken for distinguishing between interbreeding populations and hybridizing species.

Defining the supra-specific taxa:

The second stage of creating a classification is one that is causing considerable debate within the literature at present. There are two opposing stances: those who want to maintain the current 'Linnaean classification' system under the International Code of Botanical Nomenclature (Greuter *et al.*, 2000) with its hierarchical ranks base on types; and those who opt for the rankless but still hierarchical PhyloCode based upon defining clades (Cantino & de Queiroz, 2000). Lidén et al. (1997b) lists five criteria that they see as essential in a classification system: stability, high hierarchical information content, ease of use, independence from theory, high character information content. They then go on to demonstrate that Linnaean classification fits these criteria best. On the other hand, Schander (1998b) argues, with only slight variation in the emphasis of the criteria, that the PhyloCode is superior in fulfilling these criteria.

Many of the arguments concerning changing to a phylogenetic nomenclature are based around the perceived instability of the Linnaean system. The wealth of newly revised classifications based upon molecular phylogenies, as well as the arbitrary assignment of ranks to clades, has indeed created many name changes. However, both classification systems have been shown to be more stable under certain conditions compared to the opposing system (Lidén *et al.*, 1997b; Moore, 1998; Schander, 1998b; Sereno, 1999; Nixon & Carpenter, 2000; Forey, 2002). Therefore the higher visible instability in the Linnaean classification may be more a result of its longer history and that phylogenetic nomenclature is only applied once a phylogeny has been produced. The stability of the PhyloCode to changing opinions and new information will only be tested with time. The Linnaean classification should also prove to be stable once it has been applied to a fixed phylogeny (Schander, 1998b).

The other major criticism of the current nomenclatural system is the use of ranks, which have no meaning but are all too frequently used as comparable taxonomic units (e.g. Williams *et al.*, 1994; Gaston *et al.*, 1995; Mishler, 1999; La Ferla *et al.*, 2002; Berry, 2002). However, this is a mistaken approach as usually the designation of ranks was never intended as an indication of equal status but as a means of subdividing large groups into more conveniently sized ones (Stevens, 1997; Stevens, 2002). Therefore ranks should be taken as no more comparable than two taxa named with the phylogenetic nomenclatural system. However, the instability of names caused by

shifting of taxa from one rank to another is a legitimate criticism of Linnaean classification.

Of particular concern in creating a classification for *Cliffortia* is the frequent hybridization that has occurred. This has not just occurred between sister taxa, but across lineages and at deeper levels within the tree so that whole lineages have an *apparently* hybrid origin (Chapter 2). The problems resulting from hybridization for classification and nomenclature have not yet been properly addressed in the arguments concerning the two codes. This may be due to the rarity of hybridization in larger animals, as well as the difficulty of proof of its existence in deeper lineages (at which level most of the discussion has been debated).

Reticulations and phylogenetic nomenclature:

The difficulty caused by reticulations for phylogenetic nomenclature is that its purpose is to name clades (which are implicitly monophyletic), i.e. it is based directly upon cladistic analysis (Cantino, 2000). Reticulations are violations of the basic principles of clade formation (Legendre, 2000). While reticulations within a taxon are not a problem for the PhyloCode, and the hybrid can be indicated by the appropriate convention, reticulations between taxa are more troublesome. There are three options available to deal with reticulations between taxa: (1) to drop the hybrid taxon to the same position in the hierarchy as the parental taxa; (2) to include the hybrid taxon in both parental taxa; (3) to subsume the two taxa into a single monophyletic taxon. While the first option retains a single unambiguous position for the taxon, the hierarchical information is lost. Furthermore, the two parental taxa become paraphyletic as they do not include all the descendant taxa. The third option appears the most logical and retains the insistence upon monophyly for naming taxa (Schander, 1998a). It is also the solution implied, although not explicitly stated, by the PhyloCode (Cantino & de Queiroz, 2000). However, in the case of *Cliffortia* there may be as few as four subordinate monophyletic taxa that could then be named, one of which would encompass over 90% of the species and most of the diversity present. Therefore the second option, as advocated by Nelson (1973) and Funk (1985), appears the most appropriate solution. Although it creates taxa with two possible positions within the hierarchy, it does ensure that the criterion of monophyly is upheld and that the range of diversity can be classified. Disadvantages with this approach include that it quickly becomes cumbersome, especially with multiple reticulations, and citation will no longer require just a species name and a single "clade address" (sensu Cantino *et al.*, 1999). It could also result in an

overestimation of the number of taxa if care is not taken in considering all lineages together (Wiley, 1981).

However, while reticulations do not cause a problem for classifying taxa using phylogenetic nomenclature, the naming of these taxa is complicated. There are three basic methods for naming clades: node-based, stem-based or apomorphy-based. One argument against naming clades is that it is potentially far more unstable than the current system (Moore, 1998; Nixon & Carpenter, 2000; Forey, 2002). In particular, this is relevant when hybrids are present. For if a clade is circumscribed using only the minimal number of taxa, and one of the taxa used for the circumscription is of hybrid origin, then the addition of further data could cause radical changes in the taxa included within that circumscription. Choice of appropriate anchors for each clade circumscription is therefore vital (Sereno, 1999).

Reticulations and Linnaean classification:

Within the 'Linnaean classification' system the problem caused by reticulations is more controversial because of attempts to apply the principle of only classifying monophyletic clades (e.g. Freudenstein, 1998). For classifying taxa which contain reticulations many of the same arguments can be used as for phylogenetic nomenclature: reticulations within taxa do not cause a problem, but reticulations between taxa do. The more rigorous rules of the ICBN do not allow a taxon to belong to more than one higher taxon, so the solution used for phylogenetic nomenclature is not applicable here. If monophyly is to be insisted upon the only option is to include the reticulating taxa within a single encompassing one (Sosef, 1997). However, unlike the PhyloCode, monophyly is not a stipulation for recognition of taxa using Linnaean classification and the recognition of paraphyletic taxa is even advocated by some (Brummitt, 1997; Sosef, 1997; Brummitt & Sosef, 1998; Knox, 1998; Brummitt, 2002).

The creation of paraphyletic taxa provides one of the strongest arguments for using a phylogenetic nomenclature (Brummitt, 1997; Brummitt, 2002). However, the arguments against accepting paraphyletic taxa (van Welzen, 1997; Lidén, 1997; Freudenstein, 1998) are generally based against the problems caused by ancestral taxa (Brummitt, 1997; Sosef, 1997; Brummitt & Sosef, 1998; Brummitt, 2002) and not reticulations (Sosef, 1997). Freudenstein (1998) simply ignores the problem of reticulations. Lidén (1997) briefly touches on the problem of reticulation but only states that cladistics has been used successfully to identify individual incompatibility between different character sets and therefore is useful in identifying reticulation. He fails to explain how the fact

that cladistics can be used for identifying reticulate patterns removes the need for paraphyletic taxa to accommodate these reticulations. Van Welzen (1998) also is brief in his dismissal of hybrids, suggesting that the situation proposed is not real, that the species have been excessively 'split' and thus conforms to the first option that they should all be regarded as a single taxon. He then points out that all classification systems struggle with hybrids unless one "can do something classically artificial" as in wheat.

Two options are available for coping with reticulations between taxa: to drop the hybrid taxon to the same rank as its parents, or to include it within one or other of the parental taxa (but not both). Both these solutions will create paraphyletic taxa, the first one will leave both parental taxa as paraphyletic, as they do not contain all descendant taxa, while the second option only creates a single paraphyletic taxon. It therefore seems preferable to place the hybrid taxon within one or other of the parental taxa.

Methods:

The various phylogenetic trees already produced (see Chapter 2) were used to construct a revised classification. Weimarck's classification was mapped over the 5S NTS selective total evidence tree, to determine if the sections corresponded to the phylogeny. Species described since Weimarck's classification were attributed to his sections based upon his key (Weimarck, 1948).

All available lines of evidence were examined to construct both a phylogenetic classification based upon the PhyloCode and a Linnaean one. Species were evaluated based upon their morphological and genetic divergence. Species were recognised from morphology if they had more than one diagnostic character. Frequently occurring forms with just a single diagnostic character were attributed varietal status, but these are not regarded as distinct taxa for any of the analyses. Within species complexes, the degree of genetic divergence when known, as well as geographical distributions, was also taken into account to delimit species when the morphological variation between populations overlapped.

For the Linnaean classification, it is intended that subgenera and sections will be formally described, although not in the thesis, and therefore the names follow the conventions as set out in the ICBN (Greuter *et al.*, 2000). However, series are regarded as informal names used to circumscribe species groups, whose delimitations are more uncertain, following the example set by Goldblatt & Manning (1998). Therefore the

name of a series is based upon the typical member of the group and will not be described. For the phylogenetic classification there is still much debate as to whether the naming of species should be included in the classification or not (Cantino, 1998; Cantino *et al.*, 1999). Therefore, no convention for naming uninomials is followed and the species are merely named by using the specific epithet unaltered. If the PhyloCode was ever to become accepted and applicable to species then more complicated uninomials would be required.

Newly recognised species were given provisional names but are not formally described in this thesis and are indicated as manuscript names (MS) to prevent accidental publication. To decrease the proliferation of new names, especially amongst species complexes, already extant names were attributed to the taxa whenever a type appeared similar morphologically. This was done even when the type locality population had not been relocated and hence its placement within the taxon to which it was attributed could not be confirmed (i.e. *C. berberidifolia*, *C. cymbifolia*, *C. geniculata*, *C. lanata*, *C. meyeriana*, *C. rigida*, *C. semiteres*).

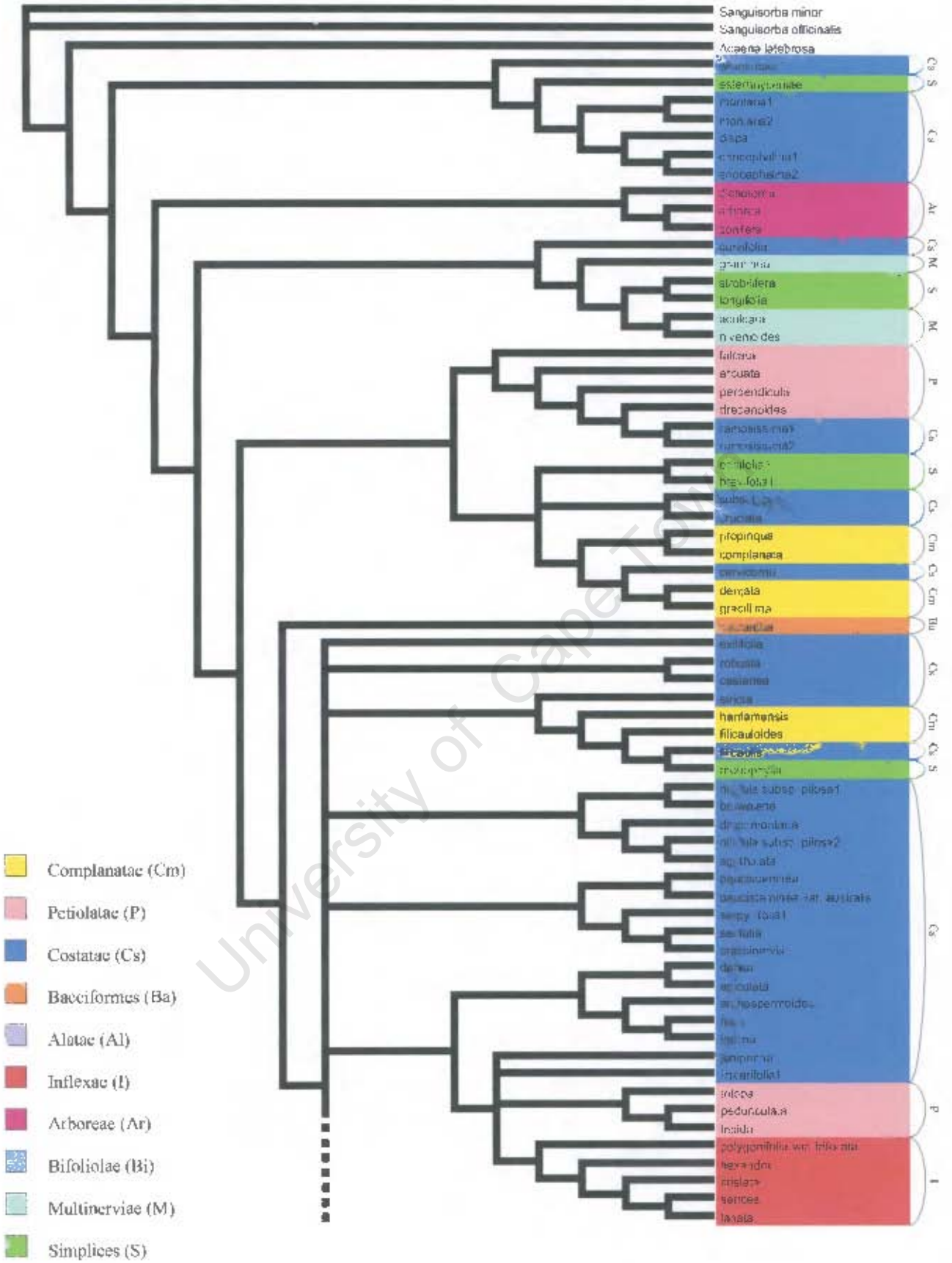


Fig. 3.1. Weimarck's sectional classification overlaid on to the 'Selective total evidence' tree, showing the polyphyletic nature of most of his sections.

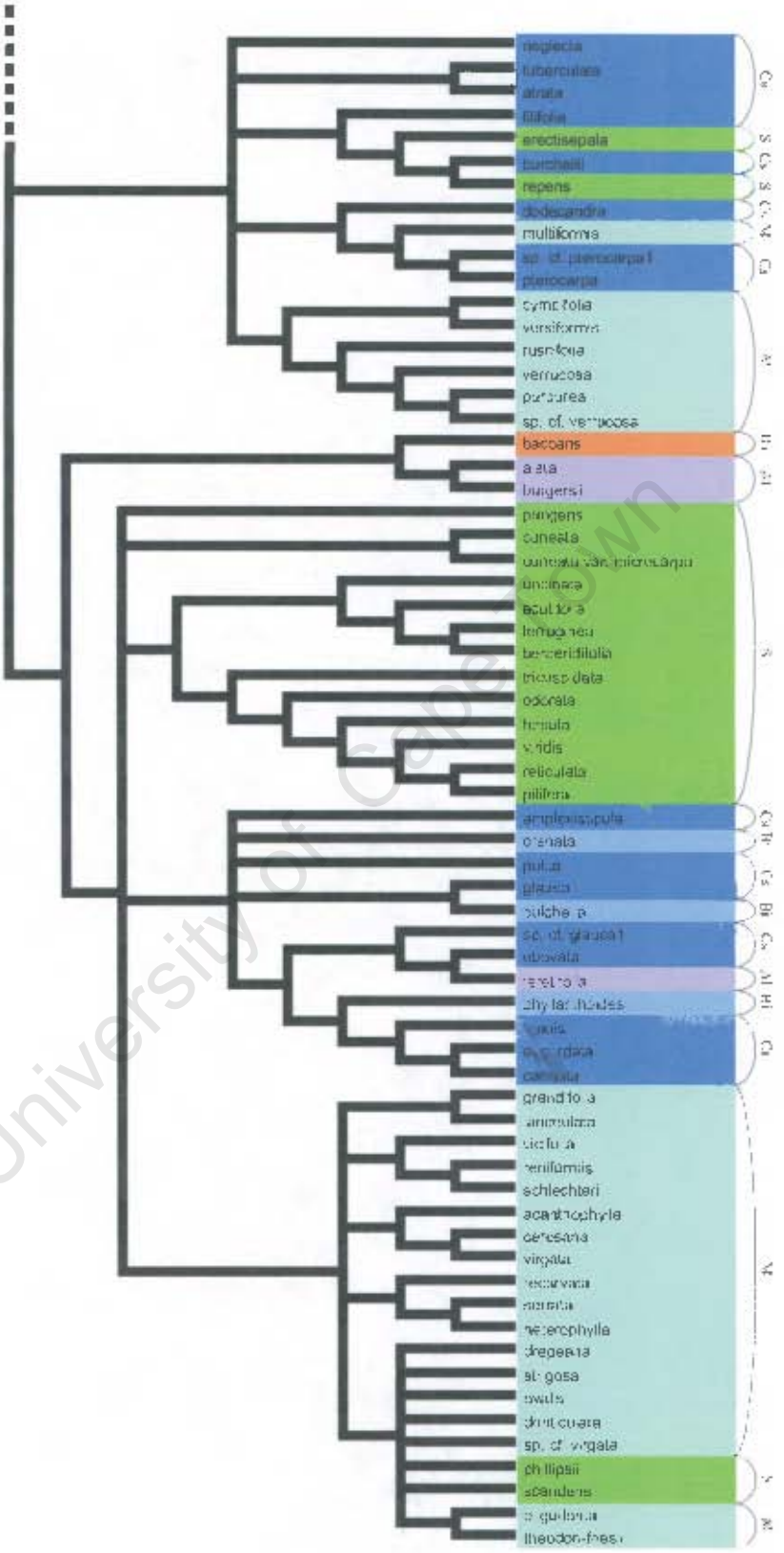


Fig. 3.1. Cont.

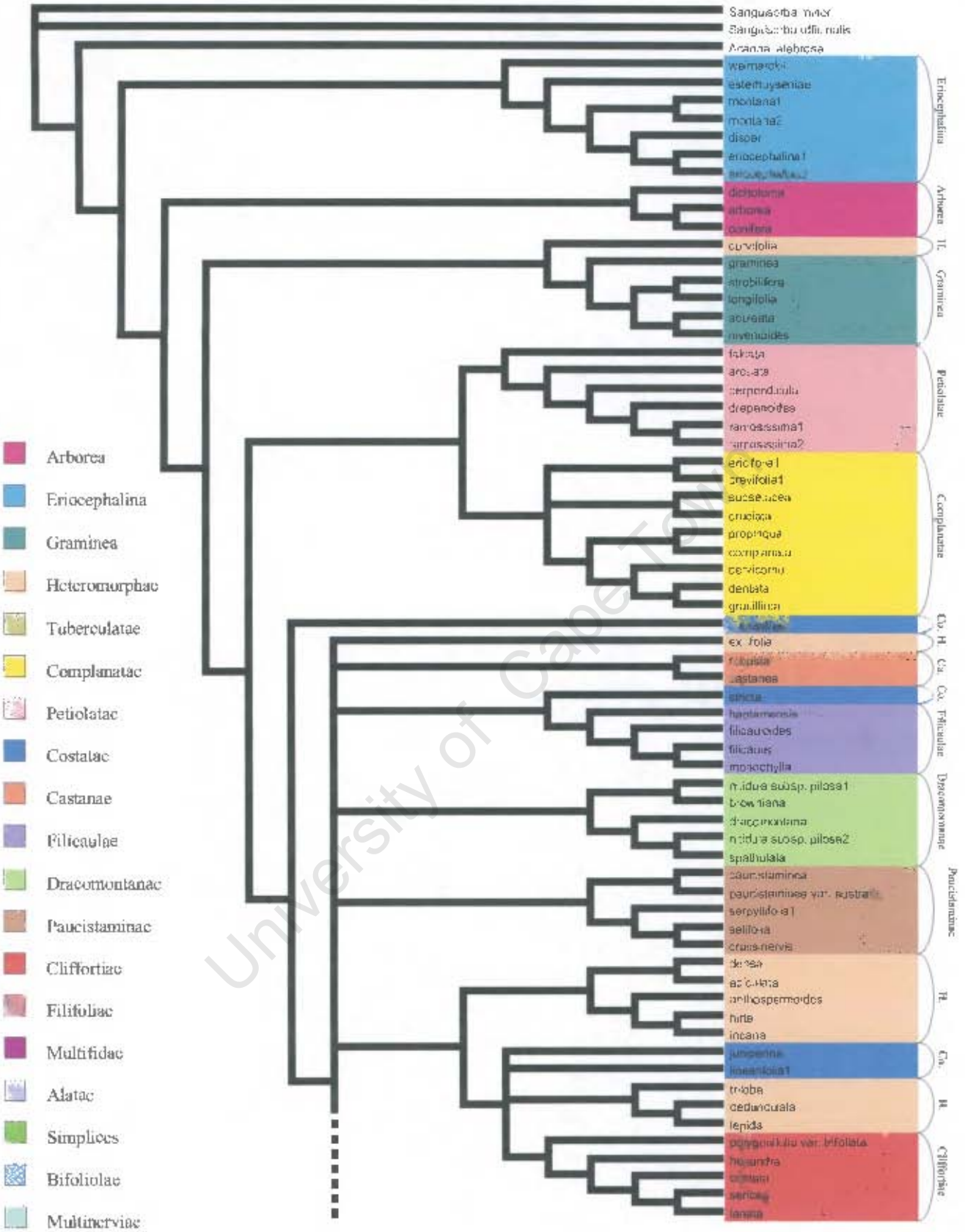


Fig. 3.2. Proposed Linnaean subgeneric and sectional classification overlaid on to the 'Selective total evidence' tree, showing the paraphyletic nature of the sections Costatae (Co.), Castanae (Ca.) and Heteromorphae (H.).

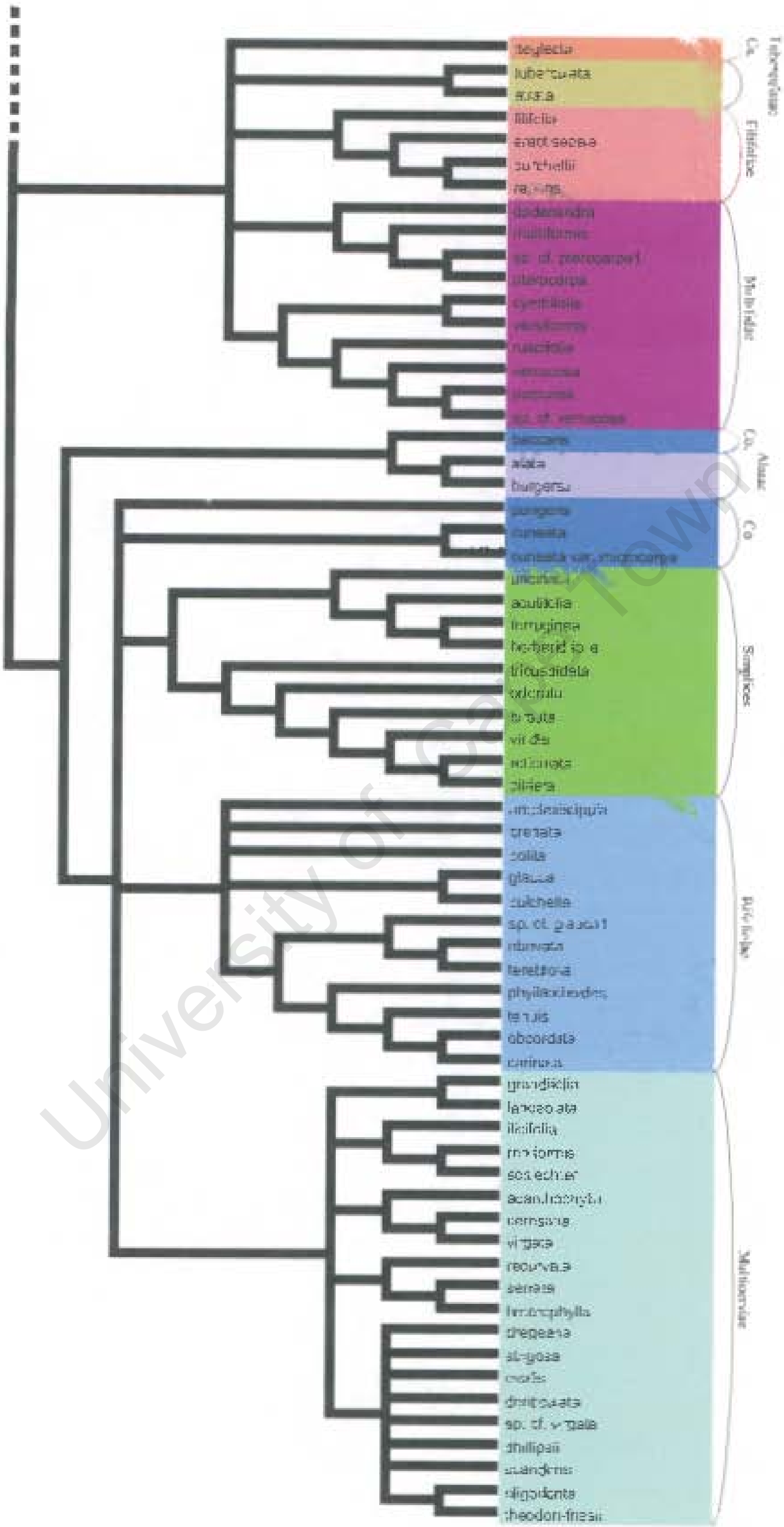


Fig. 3.2. Cont.

Table. 3.1. Classification system for *Cliffortia* based upon the PhyloCode. All species are uninomials based upon the specific epithet alone. '×' before a species or taxon indicates that it is of putative hybrid origin. '*' after a taxon indicates that circumscription of that taxon is particularly difficult as node- and stem-based definitions will not work due to it including all hybrid or only one non-hybrid species within it. Taxa in bold contain only species that are exclusive to that taxon.

Taxon name	Species included
Arborina	arborea, conifera, dichotoma, sp. cf. arborea
Eriocephalina	dispar, eriocephalina, ×esterhuyseniae, montana, ×sp. cf. eriocephalina, weimarckii
Gramina	graminea
Longifolina	
Strobiliferina	longifolia, strobilifera
Aculeatina	aculeata, nivenioides
Curvifolina*	×apiculata, curvifolia, ×densa, ×exilifolia
×**Hirtina**	anthospermoides, hirta, incana
×Pedunculatina	lepida, pedunculata, ×triloba
×Tuberculatina	atrata, ×crassinervis, ×cruciata, ×homunculi, ×pubescens, tuberculata
Cliffortina	
Complanatina	cervicornu
Subsetacina*	×cruciata, subsetacea
Ericifolina	brevifolia, ericifolia
Propinquina	complanata, propinqua
Dentatina	dentata, gracilis, gracillima
Falcatina	acocksii, ×arcuata, drepanoides, falcata, ×perpendicula, ramosissima
Cliffortiani	×apiculata, baccans, bolusii, cuneata, ×densa, ×exilifolia, linearifolia, ×meyeriana, pungens, ×cymbifolia, stricta, ×teretifolia
Juniperina	juniperina, pilosula, ×sp. cf. juniperina
Acanthophyllina*	×acanthophylla, ×ceresana, ×virgata
Castanina	castanea, ×homunculi, neglecta, robusta
Filicaulina	filicaulis, filicauloides, hantamensis, ×monophylla
Micranthina	micrantha
Dracomontanina	×browniana, dracomontana, nitidula subsp. pilosa, spathulata

Cliffortiani (cont.)	
Paucistamina	
	×crassinervis, paucistaminea, serpyllifolia, setifolia
×Hirtina	
	anthospermoides, hirta, incana
×Pedunculatina	
	lepida, pedunculata, ×triloba
Trilobina*	
	×triloba
Polygonifolina	
	cristata, hexandra, lanata, polygonifolia, ×pubescens, ×sericea, subdura
×Dodecandrina	
	dodecandra, multiformis, pterocarpa, ×sp. cf. pterocarpa
×Tuberculatina	
	atrata, ×crassinervis, ×cruciata, ×homunculi, ×pubescens, tuberculata
Filifolina	
	filifolia
Erectisepalina	
	burchellii, erectisepala, repens
×Multiformina	
×Dodecandrina	
	dodecandra, multiformis, pterocarpa, ×sp. cf. pterocarpa
Ruscifolina	
	×integerrima, ×intermedia, purpurea, ruscifolia, ×ruscifolia, ×cymbifolia, ×sp. cf. juniperina, ×versiformis, ×sp. cf. verrucosa, verrucosa
Alatina	
	alata, burgersii
Odoratina	
	tricuspidata
×Ferrugina	
	acutifolia, berberidifolia, ferruginea, uncinata, ×viridis
Piliferina	
	hirsuta, odorata, pilifera, ×reticulata, ×viridis
Glaucina	
	concinna, ×obovata, polita, ×pulchella, ?×crenulata
Crenatina	
	crenata, ?×crenulata, mirabilis, ×varians
Amplexistipulina	
	amplexistipula, ?×crenulata, glauca, longimontana, ×pulchella, ×reniformis, ×schlechteri, sp. cf. glauca2
Obcordatina	
	carinata, ×marginata, obcordata, phyllanthoides, ×recurvata, geniculata, ×sp. cf. glauca1, tenuis
Obovatina*	
	×obovata, ×sp. cf. glauca1, semiteres, ×teretifolia, ×varians
Denticulatina	
	×acanthophylla, ×ceresana, denticulata, dregeana, grandifolia, heterophylla, ×integerrima, lanceolata, ×meyeriana, oligodonta, ovalis, phillipsii, ×recurvata, scandens, ×serrata, sp. cf. virgata, strigosa, theodori-friesii, ×virgata
×Ferrugina	
	acutifolia, berberidifolia, ferruginea, uncinata, ×viridis
Ilicifolina*	
	ilicifolia, ×intermedia, ×reniformis, ×schlechteri

Table. 3.2. Outline of a Linnaean classification system for *Cliffortia*. Numbers corresponding to species names indicate:
1. Species that is morphologically distinct and does not appear to be of hybrid origin, nor does it form intermediates with other species.
2. Species that in itself is morphologically distinct but forms intermediates with other species.
3. Species that is morphologically distinct and is of putative hybrid origin.
4. Species that is part of a species complex and the morphological boundary between it and some of the other species within the section are indistinct.
5. Species that is of hybrid origin and is retained because it had previously been named.
6. Hybrids that are not named.
The type of a taxon is indicated by the subordinate taxon in bold type. Species in brackets have been assessed using accessions that are not necessarily conspecific with the type of that name. They will require renaming if the type is subsequently found to be different.

Subgenus	Section	Series	Species	
Arborea			***C. arborea***	1
			C. conifera	4
			C. dichotoma	1
			C. sp. cf. arborea	1
Eriocephalina			*C. dispar*	4
			C. eriocephalina	2
			C. esterhuyseniae	4
			C. montana	4
			C. sp. cf. eriocephalina	4
			C. weimarckii	4
Graminea	**Graminae**		***C. graminea***	1
	Longifoliae	**Longifolia**	***C. longifolia***	4
			C. strobilifera	1
		Aculeata	***C. aculeata***	1
			C. nivenioides	4
Cliffortia	Heteromorphae	Apiculata	***C. apiculata***	1
		Curvifolia	***C. curvifolia***	1
			C. densa	1
		Exilifolia	***C. exilifolia***	1
		Hirta	*C. anthospermoides*	1
			C. hirta	1
			C. incana	1
		Pedunculata	*C. lepida*	4
			C. pedunculata	2
			C. triloba	3
	Tuberculatae		*C. atrata*	2
			C. tuberculata	2
	Complanatae	Cervicornu	***C. cervicornu***	1
		Subsetacea	*C. cruciata*	3
			C. subsetacea	1
		Ericifolia	*C. brevifolia*	4
			C. ericifolia	1
		Complanata	***C. complanata***	4
			C. propinqua	1
		Dentata	***C. dentata***	1
			C. gracilis	4
			C. gracillima	1
	Petiolatae		*C. acocksii*	4
			C. arcuata	3
			C. drepanoides	1
			C. falcata	2
			C. perpendicula	3

		C. ramosissima	2
Costatae		*C. baccans*	1
		C. bolusii	1
		C. linearifolia	2
		C. micrantha	1
		C. pungens	2
		C. stricta	1
		C. cuneata	1
		C. cuneata var. microcarpa	4
		C. juniperina	2
		C. pilosula	4
		C. sp. cf. juniperina	6
Castanae		***C. castanea***	1
		C. ×homunculi	3
		C. neglecta	2
		C. robusta	1
Filicaulae		***C. filicaulis***	2
		C. filicauloides	1
		C. hantamensis	2
		C. monophylla	3
Dracomontanae		*C. browniana*	3
		C. dracomontana	1
		C. nitidula	2
		C. nitidula subsp. pilosa	4
		C. spathulata	1
Paucistaminae		*C. crassinervis*	3
		C. paucistaminea	1
		C. paucistaminea var. australis	4
		C. serpyllifolia	1
		C. setifolia	1
Cliffortiae		*C. cristata*	4
		C. hexandra	4
		(*C. lanata*)	4
		C. polygonifolia	2
		C. polygonifolia var. trifoliata	4
		C. pubescens	6
		C. sericea	4
		C. subdura	4
Filifoliae		***C. filifolia***	1
		C. burchellii	1
		C. erectisepala	1
		C. repens	1
Multifidae	Multiformis	*C. dodecandra*	4
		C. multiformis	3
		C. pterocarpa	4
		C. sp. cf. pterocarpa	4
	Ruscifolia	(*C. cymbifolia*)	4
		C. purpurea	4
		C. ruscifolia	2
		C. sp. cf. verrucosa	4
		C. verrucosa	4
		C. versiformis	4
Alatae		***C. alata***	1
		C. burgersii	1
Simplices	Tricuspidata	***C. tricuspidata***	1
	Ferruginea	*C. acutifolia*	1
		(*C. berberidifolia*)	4
		C. ferruginea	2
		C. uncinata	1
	Odorata	*C. hirsuta*	2
		C. odorata	2
		C. pilifera	2

		C. reticulata	5
Simplices	**Odorata**	*C. viridis*	3
Bifoliolae	Polita	***C. polita***	4
	Bifoliola	***C. crenata***	2
		C. crenulata	5
		C. mirabilis	5
		C. varians	5
	Glauca	*C. amplexistipula*	2
		C. concinna	4
		C. glauca	4
		C. longimontana	4
		C. pulchella	1
		C. sp. cf. glauca2	4
	Obcordata	*C. carinata*	4
		(*C. geniculata*)	4
		C. marginata	4
		C. obcordata	2
		C. phyllanthoides	1
		C. sp. cf. glauca1	6
		C. tenuis	4
	Obovata	***C. obovata***	2
		(*C. rigida*)	4
		(*C. semiteres*)	4
		C. teretifolia	3
Multinerviae	Grandifolia	***C. grandifolia***	2
		C. lanceolata	3
	Heterophylla	***C. heterophylla***	2
		C. serrata	3
	Phillipsii	***C. phillipsii***	1
	Denticulata	***C. denticulata***	2
		C. dregeana	4
		C. integerrima	5
		(*C. meyeriana*)	3
		C. oligodonta	4
		C. ovalis	2
		C. recurvata	3
		C. scandens	2
		C. sp. cf. virgata	4
		C. strigosa	2
		C. theodori-friesii	4
	Ilicifolia	***C. ilicifolia***	2
		C. intermedia	5
		C. reniformis	3
		C. schlechteri	3
	Acanthophylla	***C. acanthophylla***	4
		C. ceresana	4
		C. virgata	2

Results & Discussion:

Defining and naming the species of *Cliffortia*:

Of the 150 possible species for *Cliffortia*, 45 were easily diagnosable morphologically (Table 3.2), thus without specimens or populations that are intermediate to other species, possibly indicating the absence of hybridization. Their positions in the nuclear and chloroplast trees are congruent, suggesting that they did not have a hybrid origin.

These species would be recognised under almost any species concept. However, in the phylogenetic analysis these species were generally represented by a single accession, thus the monophyly of these species has not been tested. Therefore they may not fit the Autapomorphic Species Concept.

A further 31 species are also morphologically distinct, but appear to hybridize with other species as intermediate specimens or populations have been found occasionally. If these intermediates are found to be fertile then the Biological Species Concept would not recognise the validity of these species.

The difficulty of fertile hybrids is emphasised by the presence of 17 species within *Cliffortia* that are easily diagnosable but have different sister species in the nuclear and chloroplast trees respectively, indicating that they might be of hybrid origin. For many of these cases, they were easily diagnosable because the parent species were from widely separated lineages and so the 'intermediate' hybrid was not particularly similar to either parent or to any other species, e.g. *C. cruciata* MS. Furthermore, hybrids can also show unique characters that are not present in either parental lineage, e.g. *C. lanceolata*, C. *teretifolia*. This can be for a number of reasons, which are hard to confirm unless the original cross can be reconstructed:

(1) The parent that had that character has been lost and the species that resolves as the parental taxon instead does not have the character.

(2) Accumulation of characters through selection and drift subsequent to the hybridization event (McDade, 1995).

(3) Hybrids frequently show transgressive characters that are extreme to either parent (Rieseberg & Ellstrand, 1993; Rieseberg *et al.*, 1999).

Thus, a total of 93 species can be diagnosed reliably based upon consideration of morphological characters alone. However, intermediates are sometimes found between these morphologically distinct species, especially between hybrid species and one of their parents, e.g. *C. cymbifolia*, *C. meyeriana*. This can result in hybrid swarms and determination of species boundaries again becomes more difficult. For example, *C. denticulata*, a 2 m tall spindly shrub, *C. dregeana*, a dense spiny bush, and *C. ovalis*, a sprawling tangled mass in seepage areas, are three morphologically distinct species, but appear to form a hybrid swarm on the slopes above Jonkershoek. While the parental species remain easily recognisable, in the intermediate populations, the range of variation present means that they cannot be attributed to a new taxon, despite each

population being diagnosable individually from any one of the three potential parents, as there is no unique character to diagnose the hybrids as a group.

The recognition of a hybrid between distantly related species as a distinct taxon depends upon determining whether it will persist in the nature, or if it is just a chance novelty. This is remarkably difficult to establish, since it requires prediction. Two criteria were used here:

(1) The ability to reproduce via seed was determined. Any putative hybrid that could not resprout after fire would have to be able to reproduce from seed if it was to persist. This criterion was deemed important because the chance of the hybrid spreading beyond its point of origin was greater. This would allow establishment in new habitats away from its parents, as well as reducing the risk of extinction by localised events. Although this character might also indicate that sexual reproduction in the hybrid was possible, agamospermy may be frequent within *Cliffortia*. However, agamospermy is often facultative, indicating that although sexual reproduction may not be the means by which the hybrid persists, it may occur frequently enough to allow genetic recombination (Rieseberg & Noyes, 1998; Gornall, 1999).

(2) For clonally spreading species, those which occurred in more than one locality were identified. This was a useful criterion for establishing taxa as it enabled definition from herbarium material and cursory field observations. There are two reasons why this criterion was used:

a) It may indicate that the species was once more widespread and had been reduced subsequently in its limit, giving some indication of persistence in the environment.

b) It could also substantiate the first criterion that reproduction via seed was possible and that it had spread from its point of origin.

However, the hybrid could also be present in more than one locality because of multiple origins. In this case the species will be polyphyletic and unless gene flow can be demonstrated between the populations, defining them as the same taxon is a very dubious conclusion. However, demonstrating a multiple origin is also very difficult, entailing extensive sampling of the parental populations (Ashton & Abbott, 1992).

Finally, there was a need to define the species within the species complexes. This was a much harder task to resolve and create a satisfactory answer for. It applied in particular to sections Multinerviae, Multifidae, Cliffortiae, Glaucae and subgenus Eriocephalina.

Hybridization amongst these taxa is frequently much harder to discern because the relationships in the DNA analyses are poorly resolved. Often only a few base pairs change between the accessions and then they are frequently at highly homoplasious sites. Detailed studies at the sectional levels are needed to determine species limits and degree of genetic transfer between populations. Evidence for species delimitation was therefore based upon assessing the degree of morphological variation and combining it with geographical distributions along with the little resolution from molecular data. Species were only named when there was evidence from morphology and some degree of genetic variation combined with geographically coherent distributions.

In addition to the above, one further consideration was taken into account. If a species was already recognised for a taxon now presumed to be a hybrid or a minor form of a species complex, then that taxon was retained until new evidence comes to light that it is synonymous with a more widespread species (i.e. *C. crenulata*, *C. cymbifolia*, *C. geniculata*, *C. integerrima*, *C. intermedia*, *C. lanata*, *C. mirabilis*, *C. reticulata*, *C. rigida*, *C. semiteres*, *C. varians*). These retained species are generally only known from the type collections or single populations.

Size of the genus:

As Hull (1997) pointed out: "the two phenomena that have proved to be the most intractable for species definitions are asexual reproduction and hybridism". As both appear to be occurring in *Cliffortia*, it is not surprising that finding the most appropriate and operational species concept has proved to be remarkably difficult. The debate remains therefore as to how big the genus *Cliffortia* is. A very strict monophyletic PSC might only recognise ± eight species, as relationships beneath the subgeneric level are generally reticulate. On the other hand the same principle could be used to identify innumerable apomictically reproducing lineages.

By examining table 3.2, and taking only those species that are morphologically distinct (1–3), a very conservative estimate of around 93 species is reached. However, this would attribute all the variation found within the species complexes to just one or two species, much as Kellogg (1990) did with the agamospermous grasses in her study. At the upper end of the scale therefore, ± 140 taxa may be a more realistic figure. This would also allow flexibility for some of the lineages currently recognised to be abandoned, while others are created, without wildly changing the estimate.

The tropical species of *Cliffortia*:

All the collecting for this study has been carried out in South Africa, with the most northerly collections coming from Royal Natal National Park at the northern extreme of the Kwazulu-Natal Drakensberg escarpment. Only a single herbarium specimen was successfully used for DNA extractions from outside of South Africa, that of *C. linearifolia*(2) from the Nyanga Highlands of Zimbabwe.

Weimarck (1934) recognised three species from outside of South Africa (including Lesotho): *C. aequatorialis*, *C. linearifolia* and *C. nitidula*, of which the latter was divided into three subspecies: subsp. *pilosa* found exclusively in South Africa, subsp. *angolensis* in Angola, and subsp. *nitidula* in the remaining countries, namely Zimbabwe, Malawi and Tanzania. *C. aequatorialis* was considered by Weimarck to be endemic to Kenya, but later workers in tropical Africa have regarded this and *C. nitidula* subsp. *angolensis* as synonymous with *C. nitidula* subsp. *nitidula* (Graham, 1960; Mendes, 1978). They do not comment however on the status of the South African *C. nitidula* subsp. *pilosa* in relation to these.

C. nitidula is very variable across the whole of its range. The evidence from the molecular work conducted is that in some areas it has introgressed with *C. linearifolia* (*C. nitidula* subsp. *pilosa*(1) from Hogsback has an additive 5S NTS sequence between these two species, where they both grow in close proximity). Mendes also cites two specimens as putative hybrids. What is more, *C. browniana* may be a more ancient hybrid between these two species (it is sister to *C. nitidula* subsp. *pilosa* in the chloroplast tree but is placed basally on the same lineage as *C. linearifolia* in the nuclear tree). Considering the fact that introgression has also been shown to be currently occurring, it is possible that if *C. browniana* is of hybrid origin then it has arisen more than once.

Consequently, the claim by Mendes (1978) that *C. serpyllifolia* grows in Zimbabwe and Mozambique needs to be reconsidered. Certainly, Kwazulu-Natal specimens of *C. serpyllifolia* have occasionally been misidentified as *C. browniana* (e.g. *C. serpyllifolia*(2)) and they look morphologically similar. Hence, it could be that these records are in fact introgressed specimens between *C. nitidula* and *C. linearifolia*, or *C. browniana* itself. The other possibility is that they are just shade forms of *C. nitidula*, as Mendes also states that it has been found "frequently in the shade of forest trees". A better understanding of the geographical variation within *C. serpyllifolia* across its whole range, from the Langeberg to Kwazulu-Natal, is needed along with molecular

work. Until this has been carried out, the presence of *C. serpyllifolia* outside of South Africa must be regarded as highly dubious.

Work needs to be done on the tropical variants of *C. nitidula* to determine whether any of them can be upheld as taxonomic entities and what their relationship is to the South African subspecies. For the time being, only *C. nitidula* subsp. *nitidula* is upheld for tropical *Cliffortia* (along with *C. linearifolia* in Zimbabwe), while *C. nitidula* subsp. *pilosa* is maintained for the South African collections of this species.

Weimarck's sections:

Weimarck's subgeneric and sectional classification of *Cliffortia* (Weimarck, 1948) is clearly inconsistent with the molecular analysis (Fig. 3.1). All but three of his groupings are shown to be polyphyletic for both the nuclear and chloroplast DNA datasets. Section Hermaphroditicae and section Arboreae were monotypic when described, so there could be no conflict with Weimarck's original circumscriptions.

Section Hermaphroditicae, and its single species, *C. hermaphroditica*, is here abandoned on account of it being from a mixed collection. The BOL specimen is a form of *C. pterocarpa* sensu lato and it is presumed that the type, which has not yet been located at NBG, is yet another form of this problematic complex, and probably of hybrid origin. The type comes from Jonkershoek, which is botanically a very well-known area, having been part of extensive ecological surveys in the 1960s, and the 'species' has not been recollected. It is therefore presumed to be an odd morph or hybrid that occurred for one generation and has since been lost.

The circumscription of section Arboreae has recently been expanded by the addition of *C. conifera* (Oliver & Fellingham, 1994). Another undescribed species, *C. dichotoma* MS, has also been included (Fellingham, 1999), and a form of *C. arborea* has also been found which may constitute a new species, *C. sp. cf. arborea*. These four species were resolved as monophyletic in all three datasets.

Section Bacciformes was also monotypic, containing just *C. baccans*, when Weimarck described it originally but he then placed *C. micrantha* into it on the basis of its swollen achenes (Weimarck, 1948). Fellingham (1993a) examined the number of styles and achenes in *C. micrantha* and deemed that it would be better placed in subgenus Digraphidium in its own section, but she did not formally move it. From the phylogenetic analysis it is very clear that *C. baccans* and *C. micrantha* are not sister species, nor can *C. baccans* be placed with confidence as sister to any other species, as

it has a different position in each analysis (sister to section Alatae in the nuclear tree, but to the *C. erectisepala* clade in the chloroplast tree). Hence Weimarck's section Bacciformes could still be maintained but as a monotypic section containing just *C. baccans*, which might deserve such a status on account of its unique fleshy fruit.

The only other section of Weimarck that is monophyletic is section Incurvae. Although it is paraphyletic in the separate analyses, combination of all the datasets clearly supports its monophyly. However, it should correctly be called sect. Cliffortiae as it contains the type of the genus, *C. polygonifolia* (following Green, 1929).

Three of Weimarck's other sections are still useful at some level but need to be refined. Weimarck's circumscription of section Petiolatae contains most of the species with a petiole. The phylogeny produced here shows that it should be divided into two unrelated taxa: those with a short to long peduncle (*C. pedunculata*, *C. lepida* and *C. triloba*) and those whose flowers are sessile (*C. falcata*, *C. arcuata* and *C. drepanoides*). The latter group also includes *C. ramosissima*, which Weimarck failed to notice also has a very short petiole, as well as several other morphological features in common with *C. falcata* and *C. arcuata*. However, Weimarck correctly did not include the petiolate species *C. filifolia* and *C. filicauloides* in the section; their affinities clearly lie elsewhere.

The other sections that reflect the phylogeny to some extent are sections Bifoliolae and Multinerviae. The species of section Bifoliolae do not form a monophyletic clade but they are all placed within a small part of the tree topology in all the analyses and they are clearly closely related to one another. For section Multinerviae, *C. ruscifolia* and its relative *C. multiformis*, and the grassy *C. graminea*, *C. aculeata* and *C. nivenioides* all belong elsewhere, but the rest of the species form a well-defined clade in the nuclear and morphology analyses (and the majority in the chloroplast too).

Of the remaining sections, Complanatae and Alatae contain a number of sister species pairs, but the pairs are unrelated to each other. While the two largest sections, Costatae and Simplices, are so demonstrably polyphyletic that they are more or less meaningless. They may have been used by Weimarck to include all those species that did not fit elsewhere but had trifoliate or unifoliate leaves respectively as these were the only synapomorphies that held them together.

Phylogenetic classification of *Cliffortia*:

The PhyloCode (Cantino & de Queiroz, 2000) provides simple rules for naming clades and naming of all clades is not mandatory. Hence in theory, putting a classification to a

cladogram is a simple task, just requiring enough inventiveness to produce the names for the clades. However, in constructing a phylogenetic nomenclature for *Cliffortia*, problems with this method soon arose.

The difficulty in applying the PhyloCode came not from naming the clades, but in the circumscription of those names. Table 3.1 gives the outline for the putative classification of *Cliffortia* following the PhyloCode. To simplify naming, the epithet from an exemplar species belonging to the taxon was used as the stem for the taxon names and was given an -ina suffix (except for Cliffortina and Cliffortiana) following the convention used by Cantino (1997) for the Lamiaceae. Hybrid taxa are included at both their parental positions within the classification, including any subordinate taxa as suggested by Funk (1985). Although it creates a long-winded classification, the criterion of monophyly is still upheld and the phylogeny can be partially reconstructed from the classification.

Unfortunately, creating the definitions proved much more difficult and was abandoned. Although many clades were simple to name, usually by node-based definitions, certain ones proved impossible to name without risk of having high instability. The best example to show this is Curvifolina. A node-based definition cannot be constructed, as there is only one taxon that does not show further reticulation. Choosing one with a reticulation would mean that the circumscription of the clade would change depending upon which line of descent was chosen to locate the common ancestor. Neither will a stem-based definition work for a similar reason. Incidentally, even if a taxon does contain more than one non-hybrid species within it, it does not necessarily mean that it will be possible to circumscribe it if hybrids are present. As has been discussed, hybrids have a tendency to express plesiomorphic characters and drop to the base of the clade of their parent species (e.g. *C. recurvata*, *C. intermedia* or *C. sp. cf. juniperina*). If one circumscribes the clade using the non-hybrid species then the hybrid will be excluded from the clade that contains its parents (if one strictly adheres to the cladogram rather than the phylogeny). This makes circumscribing that clade without using the hybrid equally impossible and again instability can easily result.

One way around these problems is to choose a single genome tree to base the classification upon. In the example mentioned above, Curvifolina could be easily circumscribed using a node-based definition on the chloroplast gene tree. However, for many other taxa, using only the chloroplast tree would result in taxa with few or no morphological synapomorphies. Indeed, chloroplast capture of a distant species by part

of a population would result in that species being split into two disparate taxa. Neither can the nuclear genome be used, because bidirectional concerted evolution and recombination will mean that each part of the genome might reflect a slightly different phylogenetic history.

Cantino et al. (1997) recommended introducing contingency clauses for cases such as the above to ensure stability of the name. They admitted that the method was "cumbersome" but defended it on the grounds that it was explicit. However, Moore (1998) points out that the explicitness also places severe restrictions upon circumscription beyond what is stated and the distinction between nomenclature and taxonomy becomes obscure. Here the definitions became so 'cumbersome' that most were not even attempted.

The problems posed here raise the question as to why these nodes should be named. For example, if only Arborina, Eriocephalina, Gramina and Cliffortina were maintained, then they are easily circumscribed. This is still a useful classification, but actually no more useful than the Linnaean one (see later on), and rather limited in its division of the diversity found within *Cliffortia*. However, the purpose of classification is to give the user a handle upon which to base further scientific discussion. Almost as great a variety of form is found within Cliffortina as across the whole genus. To be unable to subdivide this clade would make discussions about it rather awkward. A classification needs to be able to convey information but also needs to remain simple enough to use. If one has to return to the original trees to understand what is going on then it is no more useful than to have a Linnaean classification and a separate phylogeny.

It may be that provisions can be made within the rules of the PhyloCode to overcome the problems presented by reticulations, but under the present version it appears to only be useful for clades above which no reticulation occurs.

Subgeneric and sectional classification of *Cliffortia*:

A Linnaean Classification with monophyletic taxa is equally problematic as using the PhyloCode because of similar arguments. Under a Linnaean classification, with enforced monophyly of supraspecific taxa, only the four subgenera can be identified within *Cliffortia*.

However, as already stated, monophyly of taxa is not a stipulation of the code, hence there is still flexibility for the application of names to taxa (Stuessy, 2001) and one reason why the classification is still "more or less theory-independent" (van Welzen,

1998). The problem with accepting paraphyletic taxa is that it then becomes very subjective as to where the boundaries are drawn and this can lead to some of the instability that has been found in Linnaean classification over the years, especially at the generic level (van Welzen, 1997). Nixon & Carpenter (2000) also highlight that one of the advantages of retaining ranks, as opposed to the 'rankless' PhyloCode, is that it is informative in the fact that it implicitly excludes taxa of the same rank. This would be negated if paraphyletic taxa are permitted and so the classification will be less informative.

The presence of paraphyly caused by reticulations is a big problem that taxonomists who encounter it have to overcome in creating a classification (Bremer & Wanntorp, 1979). However, in the vast majority of studies, reticulations above the species-level do not occur and monophyly for higher taxa can be achieved (Freudenstein, 1998), even if it requires a high degree of splitting or lumping. While monophyly for species has also been proposed, it has been shown that all this stipulation achieves is to push the level of paraphyly down to the next rank (Crisp & Chandler, 1996; Brummitt, 1997) or to create metaspecies (de Queiroz & Donoghue, 1988). Many advocates of a monophyletic classification system now accept that the species-level represents a special case and allow paraphyletic taxa to occur (Crisp & Chandler, 1996). Here we have had to go one step further and accept that the sectional and series levels are special cases and permit paraphyly at these levels too for ease and maximum informativeness of the names.

Therefore, for the classification proposed here (Table 3.2, Fig. 3.2), three steps were taken:

1. Monophyly of taxa was attempted wherever possible.

2. When this was deemed not possible, the monophyly for the taxon in at least one gene tree was sought.

3. Where hybrid taxa could have been placed in more than one taxon, they are placed in the taxon with the greatest overall morphological similarity.

Of these, Step 3 is the most problematic as it will sometimes cause Step 2 to become invalidated. If the clade chosen for the taxon comes from the gene with which it has least morphological similarity, it might entail the removal of a species from an otherwise monophyletic grouping. Ideally therefore, one should choose the clade in Step 2 that has the best morphological congruency. However, this is not possible when well-defined and morphologically supported clades from separate genes overlap. (If the

hybrid taxa were allowed to be listed in both taxa as suggested for phylogenetic nomenclature then this would not be a problem, but this is not possible under the ICBN, nor is it particularly desirable.)

Wherever possible, Weimarck's sectional names were retained where this made logical sense, as it reduces the number of new taxa to be described. Fortunately, Weimarck did not typify any of his sections, so the names can still be fixed to only part of his groupings by picking the most representative species.

There still remains a problem with species that are not included within the initial selection of clades. Three options exist: to include them in a basal paraphyletic taxon; to create several monotypic taxa; or to not assign them to a rank at that level. The debates by Sosef and Brummitt (1997; 1997; 1998) imply that the first one is preferable to the second. Others disagree, but here we have already violated their insistence upon monophyly, albeit only partially and for reasons which their arguments tended to avoid. Finally, Cantino et al. (1997) point out that it is not necessary in a Linnaean classification to assign a rank above the genus level when there is inadequate evidence for it. Ideally, one would not have to place these species into any section, giving them the status of *incertae sedis*, until a better understanding of their evolutionary history was achieved. However, below the rank of genus, Article 22.3 of the ICBN mandates that when one publishes a name of a subdivision of a genus that does not include the type of the genus, a name at the same rank based on the type is automatically created (autonym). It is this clause and the subsequent establishment of exhaustive subsidiary taxa below the generic rank in Linnaean classification that cause the problem in this classification.

To solve this a compromise was sought. The creation of eight new monotypic sections was deemed unwarranted for species whose placement within the sectional hierarchy might become clearer at a later date. (Monotypic series were regarded as an inevitable necessity when creating other more informative series.) While the third option to have nominated them as *incertae sedis* would have been the most preferable, it is not as yet permissible under the ICBN. Hence, Weimarck's section Costatae was retained as a paraphyletic taxon and all the unplaced taxa assigned to it. It can be omitted from future analysis and by indicating clearly in its description that it is an unnatural grouping other users will hopefully also be wary. *C. juniperina* was used to typify the section, as the name 'Costatae' will fit best with that taxon should it ever be taken up as a monophyletic section. It also prevents the name sect. Costatae being referred to any

newly named section on account of its priority. Thus, these taxa can be discussed as *incertae sedis* for all intents and purposes, but for those who insist upon exhaustive subsidiary taxa they can be attributed to the paraphyletic section Costatae.

Neither the phylogenetic nor the Linnaean classifications will satisfy everyone. There are some severe 'violations' of principles held dear by some with respect to what they believe a classification should entail. On the other hand, not enough attention has previously been paid to dealing with hybrids when producing classifications and hopefully the attempt herein will prompt further discussion on the problem. In the past, problems of severe reticulation have been waved aside as inapplicable to real life situations caused by unnatural splitting of species (e.g. van Welzen, 1998). This study of *Cliffortia* has shown that unless one can create a classification that can cope with these severe reticulations then it will not be universally applicable.

4. Evolution and Speciation

Introduction:

The phylogenetic hypothesis produced in Chapter 2 represents the best estimate using the data currently available for explaining the evolutionary pathways that gave rise to the immense morphological diversity found within *Cliffortia*. This phylogeny in combination with morphological, ecological and distributional data can be used to infer the processes that have influenced speciation. The frequency of the various hypothesised processes that have driven speciation events can then be used to identify the major patterns of speciation within *Cliffortia*.

Modes of speciation:

Allopatry was long regarded as the dominant or even the only possible mode of speciation (Mayr, 1942; Pielou, 1979), and thus it was also invoked for the Cape Flora (Goldblatt, 1978). More recently, mechanisms by which sympatric speciation can occur have begun to be widely accepted (Via, 2001). Indeed, work in the Cape Flora has suggested that it is just as common there as the allopatric mode, if not even dominant, and is caused by strong ecological gradients (Linder & Vlok, 1991; Kurzweil *et al.*, 1991; Cowling & Holmes, 1992a; Cowling *et al.*, 1992). However, the geographical division of speciation processes into these two modes is not necessarily the most helpful reflection of the biology of speciation (Via, 2001).

Two factors are needed for speciation to occur: interruption of gene flow and differential selection or genetic drift. In allopatric speciation, interruption of gene flow will occur before differential selection or genetic drift, while sympatric speciation generally implies that intense selection can override gene-flow. However, sympatric speciation can also occur by initial interruption of gene flow caused by polyploidization, either auto- or allo- (Thompson & Lumaret, 1992; Ramsey & Schemske, 1998), by homoploid hybrid speciation (Rieseberg, 1997) or by a shift in phenology (Linder & Midgley, 1996). Therefore, in discussing modes of speciation it is more informative to identify barriers to gene flow and selection pressures.

Gene flow between plants in the Cape Flora has been hypothesised to be spatially very limited (Linder, 1985). Consequently, its role in speciation has been considered to be minor compared to the strong ecological gradients that exist within the CFR (Linder,

1985). However, the claims of limited gene flow for the CFR have never been tested. In addition, the widely held view that gene flow is limited between populations of plants has recently been questioned and it has been postulated that even occasional long distance gene flow permits the spread of strongly advantageous alleles (Ellstrand, 1992; Rieseberg & Burke, 2001). For example, the possibility of long distance gene flow might be one explanation for the finding of high genetic variability within small isolated populations of the rare *Leucadendron elimense* (Tansley & Brown, 2000).

For differential selection, two basic models have been proposed to explain the pressures upon the direction of speciation: growth-environment driven and pollinator driven (Johnson, 1996). The uniformity of the flowers, and for the majority of species an undifferentiated inflorescence structure of scattered single flowers, means that most of the diagnostic features for *Cliffortia* species are vegetative. In addition, the remarkable similarity in leaf morphology between species of wind-pollinated *Cliffortia* and insect-pollinated *Aspalathus* (Dahlgren, 1971) implies that this diversity has been selected for, irrespective of pollination syndrome. These hypotheses predict that the major force influencing speciation within *Cliffortia* has been the strong selection for vegetative traits across ecological boundaries in the absence of significant gene flow between populations.

Morphological adaptation:

Speciation processes can be investigated by identifying character changes and their relative distributions on a phylogenetic tree (Barraclough & Nee, 2001). However, caution is needed as problems exist with this method when applied to parsimony trees. In particular it assumes equal probability of gains and losses for characters, that changes on all branches are equally likely, and that the rate of evolution is relatively slow (Cunningham *et al.*, 1998; Omland, 1999). Furthermore, vegetative characters are often continuous, rather than discrete (Stevens, 1991). Reversals in character states are then more likely, as they are not caused by loss or gain of a more complex character but by selection upon quantitative variation.

Despite the wide variation in growth form, the number of discrete morphological characters within *Cliffortia* is surprisingly low (see appendix 4). In addition, the continuous nature of many of these supposedly discrete characters is supported by the very poor consistency index of the morphological phylogeny and all vegetative characters are homoplasious when optimised onto the molecular or total evidence trees.

Therefore, there are only a few reliably discrete characters that can be investigated for *Cliffortia*.

In addition to this, the hypothesised reticulate nature of the phylogeny of *Cliffortia* complicates the interpretation of character evolution. Character optimisation methods are designed to work with fully dichotomised trees. Reticulations will produce polytomies in a consensus tree between the clades in which the parental lineages occur (Bremer & Wanntorp, 1979; Funk, 1985). As character optimisation algorithms are inappropriate when used upon polytomies (Maddison, 1989), producing a consensus tree from reticulating phylogenies is unsatisfactory. Producing a total evidence tree is also unsatisfactory as the intermediate nature of the reticulating taxa could drop the hybrid to an ancestral position relative to the parental clades (Funk, 1985). This would cause any derived character in either of the two parental taxa that is now shared by the hybrid to be optimised as ancestral. Therefore any statistical tests are at present inappropriate to use for investigating character optimisation in *Cliffortia*, as basic assumptions will always be violated and cannot be circumvented with the algorithms available.

However, although the expression of morphological characters of hybrids on a phylogenetic tree is unpredictable, they do not tend to influence the position of non-hybrid taxa (Rieseberg & Ellstrand, 1993). Therefore, one option would be to remove the hybrid taxa from the tree before carrying out any optimisation analysis. Unfortunately, for *Cliffortia* the number of species showing signs of putative hybridization events is so high (61.6% have incongruent positions of which 38.3% show strong incongruence, Table 2.9) that the informativeness of any optimisation analysis on the remaining taxa is questionable. Furthermore, there is frequently ambiguity as to which of two species represent the hybrid taxon and this would further reduce any confidence in the taxa left in the analysis.

The lack of discrete unique character changes as well as the presence of possible reticulations means that detecting shifts in the morphology that have increased rates of speciation in the different parts of the tree is difficult if not impossible. To overcome these problems, pairs of sister species can be examined to determine the factors that have influenced their speciation and then repeated patterns in the shifts can be looked for among them. However, caution is still needed when determining the factors that have promoted speciation because observed patterns may be incidental due to independent evolutionary histories since speciation (Barraclough & Nee, 2001).

Ecological divergence:

Except for allopatric speciation coupled with genetic drift, speciation events will be accompanied by a shift in ecology (Wiley, 1981; Templeton, 1981; Orr & Smith, 1998; Schluter, 2001). Speciation can only occur in sympatry if a new morph of a species has an associated ecological change. Otherwise, the new morph will either not become established or in rare cases will eventually replace the parental species, and neither result increases the number of species present. These ecological shifts can be used to detect associations between particular character traits and ecology (Orr & Smith, 1998). Furthermore, parallel speciation events, where traits evolve comparably in different populations experiencing similar environments, provide good evidence for connecting speciation to a particular character trait change (Schluter, 2001).

Examples of ecological shifts that affect the growth-environment driven model include substrate, altitude, water dependence and life-history aspects of the species (see Carson, 1985):

Substrate shifts:

The majority of *Cliffortia* species are found in the mountains of the south-western Cape, where they grow on the acid sands and shales derived from the Table Mountain Series (TMS) of rocks. However, several other substrate types are found within the CFR, and some *Cliffortia* species have either succeeded in establishing themselves on them or are even restricted to them.

Altitude shifts:

Cliffortia species are found from sea-level to the top of the Drakensberg Escarpment in Lesotho at 3,200 m. Altitude is an easy environmental factor to measure and so frequently recorded, but may be better defined as a surrogate for average temperature as altitudinal ranges of species often vary with latitude or distance from the coast.

Phenological shifts:

Phenology is associated with pollinator-driven models of speciation (Johnson, 1996) but shifts in flowering times between species have been recorded for groups that appear to be primarily growth-environment driven, e.g. *Ceratocaryum* (Linder, 2001d), *Rhodocoma* (Linder & Vlok, 1991). However, detecting these shifts can be difficult as flowering time can vary across the range of the species. Therefore, two species that grow sympatrically may exhibit different flowering times when found together but flower at the same time in other parts of their ranges (Kurzweil *et al.*, 1991).

Water related shifts:

The water needs of a species can be divided into two separate factors: the overall amount of water and its supply throughout the year. The distribution of *Cliffortia* covers a wide range of water regimes from wet margins of afromontane forest through to the borders of the arid Karoo region, and winter rainfall to all year or summer rainfall. Within these general climatic zones water supply can vary due to many other factors:

1) Altitude can affect the amount of moisture due to orographic rainfall and creation of rain-shadows.

2) Aspect can increase moisture on slopes facing the prevailing winds that bring clouds and rain from the sea.

3) Topography increases water supply in valleys and gulleys, with drier ridges in between.

4) Drainage for most parts of the CFR is very quick because of the deep sandy soils, but some flat areas can have impeded drainage because of clayish soils or underlying bedrock.

Fire survival shifts:

Fire is one of the most important ecological factors in the fynbos, as well as the montane grasslands of Kwazulu-Natal. It is the primary factor that causes disturbance within these habitats (Cowling, 1987; van Wilgen, 1987; Manders & Cunliffe, 1987), and hence enables selection for species to occupy different regeneration niches (Grubb, 1977; Bond & Midgley, 2001). Two strategies, resprouting or obligate seeding, are available for plants to survive severe disturbance (Schutte *et al.*, 1995; Bellingham & Sparrow, 2000), and *Cliffortia* exhibits examples of both of these strategies. Resprouters rely upon increasing the levels of resources stored below ground, or occasionally protected by the bark, for regrowth after loss of above-ground biomass (Pate *et al.*, 1989; Pate *et al.*, 1991; Bell & Ojeda, 1999; Verdaguer & Ojeda, 2002). On the other hand, seeders increase the levels of resources devoted to above-ground vegetative growth and consequently seed production to maximise their chance of recolonisation after fire (le Maitre & Midgley, 1992; Bellingham & Sparrow, 2000). Both these strategies can coexist in the same habitat within the fynbos (Smith *et al.*, 1992; le Maitre, 1992), hence one potential ecological shift for speciation is a change in life-history from resprouter to seeder or vice versa.

Genetic drift:

While sympatric, and even parapatric, speciation events allow examination of the ecological factors that have influenced selection, in the case of allopatric speciation this is more difficult because of the problem in separating ecological selective forces from genetic drift (Orr & Smith, 1998; Schluter, 2001). Geographic isolation of a population will allow it to evolve independently of the parental species, as gene flow between the two has been broken. Although edaphic or other ecological differences arising from *distributional* breaks may influence the direction of speciation and help to reinforce reproductive isolation (Schluter, 2001), an ecological shift is not a necessity for speciation. In allopatric speciation, genetic drift can still be the primary factor influencing morphological change. As a result, allopatric species are often more similar to each other than sympatric species (Wiley, 1981) and reproductive isolation can fail to develop in species even after comparatively long periods of time since separation (e.g. Grant & Grant, 2002).

Hybrid speciation:

While the mechanism usually invoked for speciation is intraspecific variation followed by selection or genetic drift in the absence of gene flow, hybridization events can produce new genetic combinations through either allopolyploidy (Rieseberg, 1997; Ramsey & Schemske, 1998) or homoploid hybridization (Rieseberg & Noyes, 1998). Although hybrids are often dismissed as being genetically inferior, they have in fact been shown to display fitness levels relative to their parental species ranging from sterility or inviability to marked hybrid vigour (Arnold & Hodges, 1995; Arnold *et al.*, 2001; Barton, 2001). Even if the original F1 hybrids are unfit relative to their parents, new alleles can still be incorporated into parental genomes via backcrossing and introgression (Rieseberg & Wendel, 1993; Rieseberg & Carney, 1998). Although this might be considered rare if the hybrid does not establish itself, work on hybrid zones in irises has shown that once an initial hybrid is formed, later-generation hybrids are more common (Hodges *et al.*, 1996). This could be in part due to the formation of triploid bridges promoting allopolyploidization (Ramsey & Schemske, 1998).

By whatever means a hybrid arises, it must become reproductively isolated from both its parents soon after establishment if the recombinant phenotype is not to be swamped by gene flow (Templeton, 1981). Polyploidization will produce instantaneous isolation (Rieseberg, 1997) and is the commonest type of hybrid speciation event to be observed (Raymond *et al.*, 2002). Reproductive isolation can also occur between hybrids and

parents with equal ploidy levels by chromosomal or genic rearrangement, which can lead to partial sterility of the hybrid to the parents (Rieseberg, 1997). However, there is also the possibility of isolation of the hybrid from its parents by prezygotic barriers, such as habitat preference, flowering time or pollinator behaviour (Rieseberg & Carney, 1998). Therefore, whether or not reproductive isolation by sterility or inviability is attained between a hybrid and its parents, novelties that arise in the hybrid phenotype enabling an ecological shift can lead to speciation. Indeed, several examples of these prezygotic barriers in hybrids are now being tested, e.g. salt tolerance in *Helianthus paradoxus* (Welch & Rieseberg, 2002) and intermediate habitat selection in *Polystichum* hybrids (Kentner & Mesler, 2000).

Detecting the role of hybridization in the process of speciation is a difficult task. Although one can hypothesise that a species has had a hybrid origin (see chapter 2), only by reconstructing the original cross can it be determined whether a novelty that brought about speciation was as the result of hybridization or through selection at a later date (e.g. Rieseberg *et al.*, 1996; Burke *et al.*, 1998). However, traits in both parents can be compared to investigate if there was a selective advantage in combining the two contrasting phenotypes. In addition, these traits can often appear to be extreme (transgressive) when compared to the parents (Rieseberg & Ellstrand, 1993; Rieseberg *et al.*, 1999), enhancing the possibility of hybrid establishment in more divergent habitats (Welch & Rieseberg, 2002). These experiments can naturally only be tested upon recent hybrids where both parents are still extant. If one parent is absent it will be impossible to tell if the adaptive characters were gained from the absent parent, the result of hybridization or a more recently acquired trait.

Previous Evolutionary Hypotheses:

Previous explanations for the morphological evolution of *Cliffortia* had been attempted by both Weimarck (1934) and Dahlgren (1971). Both men used the outgroup method to identify hypothetical plesiomorphic states for characters within *Cliffortia*. Weimarck identified the other genera of the *Sanguisorba apetalae*, and in particular *Polylepis*, as the closest relatives morphologically to *Cliffortia*. Dahlgren agreed with Weimarck's choice of outgroup taxa, in particular suggesting *Acaena* as the sister taxon, but chose to use the petaloid genus *Leucosidea*, which has an almost identical basic leaf-type. Recent genetic work upon the Rosaceae (Morgan *et al.*, 1994; Eriksson *et al.*, 1998; Potter *et al.*, 2002) and in particular the Sanguisorbeae (Bateman, 1999; Helfgott *et al.*, 2000; M. Hibbs, pers. comm.) has supported their identification of the genera closest to *Cliffortia*,

i.e. *Sanguisorba*, *Polylepis*, *Acaena* and the Macaronesian *Bencomia* alliance, with the petaloid *Agrimonia*, *Leucosidea*, and *Hagenia* more distantly related. Therefore, their assumptions were theoretically sound and the conclusions they drew can be used as a basis for investigating the evolution of characters within *Cliffortia*.

Weimarck (1934) used his outgroup taxa to identify certain features as 'original characters of the Sanguisorbeae'. These putative plesiomorphic characters were: the presence of more than one achene in the receptacle, tetramerous flowers, trifoliate leaves, and presence of a petiole. The first three of these characters he regarded as important enough to base his subgeneric (thus basal) split upon, even though for the most part the petiole was absent.

Dahlgren (1971), on the other hand, was more concerned with comparing the remarkable morphological similarity of leaf-types between *Cliffortia* and *Aspalathus* in the Leguminosae. Therefore, he only hypothesised a possible evolutionary pathway for the variety of leaf morphology found within *Cliffortia*. Using *Leucosidea sericea* as the exemplar species for his ancestral leaf form, he suggested that reduction to trifoliate leaves with a petiole was followed by either loss of leaflets or loss of petiole, and further reduction was caused by coalescence of leaflets into a single leaf. He also suggested similar patterns between different South African genera in the Leguminosae (Dahlgren, 1970).

Methods:

The evolution of character traits was examined by optimising the chosen characters onto the selective total evidence tree (Fig. 2.10) using Paup* 4.0b10 (Swofford, 2001) and ACCTRAN optimisation. This tree was used as the best approximation of the reticulate phylogeny, but the distribution of character states on the separate molecular trees was also noted to ensure that conclusions drawn were not unduly influenced by choice of this tree. The characters chosen were selected upon the basis that they had been interpreted by Weimarck (1934) as important steps in the evolution of the genus: the number of carpels, the number of sepals, the presence of a petiole and the number and form of the leaflets.

For ecological shifts, environmental factors were compiled from label data on herbarium specimens, distribution patterns and field observations. Two methods were used to investigate potential shifts that had driven speciation: selection of sister species

with contrasting ecological requirements and optimisation of ecological characters on to the phylogeny.

To identify the species pairs, the selective total evidence tree was examined for sister species. The species within each pair were then compared for evidence of difference in substrate preference, altitudinal range or phenology. Altitudes and flowering times were extracted from the specimen database, as well as corroboration from field observations. As both are linear and directly quantifiable in their variation, the presence or absence of discrete gaps between species could be easily substantiated. This was not possible with substrates and so each soil-type needed to be classified. Hence, substrate preference was divided into five broad categories following Dahlgren (Dahlgren, 1968): the clayish soils of the Bokkeveld and Malmesbury Series of the lowlands; the acidic soils of the Table Mountain Series; the sand and shales of the Witteberg Series; and the more alkali recent deposits of marine aeolian sands or limestones. However, for this study the Malmesbury Series was combined with the Bokkeveld Series as the former has very few species that grow on it and none of them are endemic (unless *C. acocksii* is recognised as a distinct species, see below). In addition, the Cape Granites were also scored to determine whether any species demonstrated substrate selection for them. Substrates that occurred outside the CFR were not considered for this analysis. While finer subdivision of the geological categories is possible, this would have also increased the level of polymorphisms present, making it harder to detect any evolutionary shifts.

The other two ecological characters investigated, water supply and life-history in response to fire, were optimised onto the selective total evidence tree in the same manner as for the morphological characters. The multiple factors that affect the water supply to any species means that only broad categories could be defined based upon the observable nature of the habitat in which they grow. Three categories were used to define the dependence of the species upon being inundated by water around their roots: no discernible preference for water around the roots, constant supply of water to the roots, and a tolerance for seasonal inundation of water at the roots.

Classifying life-history into resprouting and seeding strategies is not a straight dichotomy, as some species can vary their strategy depending upon severity and frequency of the disturbance (Bellingham & Sparrow, 2000), e.g. *C. ruscifolia* (van Wilgen & Forsyth, 1992). Within the Cape Flora it is also complicated by different ecotypes of species existing, which in some parts of their range resprout while elsewhere they are seeders (Ojeda, 1998). For *Cliffortia*, the distinction is further

complicated by having two different resprouting strategies. Species resprout either from a central crown with a thickened lignotuber or from an underground network of roots producing new stems at intervals along their length. The classification of these two resprouting strategies is not always well defined and determination is often difficult unless regrowth is observed soon after a fire. The scoring for each species can only be done from field observations and these are frequently from a single suitable sighting or extrapolated from observations on the growth form. Therefore, it is possible that more species are polymorphic than recorded and some may be wrongly typed.

Although geographical speciation modes are of questionable use, because ranges can change subsequent to speciation (Barraclough & Vogler, 2000), they are easier to determine than the presence or absence of gene flow. Hence, species pairs were divided into three categories based upon their degree of overlap between current distribution ranges. No overlap in ranges was regarded as allopatric, slight overlap or abutting ranges as parapatric, and overlap for the greater part of their distributions as sympatric.

Results and Discussion:

Morphological evolution:

Examination of the circumscription of Weimarck's subgenus Digraphidium (= sect. Complanatae) (Fig. 3.1) shows that he was incorrect to give such high prominence to the presence of two achenes in the receptacle. This feature has clearly arisen more than once, and is even variable within species previously thought to have been exclusively unicarpellate, e.g. *C. micrantha* (Fellingham, 1993a) and *C. juniperina*. There have also been observations that some of his species, which he regarded as bicarpellate, are sometimes unicarpellate, e.g. *C. complanata* and *C. hantamensis*, indicating that this character may not only be labile, but also variable within species.

Likewise tetramerous flowers are shown to have had at least six origins (Fig. 4.1) and tetramery is a derived character within *Cliffortia*. On the other hand, it is not as variable as the number of achenes and there are no cases of reversion from tetramerous back to trimerous under parsimony arguments. The only possible exception is in *C. paucistaminea*, which has a confirmed case of a single population with trimerous flowers. It is therefore a good character for defining clades, and hence has been useful in the classification of the sections (see Appendix 1). The presence of four sepals is always associated with small flowers (male flower sepals <5 mm long) and either four or eight stamens, while the species with three sepals have a greater range of flower sizes

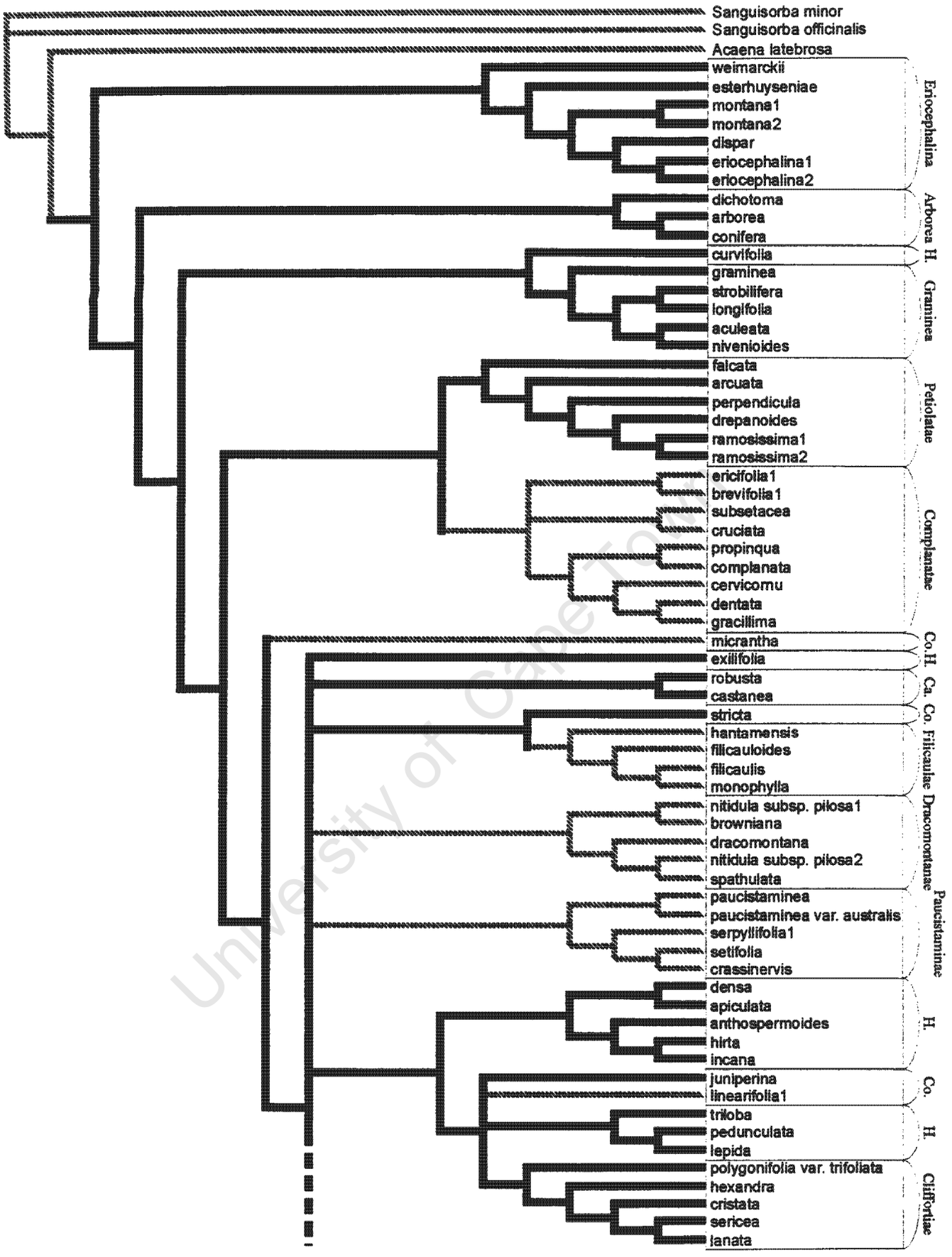
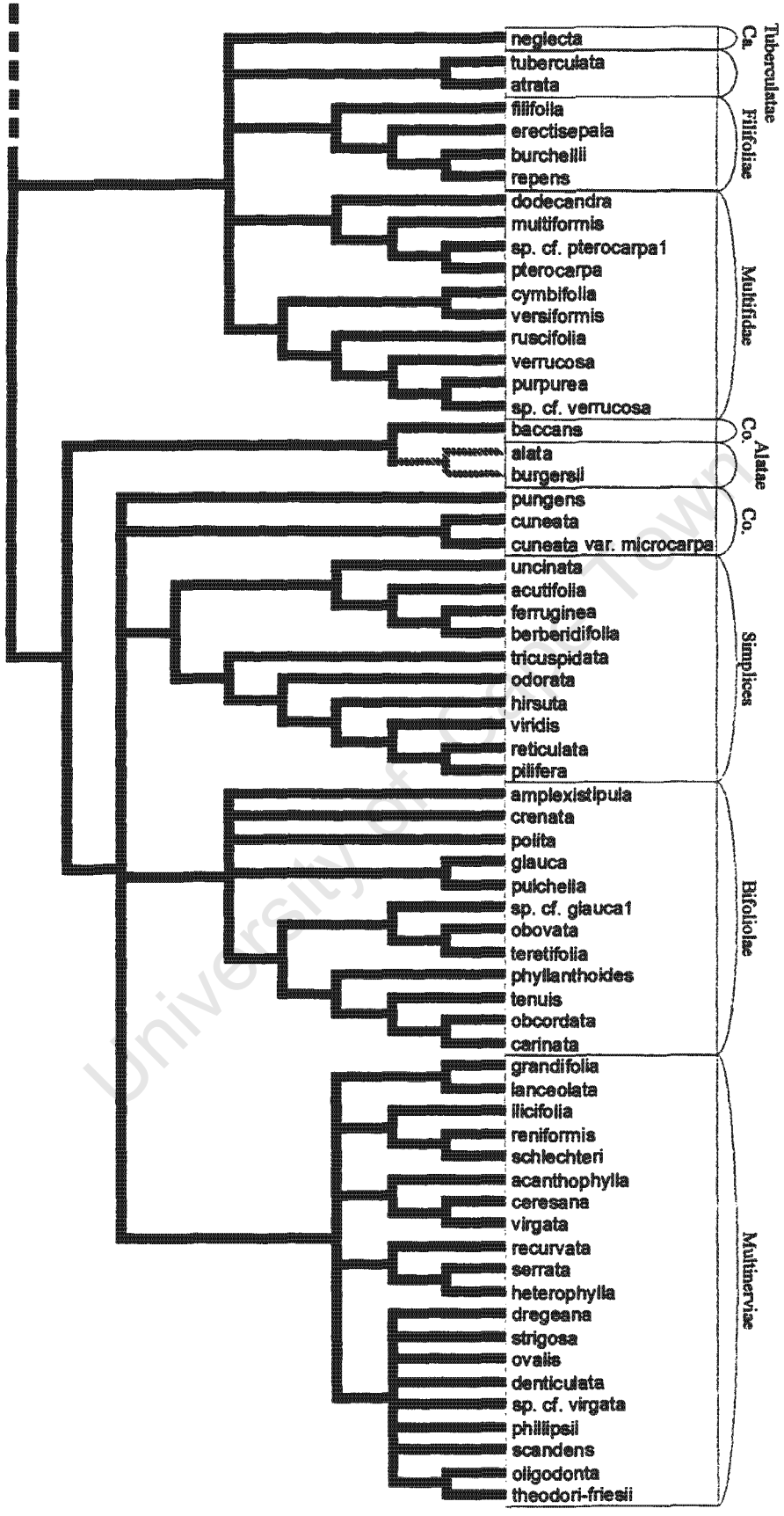


Fig. 4.1. Sepal number optimised onto the selective total evidence tree. Solid black lines indicate species with three sepals, diagonal lines four sepals.


neglecta
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filifolia
erectisepala
burchellii
repens
dodecandra
multiformis
sp. cf. pterocarpa1
pterocarpa
cymbifolia
versiformis
ruscifolia
verrucosa
purpurea
sp. cf. verrucosa
baccans
alata
burgersii
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata
hirsuta
viridis
reticulata
pilifera
amplexistipula
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii
Tuberculatae
Ca.
Filifoliae
Multifidae
Alatae
Co.
Co.
Simplices
Bifoliolae
Multinerviae

Fig. 4.1 cont.

and stamen number but can be as small as four-sepalled flowers and have only 6 stamens. However, it is not clear what pressures select for small flowers with few stamens and how this influences the sepal number.

Both Dahlgren and Weimarck regarded trifoliate leaves and the presence of a petiole as ancestral states. While trifoliate leaves appear to be supported as an ancestral condition (Fig. 4.2), the presence of a petiole does not (Fig. 4.3). However, indications of ancestral states can sometimes be intimated by examining the ontogeny of particular organs (Nelson, 1978; Nelson & Platnick, 1981; Nelson, 1985). Seedlings have only been found for a few species in the wild, and while others have been successfully germinated in cultivation, the coverage of species is still sparse (Table 4.1). From what is known, several of the species (e.g. *C. burchellii*, *C. conifera*, *C. dichotoma* MS) show some evidence of a petiole in their seedling leaves, which is subsequently lost as the leaf matures. (The juvenile leaves are also often more toothed than the adult leaves.) The extreme example of this appears to be in the needle-leaved *C. nitidula* subsp. *pilosa*, which has often been misidentified as the broad-leaved petiolate *C. filicauloides* when only juvenile leaves are present. On the other hand, the number of leaflets in the seedlings is more variable. For example, sometimes a species with trifoliate mature leaves will have unifoliate juvenile ones, e.g. *C. atrata*, *C. multiformis*, while unifoliate species may have juvenile trifoliate leaves, e.g. *C. dichotoma* MS, *C. repens*.

Table 4.1. Comparison of number of leaflets in seedlings and resprouting leaves to the mature foliage for species where it is known.

		Seedling or resprouting leaves	
		Trifoliate	Unifoliate
Mature	Trifoliate	*C. browniana*, *C. burchellii*, *C. cervicornu*, *C. complanata*, *C. conifera*, *C. dentata*, *C. dracomontana*, *C. exilifolia*, *C. falcata*, *C. filicaulis*, *C. filicauloides*, *C. montana*, *C. nitidula*, *C. pedunculata*, *C. ramosissima*, *C. sericea*, *C. serpyllifolia*, *C. spathulata*, *C. stricta*	*C. atrata*, *C. dodecandra*, *C. multiformis*, *C. robusta*
	Unifoliate	*C. dichotoma*, *C. dispar**, *C. linearifolia**, *C. monophylla*, *C. repens*	*C. acanthophylla*, *C. brevifolia*, *C. cuneata*, *C. dregeana*, *C. grandifolia*, *C. hirsuta*, *C. reticulata*, *C. ruscifolia*, *C. tricuspidata*

*Species also sometimes have trifoliate mature leaves.

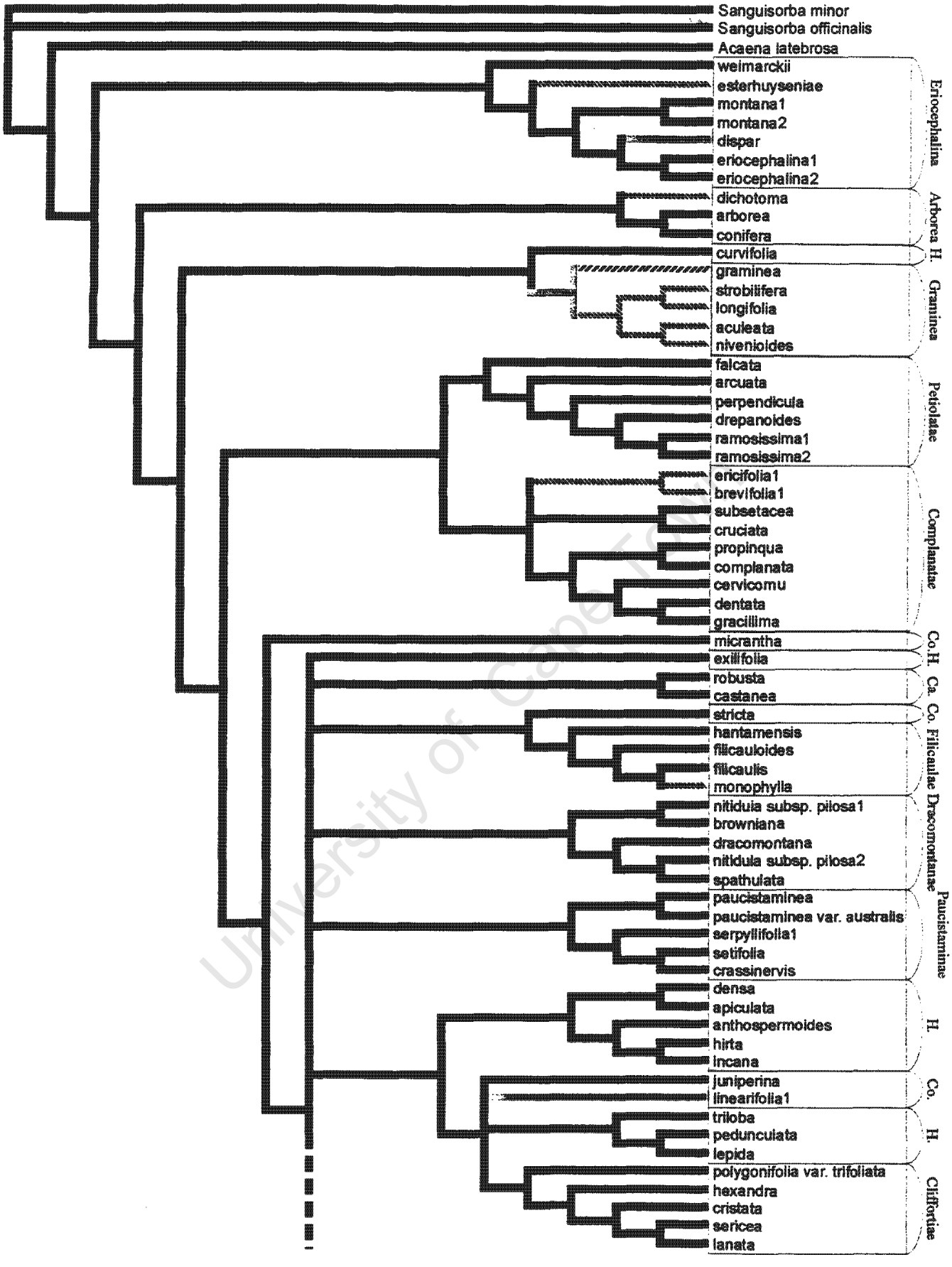


Fig. 4.2. Leaflet number optimised on to selective total evidence tree. Solid black branches indicate trifoliate leaves, vertical hatching bifoliate and diagonal hatching unifoliate. Broad forward diagonal hatching indicates multinerved simple leaves, while narrow backward indicates single-nerved simple leaves. Grey indicates polymorphic or equivocal.

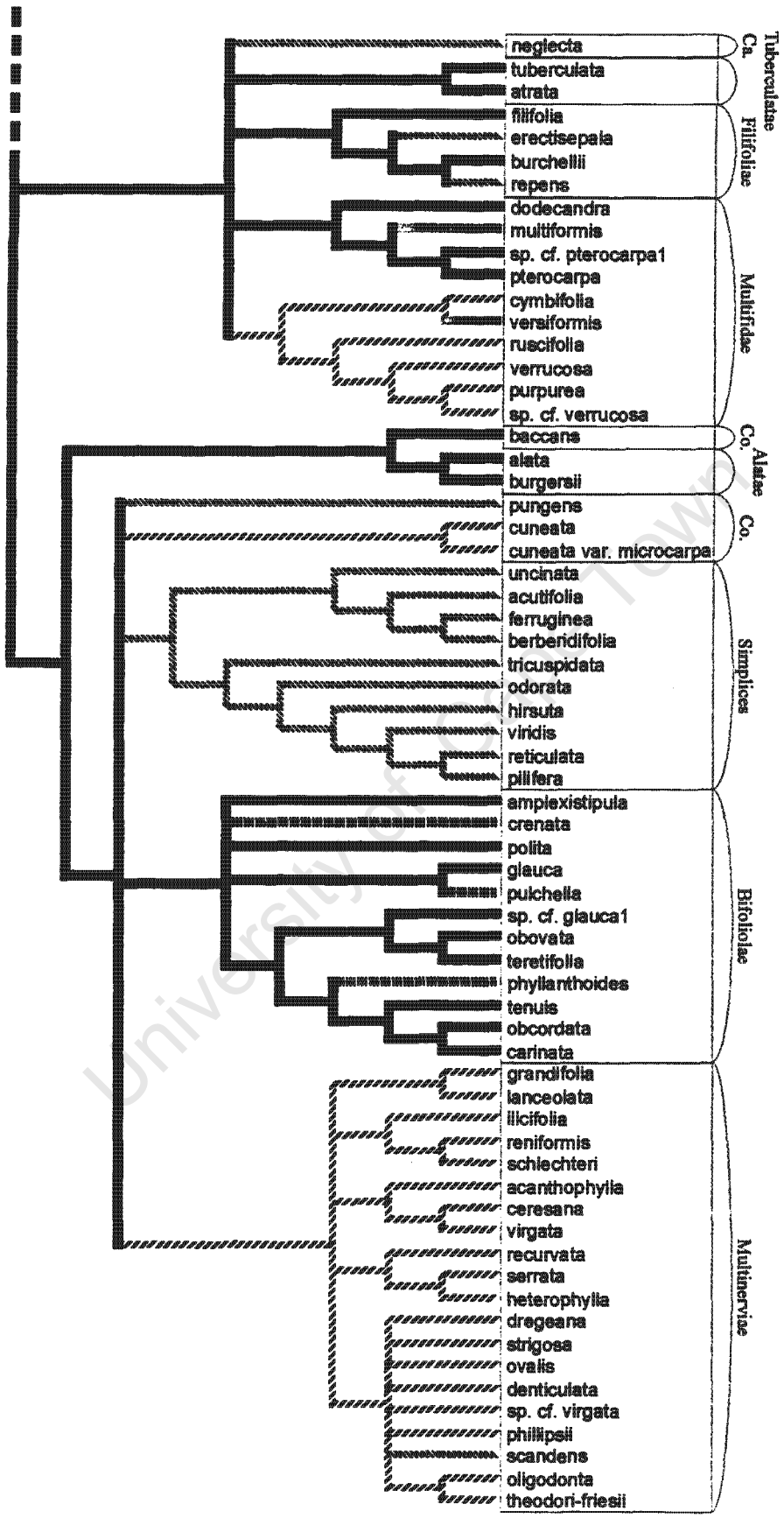

neglecta
tuberculata
atrata
filifolia
erectisepala
burchellii
repens
dodecandra
multiformis
sp. cf. pterocarpa1
pterocarpa
cymbifolia
versiformis
ruscifolia
verrucosa
purpurea
sp. cf. verrucosa
baccans
alata
burgersii
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata
hirsuta
viridis
reticulata
pilifera
amplexistipula
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii
Tuberculatae
Ca.
Filifoliae
Multifidae
Alatae
Co.
Co.
Simplices
Bifoliolae
Multinerviae

Fig. 4.2 cont.

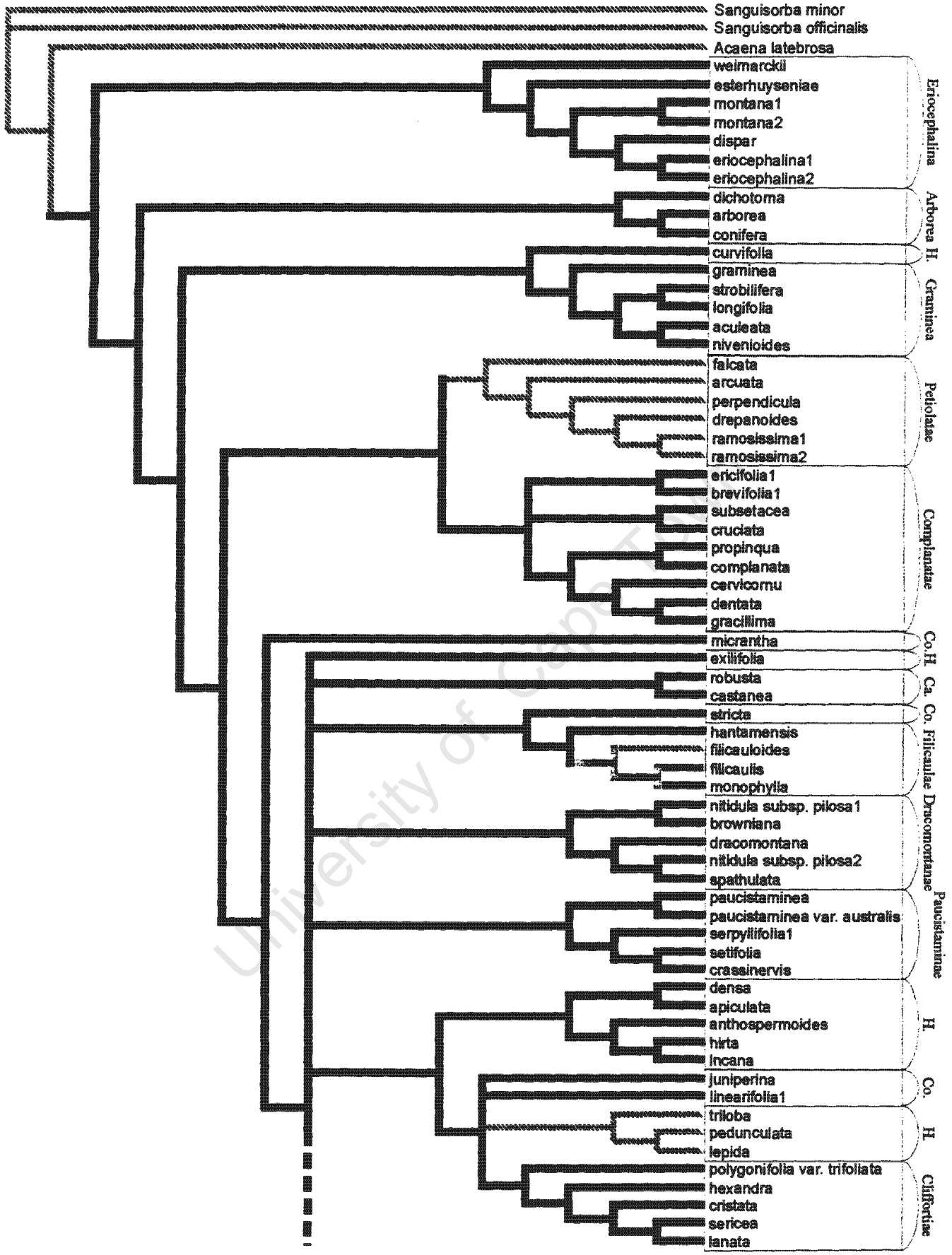


Fig. 4.3. Petiole presence optimised on to selective total evidence tree. Solid black lines indicate no petiole, diagonal hatching indicates that leaves are petiolate, grey lines indicate equivocal or polymorphic.

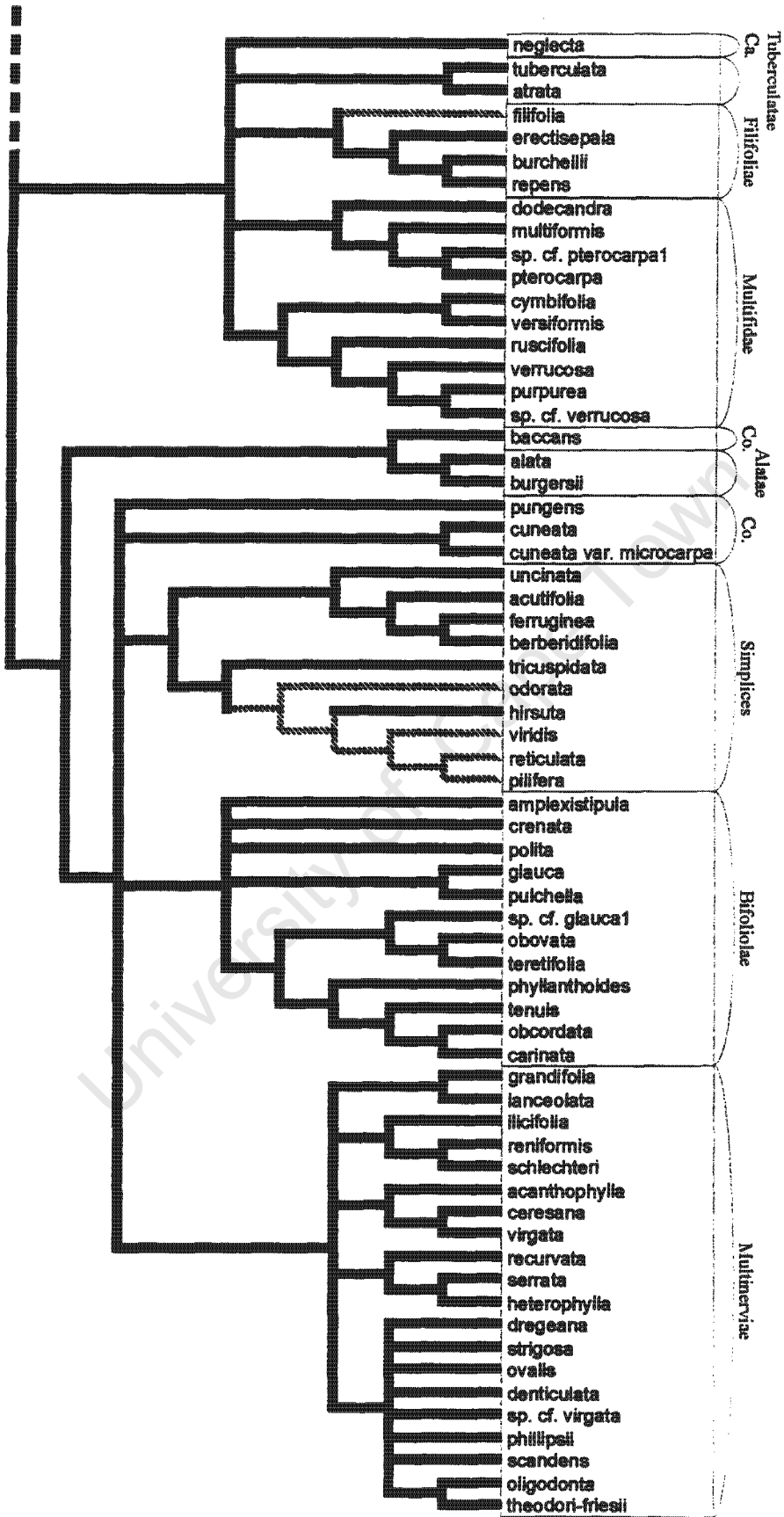

neglecta
tuberculata
atrata
filifolia
erectisepala
burcheilii
repens
dodecandra
multiformis
sp. cf. pterocarpa1
pterocarpa
cymbifolia
versiformis
ruscifolia
verrucosa
purpurea
sp. cf. verrucosa
baccans
alata
burgersii
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata
hirsuta
viridis
reticulata
pilifera
amplexistipula
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii
Tuberculatae
Ca.
Filifoliae
Multifidae
Alatae
Co.
Co.
Simplices
Bifoliolae
Multimerviae

Fig. 4.3 cont.

Table 4.2. Number of morphological changes in leaf shape in agreement with Dahlgren's hypothesis and number of reversals that oppose the hypothesis. Figures are based upon Figs. 4.2 & 4.3. Polymorphisms in species are excluded from the counts.

	Changes	Reversals
Petiole present to petiole absent	3	5
Trifoliate to bifoliate	3	0
Trifoliate to unifoliate with single vein	10	0
Trifoliate to unifoliate with multiple veins	4	0

This appears to imply that while trifoliate leaves may be the ancestral form for the genus, for some species it is a derived character, having reverted to the trifoliate state from a unifoliate leaf. On the other hand, although a petiole appears to be a derived character, its expression is possible in some species early in a plant's development, suggesting that it might be the ancestral state. It is therefore not surprising that the leaf-form has 'reverted' to being petiolate on more than one occasion. With this exception in mind, Table 4.2 indicates that Dahlgren's hypothesised evolutionary pathway for leaf morphology is applicable for the most part. The ancestral *Cliffortia* can be seen as having a leaf-type akin to *C. pedunculata*, with an early reduction of the petiole, and from that basic type the various modifications have arisen. However, Weimarck's implication from his sectional classification that there was a single origin of the unifoliate leaf-type and the multinerved leaf-type is shown to be false. Both these modifications have arisen more than once: the unifoliate leaf at least ten times, the multinerved only four (Table 4.2 & Fig. 4.2). This also matches Dahlgren's (Dahlgren, 1970) findings of convergent leaf-types amongst the legumes.

It is hard to associate the change in leaflet number to any particular habitat shift. While it might be expected that unifoliate leaves would be found in more arid areas, as less leaf surface will be exposed for transpiration of water, the distribution of unifoliate leaves within *Cliffortia*, both multinerved and single-nerved, does not support this. For example, within the unifoliate section Simplices, both needle-shaped leaves, *C. uncinata*, and the broadest leaves in the genus, *C. odorata*, are found. Similarly, the multinerved section Multinerviae has species that grow in arid well-drained sand through to wet south-facing slopes or in seeps. However, of interest is the fact that bifoliate leaves only occur in section Bifoliolae yet have evolved independently three times (and a further species, *C. obcordata*, has a highly modified middle leaflet). This might indicate some developmental tendency within the section for reduction of the middle leaflet.

Speciation:

The diversity of species in *Cliffortia* has arisen through a number of different mechanisms (Table 4.3). Over half of the speciation events (52.8%) identified have been sympatric, agreeing with the view of Linder (1985) that selection across strong ecological gradients can frequently override the limited degree of infraspecific gene flow that is suspected for Cape taxa. However, parapatric (25%) and allopatric (22.2%) speciation events are also very evident. In addition, hybridization leading to speciation contribute 27.8% of the sympatric events (Table 4.3). If these hybrid speciation events are the result of allopolyploidy or recombinational speciation, then it can also be stated that 75% of the speciation events have a barrier to gene flow instigated prior to selection. Nevertheless, caution must be used with all these assessments of the role of geography in speciation, as distribution ranges can change subsequent to the speciation event (Barraclough & Vogler, 2000).

Table 4.3. List of species pairs and their putative modes of speciation, with possible shifts in ecology that have permitted sympatric speciation to occur. Allopatry is indicated where current species ranges do not overlap, parapatry for ranges that are contiguous or only slightly overlap, and sympatry for ranges that are concurrent or overlap for the greater part.

Species pair	Mode & mechanism of speciation	Ecological shift
C. acanthophylla & *C. ceresana*	parapatric and hybridization	fire survival and rainfall
C. aculeata & *C. nivenioides*	parapatric and neoteny	unknown
C. alata & *C. burgersii*	allopatric	substrate
C. arborea & *C. sp. cf. arborea*	allopatric	?substrate
C. atrata & *C. tuberculata*	sympatric	fire survival or tolerance
C. berberidifolia & *C. ferruginea*	sympatric and neoteny	?water dependence
C. brevifolia & *C. ericifolia*	sympatric	?water dependence
C. burchellii & *C. repens*	allopatric	fire survival
C. carinata & *C. obcordata*	sympatric and hybridization	unknown
C. castanea & *C. robusta*	parapatric	substrate or fire tolerance
C. ceresana & *C. virgata*	sympatric and hybridization	?altitude or substrate
C. complanata & *C. propinqua*	parapatric	altitude and rainfall
C. crassifolia & *C. setifolia*	sympatric and hybridization	unknown
C. cruciata & *C. subsetacea*	allopatric and hybridization	?altitude and rainfall
C. dentata & *C. gracilis*	parapatric	unknown
C. dentata & *C. gracillima*	allopatric	unknown
C. dispar & *C. montana*	allopatric	rainfall
C. dodecandra & *C. multiformis*	allopatric	?fire survival
C. drepanoides & *C. ramosissima*	sympatric	fire survival and ?rainfall
C. eriocephalina & *C. dispar*	sympatric	fire survival
C. eriocephalina & *C. montana*	sympatric	fire survival
C. esterhuyseniae & *C. weimarckii*	sympatric	fire survival
C. filicaulis & *C. monophylla*	sympatric and hybridization	unknown
C. grandifolia & *C. lanceolata*	sympatric and hybridization	fire survival
C. heterophylla & *C. serrata*	sympatric and hybridization	fire survival
C. hexandra & *C. polygonifolia*	sympatric	rainfall
C. hirsuta & *C. viridis*	sympatric and hybridization	unknown
C. lepida & *C. pedunculata*	parapatric	?altitude
C. lepida & *C. triloba*	parapatric and hybridization	fire survival and water dependence
C. longifolia & *C. strobilifera*	sympatric and neoteny	substrate
C. neglecta, *C. tuberculata* & *C. ×homunculi*	sympatric and hybridization	fire survival
C. obovata & *C. teretifolia*	allopatric and hybridization	rainfall
C. odorata & *C. reticulata*	parapatric and hybridization	?altitude
C. perpendicula & *C. ramosissima*	sympatric and hybridization	unknown
C. reniformis & *C. schlechteri*	parapatric and hybridization	?substrate
C. ruscifolia & *C. cymbifolia*, *C. verrucosa*, or *C. versiformis*	sympatric and hybridization	unknown

Substrate

The majority of *Cliffortia* species grow on sandstone-derived soils (Fig. 4.4). It is also evidently the ancestral substrate in the genus. For the most part, only isolated terminal species have successfully shifted onto other soil-types. This indicates that *Cliffortia* could successfully occupy these habitats only relatively recently, and that most of the early evolution in the genus occurred on sandstone soils. However, several species do have preferences for the shale slopes of the TMS mountains and are also found

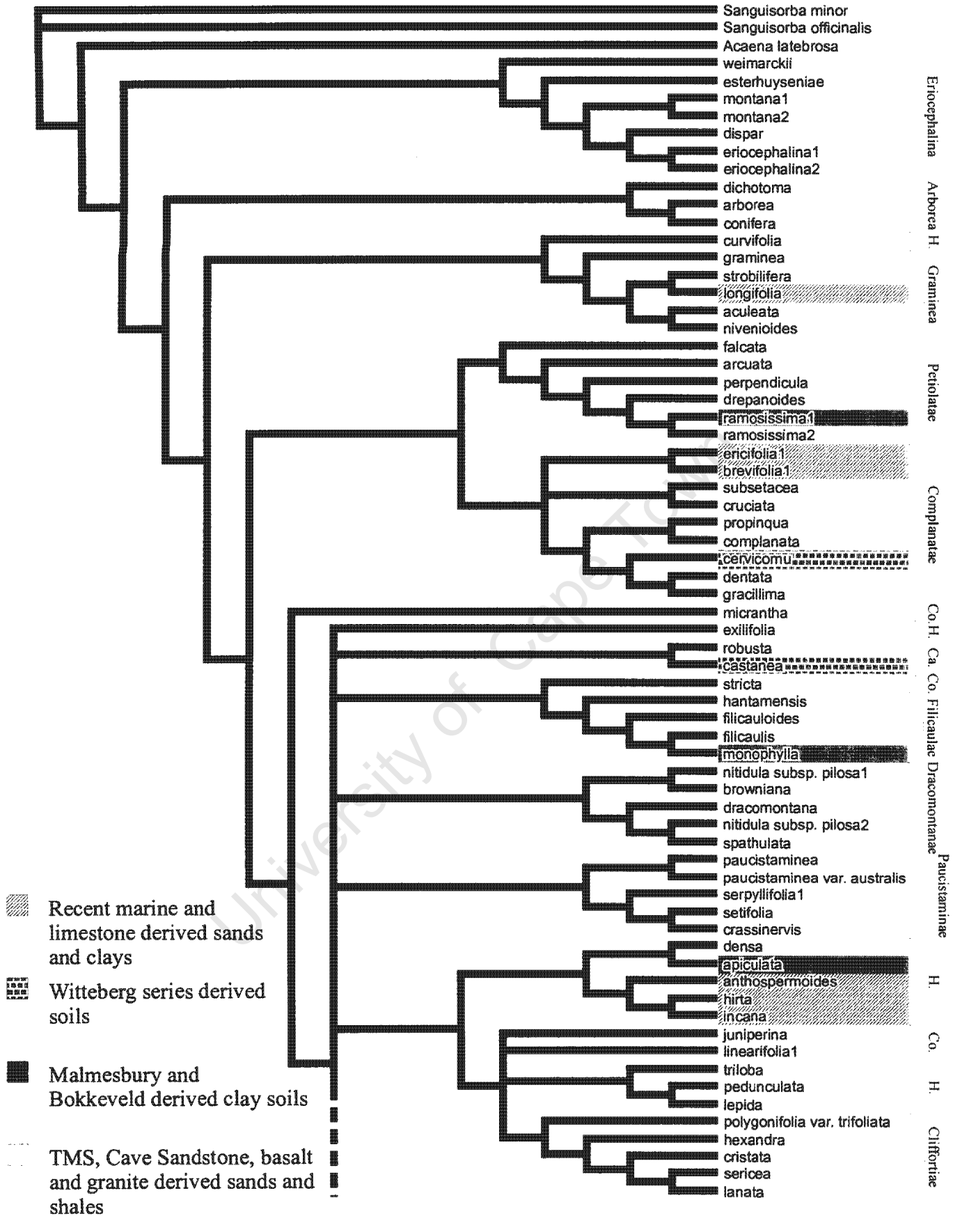
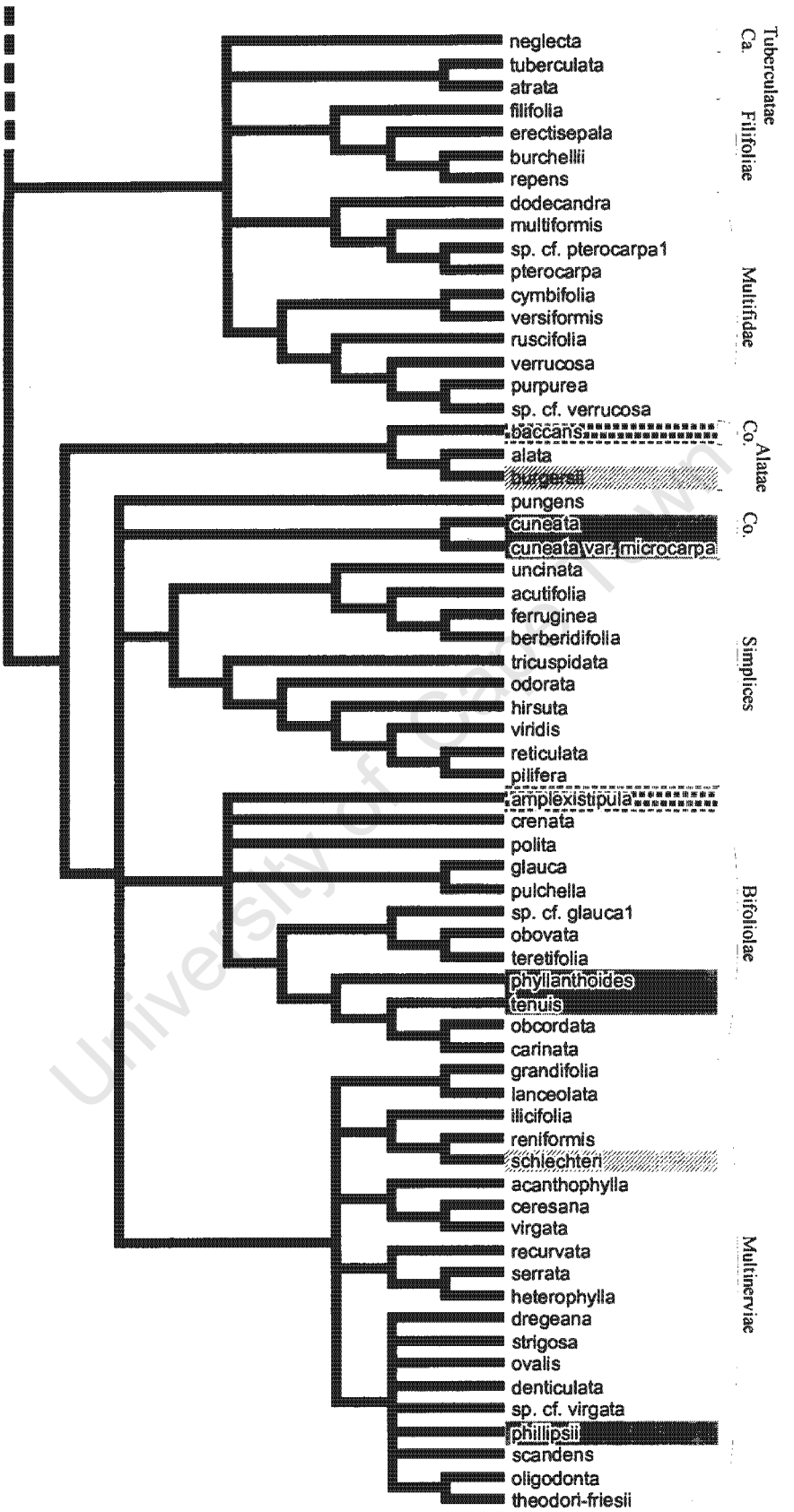


Fig. 4.4. Species endemism to certain soil types. Species polymorphic for more than one soil type are attributed to the general category 'TMS, Cave Sandstone, basalt and granite derived sands and shales'.


neglecta
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filifolia
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dodecandra
multiformis
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cymbifolia
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purpurea
sp. cf. verrucosa
baccans
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burgeri
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata
hirsuta
viridis
reticulata
pilifera
amplexistipula
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii
Ca. Tuberculatae
Filifoliae
Multifidae
Co. Alatae
Co.
Simplices
Bifoliolae
Multinerviae

Fig. 4.4 cont.

frequently on the clayish soils of the Bokkeveld or Malmesbury Series (Dahlgren, 1968), e.g. *C. filicaulis*, *C. juniperina*, *C. polygonifolia* (Table 4.4). The shift from the sandstone-derived soils to the recent deposits is difficult physiologically for a species because it involves a swing in pH from acid to alkaline. However, four species show the ability to persist on both acidic and alkali sands, *C. falcata*, *C. ferruginea*, *C. filifolia* and *C. obcordata* (Table 4.4). *C. falcata* is particularly notable as the only species that has been found growing directly out of limestone itself (*pers. obs.*, e.g. C.M. Whitehouse 256, Bredasdorp District, De Hoop Nature Reserve). While both *C. falcata* and *C. obcordata* appear to have a preference for recent marine sand deposits, they have been found on the lower slopes of TMS mountains near the sea, such as above Simonstown or Hermanus, demonstrating that they can tolerate acidic soils too (see below for *C. filifolia*). *C. ferruginea* is more particular about water requirements and the soil type does not appear to be such a determinant in its distribution. Indeed, *C. ferruginea* will grow on TMS sands, clayish shale or recent sand deposits as long as it is seasonally inundated with water. Similarly, *C. strobilifera* is also very catholic in its soil types providing that it is beside running water, except that on the alkali sands of marine and limestone deposits it is replaced by *C. longifolia* (see below).

Table 4.4. List of species within the Cape Floristic Region that grow on or are restricted to a particular substrate. Species in bold are endemic to that substrate.

Substrate	Species known to grow on that substrate
Malmesbury and Bokkeveld Series derived clay soils	***C. acocksii***, ***C. apiculata***, ***C. cuneata***, *C. ferruginea*, *C. filicaulis*, *C. juniperina*, ***C. monophylla***, ***C. phillipsii***, ***C. phyllanthoides***, *C. polygonifolia*, *C. ramosissima*, *C. ruscifolia*, *C. strobilifera*, ***C. tenuis***
Recent marine and limestone derived soils	***C. anthospermoides***, ***C. brevifolia***, ***C. burgersii***, ***C. ericifolia***, *C. falcata*, *C. ferruginea*, *C. filifolia*, ***C. hirta***, ***C. incana***, ***C. longifolia***, *C. obcordata*, ***C. schlechteri***
Witteberg Series derived soils	***C. amplexistipula***, ***C. baccans***, ***C. castanea***, ***C. sp. cf. arborea***, ***C. cervicornu***, *C. hantamensis*, *C. ruscifolia*, *C. teretifolia*, *C. tuberculata*
Granite derived soils	*C. erectisepala*, *C. filicaulis*, *C. filifolia*, *C. juniperina*, *C. pterocarpa*, *C. ruscifolia*, *C. strobilifera*, *C. teretifolia*

Two species, *C. filifolia* and *C. ramosissima* appear to shift their preference for soil type across their ranges. In the south-western part of its distribution, *C. ramosissima* is found exclusively on the Bokkeveld Series clayish soils, but in the south-east it is found on TMS-derived sands and in the north-east of its range on the Cave Sandstones of the Free State. This species has a most unusual distribution (Weimarck, 1934; Weimarck, 1941; Weimarck, 1946, Fig. 5.16) and it is possible that more than one entity is involved here. Similarly, *C. filifolia* is found on TMS derived shale slopes in the south-west of its range but exclusively on the marine sands of dune slacks east of Cape Agulhas. Again,

the chance that two separate entities are involved is possible. (*C. acocksii* is a doubtfully distinct species that grows on the clay soils of the Malmesbury Series. Unfortunately, it has not been recollected recently and so has not been assessed by molecular techniques, but it is morphologically very similar to a stunted form of *C. filifolia*.). Alternatively, in both these cases the soils could affect factors that the species is more dependent upon, such as water availability, so that different soil types provide a more appropriate regime for the species across the varying climatic patterns of its range.

Of the species restricted to a particular soil type the most noteworthy clade belongs to Series Hirta. All three species of this section are narrow endemics and restricted to soils at the border between acid and alkaline sands. This clade therefore has a very specialised habitat requirement, which partly explains its disjunctive distribution and the narrow endemism of its species. However, the sister clade cannot be readily identified as the whole clade appears to be of hybrid origin and the closest relative for the nuclear genome is uncertain (*C. cuneata* being a very isolated species morphologically and whose relationship to other species from molecular evidence is equally unclear). This is unfortunate as otherwise it might be possible to have had a tentative internal node for dating the phylogeny, since the tertiary deposits of the limestone and marine sands can be dated as about 3–5 million years old.

Only one species pair shows a clear ecological shift of soil types, namely *C. longifolia* and *C. strobilifera*. *C. strobilifera* is a ubiquitous species while *C. longifolia* is confined to water edges on marine and limestone deposits. Although distinction between the two species is unambiguous (Fellingham, 1994), they remain morphologically very similar. The distribution range of *C. longifolia* is quite large, from Langebaan to the Gouritz R mouth, but the populations are very widely scattered within it (Fellingham, 1994) and *C. strobilifera* is sympatric throughout its entire range. It is therefore possible that *C. longifolia*, and its affinity for more alkali conditions, was the result of more than one speciation event. If this was the case it would provide strong evidence for a link between particular morphological traits and an ecological shift (Orr & Smith, 1998; Schluter, 2001). However, detection of this will be difficult as genetic variation in the samples of the two species was very low and this will be confounded if there is still some level of introgression occurring.

The other sister species with differing soil types do not show evidence of soil preference as a speciation event. *C. monophylla* is restricted to Bokkeveld Shales, but *C. filicaulis* also has a preference for shale soils, only rarely being found on sandstone (e.g. Caledon

Swartberg) or granite (e.g. Perdeberg) derived soils. Indeed, both species are often found growing in the same place (e.g. Drayton Siding, Caledon, *Goldblatt* 2514, a mixed collection of both species) and it is difficult to detect any selection of ecological niche between them. *C. castanea* and *C. robusta* do show evidence of soil preference but they also differ in other habitat preferences, which make it difficult to determine which factor has influenced the speciation process (i.e. *C. castanea* grows at medium altitudes in gulleys on shale bands which are subject to burning, while *C. robusta* grows at very high altitudes amongst sandstone rocks where it escapes fire). The remaining species pairs, *C. alata* and *C. burgersii*, *C. arborea* and *C. sp. cf. arborea*, and *C. reniformis* and *C. schlechteri*, have one species that is only known from a single population so that it is not possible to determine whether its distribution is restricted by soil type or some other factor.

Species that grow on soil types other than TMS but are not endemic to them are widespread and common species that often grow in a diverse range of habitats (Table 4.4). Only *C. hantamensis* is rare and scattered in its distribution (Fig. 5.19), but it is still a widespread species and its rarity may be more due to lack of knowledge as it grows in areas that are poorly known botanically. Granite has no endemic species and only ubiquitous species have been found growing on it (Table 4.4).

Altitude

Several widespread species show altitude variation across their range. For example, *C. linearifolia* is found at sea-level in the far west of its range around George, but is not found beneath 1000 m in Kwazulu-Natal. This is in contrast to *C. serpyllifolia*, which has a similar distribution from Swellendam to Kwazulu-Natal, but even in the north of its range it is only found on the sandstones towards the coast and below 750 m. One can hypothesise from this that the distribution of *C. linearifolia* is affected by temperature while *C. serpyllifolia* is more dependent upon substrate.

However, one species pair, *C. complanata* and *C. propinqua*, shows strong evidence of altitudinal replacement. *C. complanata* is distributed from the Riviersonderend Mts and Kogelberg, north as far as the Bains Kloof Mts. In the south and east of its range it grows around the 300–700 m level but north of Jonkershoek it is rarely found below 900 m. *C. propinqua* on the other hand is found from the Wemmershoek valley north to the Cederberg, with outlying populations in the east on the Hex River Mts and Langeberg. In the Cederberg and the outlying populations it is found between 900–1500 m altitude, but in the south, where its range overlaps with that of *C. complanata*, it only

grows at around 500–900 m. In Bains Kloof, where both species grow, replacement of one species with the other is apparent across the altitudinal range. Both species have a particular habitat requirement that is also very unusual for *Cliffortia* species: shady rock crevices (although in the south of their ranges both species are sometimes able to survive on open slopes). Hence their spatial distribution can best be explained in terms of altitudinal variation and the environmental factors that go with that shift.

Other species pairs (e.g. *C. pedunculata* and *C. lepida*) may also have experienced altitude shifts but the variation within and between these species is poorly understood or one species is known from a single population. As a result, no conclusions can be drawn until the delimitation of the species is made clearer and the distribution records confirmed.

Water

There are no cases of species pairs that show ecological separation from one water dependence regime to another (Fig. 4.5). It appears instead that adaptation to a different supply of water generally leads to diversification within that lineage. Four separate clades, subgenus Graminea, section Simplices, and series Ericifolia and Pedunculatae, as well as a single species *C. ovalis* (which lacks an unambiguous sister relationship), show an ecological preference for wet conditions around their roots. Sect. Simplices is further subdivided into two clades, one which requires year round moisture, while the other is more seasonal. Only sect. Pedunculatae shows evidence of a shift in water requirements between sister species, *C. triloba* and *C. pedunculata*, but this is also a case of putative hybrid speciation, as they are not sister species in the chloroplast phylogeny.

Fire-survival

The ability to resprout is regarded as an ancestral state within angiosperms (Wells, 1969; Lloret *et al.*, 1999). However, reconstruction of fire survival strategy onto the cladogram optimizes a seeder strategy as the ancestral state (Fig. 4.6). On the other hand, fire-survival strategy is the most homoplasious (CI = 0.036) of all the morphological characters and hence reconstruction of nodes deep within the tree is highly dubious (Cunningham *et al.*, 1998). Changes in the resolution at the base of the tree could easily alter the ancestral reconstruction for the character state. In addition, the extinction or exclusion of a resprouting form or the misidentification of a resprouter as a seeder in one of the basal clades could radically change the reconstruction of the deeper nodes. For example, if *C. curvifolia* is excluded from the analysis (or placed in a more

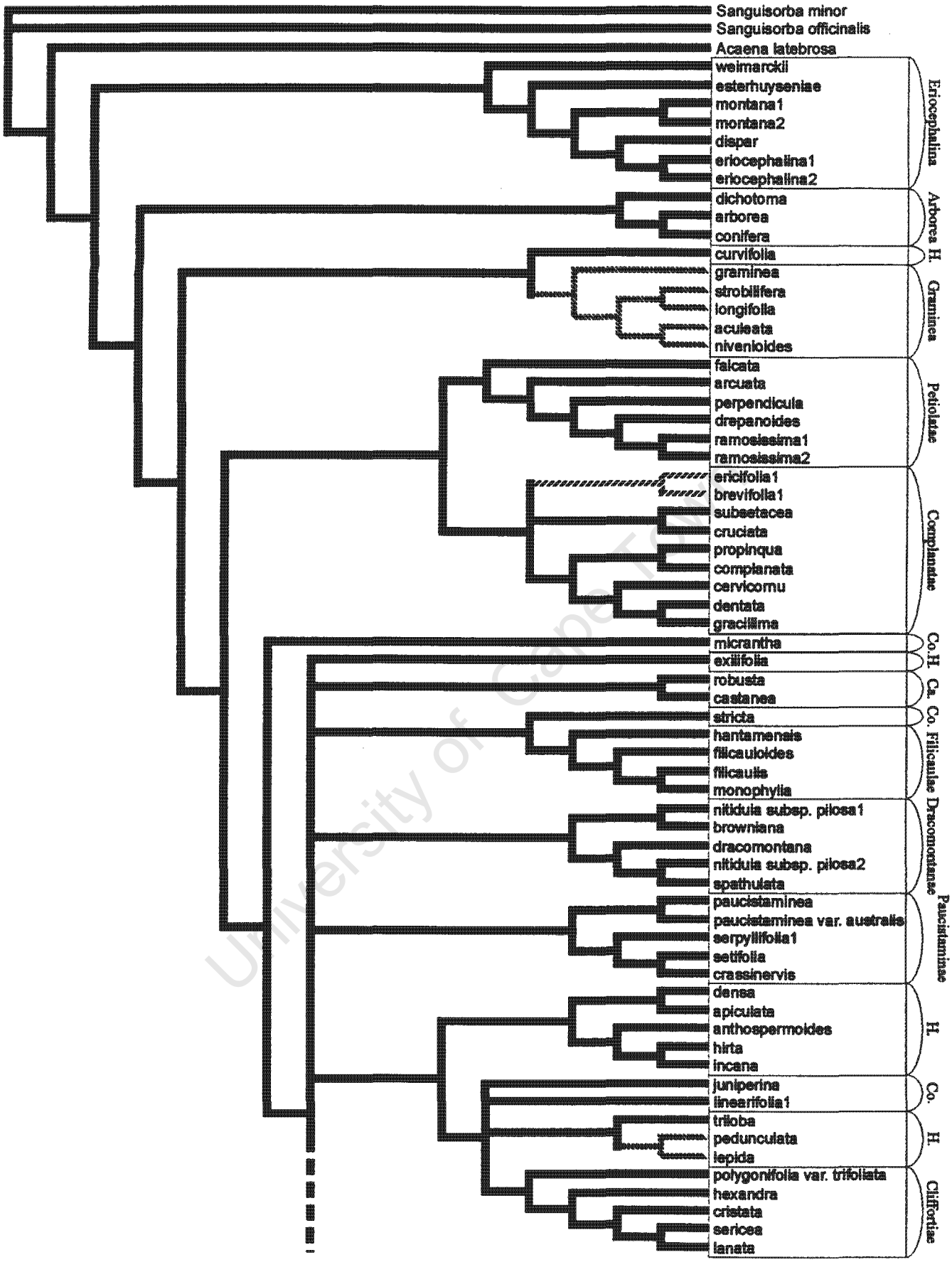

Fig. 4.5. Dependence of species upon water supply to their roots optimised on to the selective total evidence tree. Solid black lines indicate no clear dependence, diagonal hatching indicates that species are tolerant of seasonal water supply (broad forward) or need a constant water supply (narrow backward)

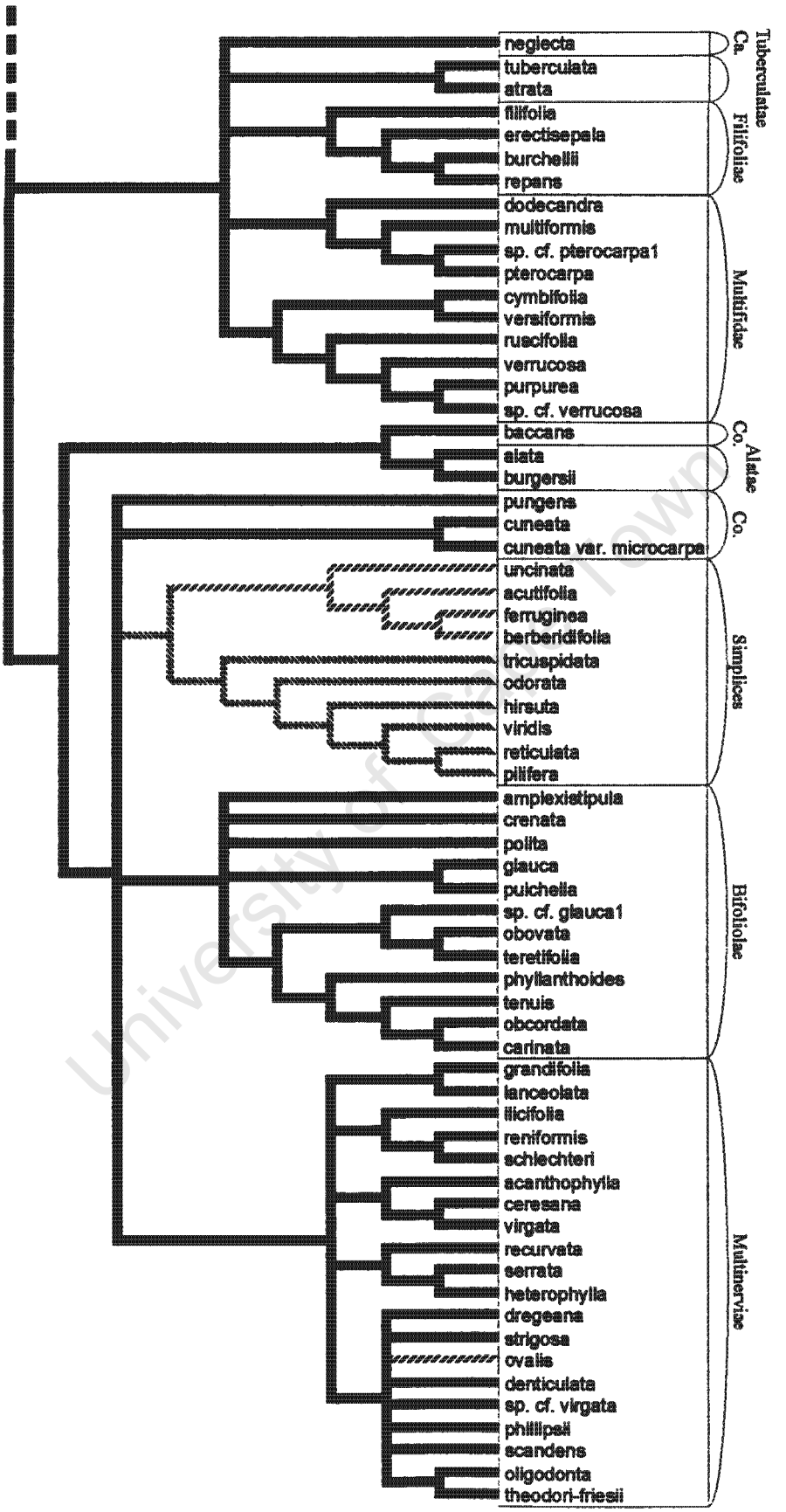
neglecta
tuberculata
atrata
filifolia
erectisepala
burchellii
repens
dodecandra
multiformis
sp. cf. pterocarpa1
pterocarpa
cymbifolia
versiformis
ruscifolia
verrucosa
purpurea
sp. cf. verrucosa
baccans
alata
burgersii
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata
hirsuta
viridis
reticulata
pilifera
amplexistipula
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii
Ca.
Tuberculatae
Filifoliae
Multifidae
Co.
Alatae
Co.
Simplices
Bifoliolae
Multinerviae

Fig. 4.5 cont.

derived position) and either *C. dichotoma* or *C. esterhuyseniae* is attributed the status of a resprouter then resprouting is optimised as the ancestral state.

Furthermore, reconstruction of ancestral states depends upon equal chance of gain and loss of the character state (Cunningham *et al.*, 1998; Omland, 1999) and this may not be so considering that the proportion of seeder species is generally higher in other taxa (le Maitre & Midgley, 1992; Schutte *et al.*, 1995; Ojeda, 1998). The problem of determining the ancestral condition is further complicated because the outgroups selected, as well as the majority of outgroup species, are not subject to a fire regime in their natural habitats, so that determining their strategy is inapplicable. In contrast, clonal underground spread by the roots is a rare character amongst fynbos woody shrubs, although it is known in more herbaceous taxa, e.g. Restionaceae and *Salvia*, while being a well-known character in other Rosaceae, such as *Rosa* and *Rubus*. Therefore, although reconstruction using parsimony implies that the seeder state was the ancestral condition for *Cliffortia*, the evidence to support it is very weak and external evidence points towards a resprouting ancestral state. However, within subgenus *Cliffortia* there is more convincing evidence for an ancestor with a seeder strategy.

The presence of species in the fynbos that are polymorphic for fire survival strategy implies that the switch from resprouting to seeding has occurred repeatedly (Bond & Midgley, 2001). Within *Cliffortia* those species that resprout from a central crown, except for *C. pterocarpa*, are derived from sister species that have a seeder strategy (Table 4.5, Fig. 4.6). In addition, many crown resprouters show a sensitivity to fire, preferring protected places such as between rocks, e.g. *C. neglecta*, *C. tuberculata*, *C. complanata* and *C. propinqua*, wet areas, e.g. *C. aculeata*, *C. graminea*, *C. nivenioides* and possibly *C. strobilifera*, or the less intense fires of montane grasslands, e.g. *C. linearifolia*, *C. nitidula* subsp. *pilosa*, *C. repens*, *C. spathulata*. Hence it appears as if they cannot tolerate intense or prolonged heat at or just below ground-level and some behave as facultative resprouters depending upon the habitat in which they grow (e.g. *C. ruscifolia*). These observations may support the hypothesis that crown resprouters derive from a seeder strategy rather than a clonal resprouter.

Table 4.5. Number of shifts between different life-histories in response to fire. Figures taken from Fig. 4.6.

	Changes	Reversals
Seeder to crown resprouter	7	0
Seeder to clonal resprouter	12	8
Clonal resprouter to crown resprouter	1	0

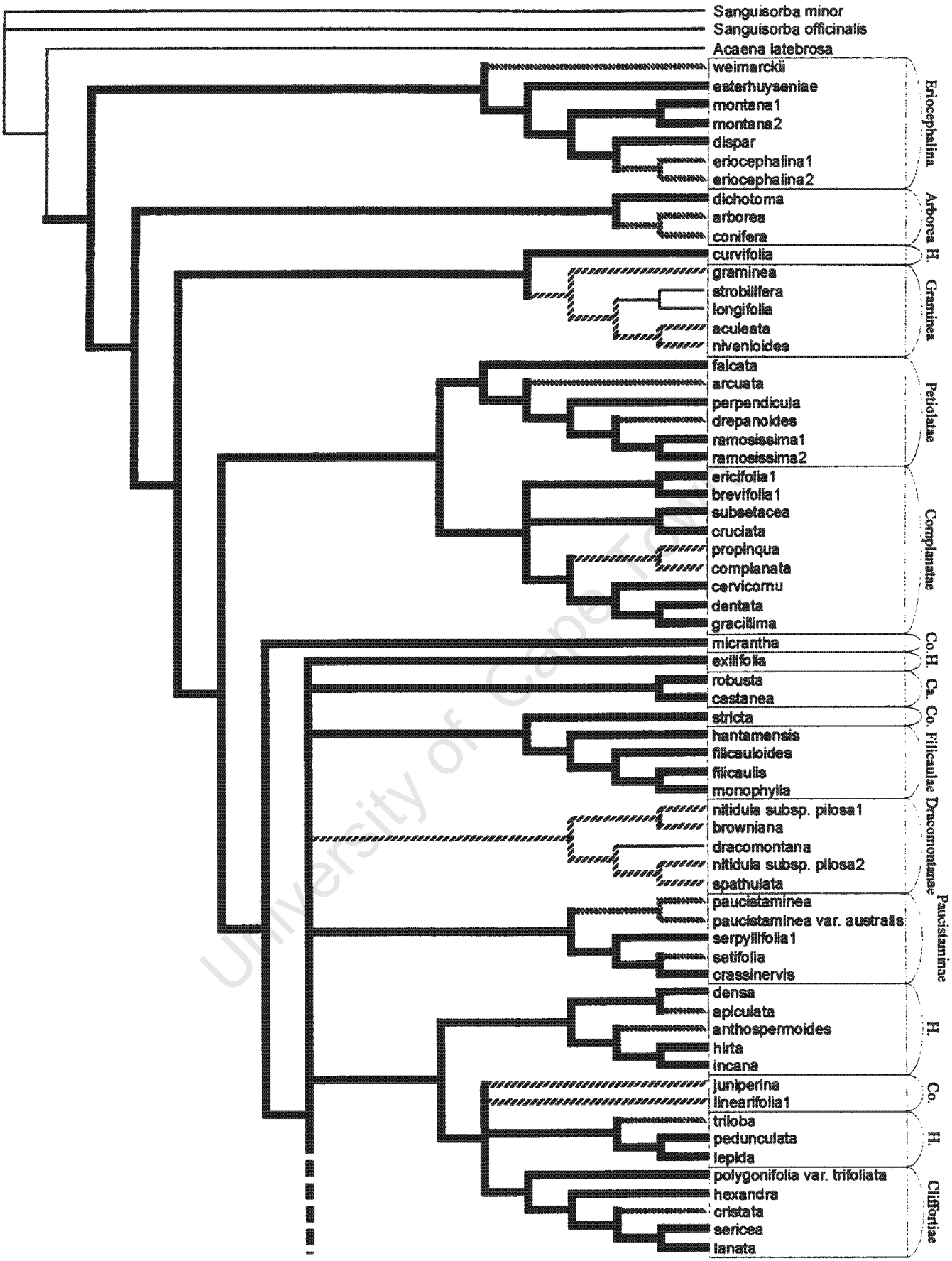

Fig. 4.6. Fire survival strategy optimised onto the selective total evidence tree. Solid black lines indicate seeder strategy, narrow backward diagonal indicate clonal resprouter, broad forward diagonal indicate crown resprouter, grey line indicates polymorphic and narrow lines indicate uncertain strategy.

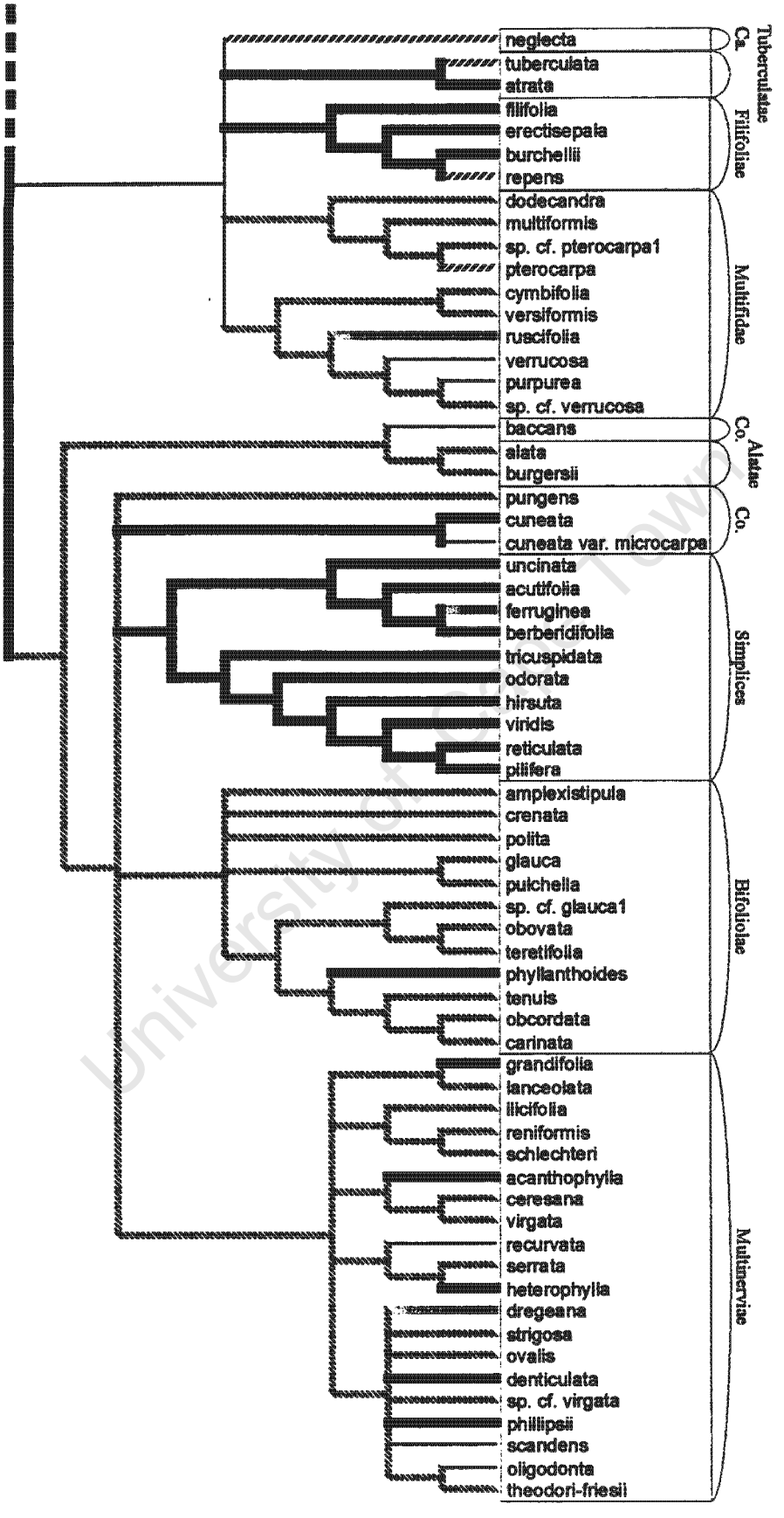
neglecta
tuberculata
atrata
filifolia
erectisepala
burchellii
repens
dodecandra
multiformis
sp. cf. pterocarpa1
pterocarpa
cymbifolia
versiformis
ruscifolia
verrucosa
purpurea
sp. cf. verrucosa
baccans
alata
burgersii
pungens
cuneata
cuneata var. microcarpa
uncinata
acutifolia
ferruginea
berberidifolia
tricuspidata
odorata
hirsuta
viridis
reticulata
pilifera
amplexistipula
crenata
polita
glauca
pulchella
sp. cf. glauca1
obovata
teretifolia
phyllanthoides
tenuis
obcordata
carinata
grandifolia
lanceolata
ilicifolia
reniformis
schlechteri
acanthophylla
ceresana
virgata
recurvata
serrata
heterophylla
dregeana
strigosa
ovalis
denticulata
sp. cf. virgata
phillipsii
scandens
oligodonta
theodori-friesii
Tuberculatae
Ca.
Filifoliae
Multifidae
Alatae
Co.
Co.
Simplices
Bifolioliae
Multinerviae

Fig. 4.6 cont.

Table 4.6. Number and percentage of taxa that belong to particular fire survival strategies.

	Seeders	Crown resprouters	Clonal resprouters	Mixed	Unknown
Total	55	17	45	3	20
Percent (of known taxa)	45.8%	14.2%	37.5%	2.5%	
Cape Floristic Region	54	12	44	3	18
Percent (of known taxa)	47.8%	10.6%	38.9%	2.7%	

Le Maitre & Midgeley (1992) showed that several of the largest families within fynbos had between 50–90% seeders. *Cliffortia* is at the lower end of this with only 47.8% of taxa within the CFR being seeders (Table 4.6). The reason for this low number can be attributed in a large part to three sections, which are predominantly clonal resprouters, sect. Bifoliolae, sect. Multifidae, and sect. Multinerviae. Together these clades have 30 accessions that clonally resprout (including two mixed strategy species) out of 37 for which the strategy is known. Most theories predict that the seeding strategy leads to higher rates of speciation (Wells, 1969; Cowling & Holmes, 1992a; Schutte *et al.*, 1995; Ojeda, 1998; Cowling & Pressey, 2001). This is based upon a higher risk of extinction and hence lineage turnover, and it is not necessarily a selective advantage (Cowling & Holmes, 1992a). However, for these three groups the reverse appears to be true and clonality has promoted speciation. The reason for this needs to be investigated further (see hybridization below).

Despite the almost even split between seeder and resprouter species there are only a few cases where a shift in fire survival has occurred between two sister species. One of the most widespread species pairs with differing life histories is *C. atrata* and *C. tuberculata*. *C. atrata* has a greater altitudinal range, from sea-level to over 1000 m, but *C. tuberculata* is more widespread at higher altitudes from Piketberg to Uitenhage, although absent from the Cape Peninsula. Between Groot Winterhoek and Franschhoek both species grow together in similar habitats and are very similar in general appearance, but separable by their life-histories and the warty fruits of *C. tuberculata*. Similar species pairs include *C. eriocephalina* and *C. dispar* or *C. montana*, depending upon the mountain range. In these species, *C. eriocephalina* is always a low-growing clonal resprouter, while the other two generally grow in wetter and less exposed areas such as stream gulleys or south-facing slopes and are taller single-stemmed seeder species. However, there are also usually indications that introgression has occurred and intermediates often exist that blur the boundaries between the species pairs, their habitats and life-histories.

An unusual pair of species pairs includes *C. grandifolia* and *C. lanceolata* from the Langeberg and *C. heterophylla* and *C. serrata* MS from the Kogelberg, all the species having a preference for wet south-facing slopes. Both *C. grandifolia* and *C. heterophylla* have a very peculiar growth form of a tall spindly branched seeder shrub, while their sister species form a medium, more densely branched, clonal resprouter. Each species of the pair has been found growing intermingled with the other and they have almost exactly the same distribution. This is especially unusual in *C. grandifolia* and *C. lanceolata*, which includes a big disjunction between the Swellendam Mts and Garcias Pass. The similarity in habitats, growth form and life-histories between the two species pairs would make them very good subjects for further studies of speciation processes. In particular, these species pairs could be used to examine the link between resprouting and seeding and that seeders appear to be taller than resprouters, as hypothesised by Midgeley (1996).

Genetic drift

Although several species pairs have allopatric distributions, demonstrating that speciation is entirely the result of genetic drift is difficult. It would only be possible in situations where no other ecological shift has been found, otherwise morphological changes may be more appropriately attributed to different selection pressures. But even in these cases, it is possible that undetected selection pressures may have driven speciation.

The best example of possible genetic drift in allopatric speciation is between the three species *C. dentata*, *C. gracillima* MS and *C. gracilis*. *C. gracilis* is only tentatively distinct, being a narrow endemic that abuts onto the periphery of the more widespread *C. dentata*, and only varying in the number of teeth at the apex of the leaf. *C. gracillima* MS on the other hand is distinctly smaller in all its parts as well as having fewer teeth than *C. dentata*, and there is a broad disjunction between itself and the other two species. However, all three species occupy a similar habitat of damp shady gulleys in mountains and share a common life-history. Even so, their alternative distributions mean that they are naturally subject to different climatic factors and these could have acted as selection pressures behind the speciation events.

The only other tentative example of allopatric speciation might be between *C. dispar* and *C. montana*. However, this is also associated with a clear shift in rainfall, *C. montana* occupying the drier mountains of the Swartberg and Graaff Reinet Sneeuberg,

and it might be more accurate to interpret them as two separate speciation events from the resprouting *C. eriocephalina* to the seeder strategy (see above).

Hybridization

Hybridization without an ecological shift could result in the replacement of one or both parental species. It is therefore interesting to investigate the ecological consequences to the species of hybridization. Unfortunately, only a few examples can be demonstrated where both putative parents and the hybrid are known and this is associated with an ecological shift (Table 4.3). One such case is *C.* ×*homunculi* MS, whose parents both grow nearby. However, the parents are restricted to rocky outcrops where they are partially protected from fire and form crown resprouting plants. The hybrid on the other hand, grows in the deep TMS sand of a grassy plateau where it spreads clonally. As it is only known from one locality in the Kammanassie Mts and its ability to spread other than vegetatively has not been ascertained, attributing it specific status could be regarded as dubious. However, it might be worthy of such recognition considering that it differs so markedly in ecology, as well as morphology, from its two parent species.

The shift in fire survival strategy may also corroborate the hypothesis that clonal resprouting is an ancestral trait for *Cliffortia*, as it is common for hybrids to revert to a plesiomorphic state (Funk, 1985). Here clonal resprouting has arisen from two parents that did not express that trait. A similar example can be found in *C. arcuata* (although the hybrid nature of this species is not so clear, one parent does not grow in the same area and the species has clearly spread subsequently, and only one confirmed case of clonal resprouting has been observed). Indeed, except for *C. crassinervis*, which is thought to be a seeder species, all hybrids whose parents have differing fire survival strategies are clonal resprouters (Table 4.7).

However, this trend could also be attributed to the higher proportion of hybrid species being clonal resprouters (Fig. 4.7). Although this is not significant ($\chi^2 = 4.99$, $P = 0.083$), a higher probability of clonal resprouters should also be predicted on biological principles, as their ability to spread and persist in their environment would be greater than hybrids with a seeder or crown sprouting strategy. Therefore, the non-significant result may be more attributable to the undersampling of hybrid plants ($n = 27$). Certainly, the greatest proportion of unsampled putative hybrids has been observed (pers. obs.) in the sections Multinerviae, Multifidae and Bifoliolae, which generally have a clonally resprouting strategy (see above, Fig. 4.6).

This would also explain the disproportionate degree of speciation found amongst these three sections. Resprouter strategies are generally thought to lead to less speciation than the seeder strategy, due to reduced risk of extinction (Cowling, 1987) and gene flow between overlapping generations (Schutte *et al.*, 1995). However, resprouting also encourages persistence of odd forms or hybrids in the environment. These hybrids and forms can then backcross with the parents, resulting in introgression, or by polyploidization form new species themselves. Persistence by resprouting coupled with vegetative spread by clonal growth, gives these novelties a much longer period for potential gene flow or speciation. This is in contrast to a new hybrid that cannot resprout. In that case the chance of gene flow, if it cannot produce viable seed, is limited to backcrossing in the few years before the first fire. Even if viable seed is produced, the chance of it persisting for more than a few generations is limited unless there is markedly improved fitness in the hybrid or it occupies a different niche.

Table 4.7. List of putative hybrids and their possible parents, showing fire survival strategy where known or hypothesised from character reconstruction on tree (Fig.4.6).

Taxon	life history	male parent	life history	female parent	life history
arcuata	clonal	ramosissima	seeder	falcata	seeder
browniana	crown	?linearifolia	crown	nitidula	crown
ceresana	clonal	virgata	clonal	acanthophylla	seeder
crassinervis	?seeder	setifolia	clonal	tuberculata	crown
cruciata	seeder	?subsetacea	seeder	atrata	seeder
cymbifolia	clonal	pungens	clonal	ruscifolia	mixed
×homunculi	clonal	tuberculata	crown	neglecta	crown
integerrima	clonal	?dregeana	clonal	ruscifolia	mixed
intermedia	clonal	ilicifolia	clonal	ruscifolia	mixed
marginata	?clonal	?phyllanthoides	seeder	tenuis	clonal
meyeriana	clonal	?theodori-friesii	clonal	pungens	clonal
monophylla	seeder	hantamensis	seeder	filicaulis	seeder
nitidula (cmw65)	crown	linearifolia	crown	nitidula	crown
perpendicula	seeder	ramosissima	seeder	falcata	seeder
pubescens	seeder	atrata	seeder	sericea	seeder
reniformis	clonal	ilicifolia	clonal	?glauca	clonal
reticulata	seeder	odorata	seeder	pilifera	seeder
schlechteri	clonal	ilicifolia	clonal	?glauca	clonal
sericea	seeder	lanata	?seeder	polygonifolia	seeder
sp. cf. eriocephalina	clonal	eriocephalina	clonal	weimarckii	clonal
sp. cf. glauca1	clonal	tenuis	clonal	obovata	clonal
sp. cf. juniperina	?crown	juniperina	crown	ruscifolia	mixed
sp. cf. verrucosa	clonal	purpurea	?clonal	verrucosa	?mixed
triloba	clonal	pedunculata	seeder	?polygonifolia	?seeder
varians	clonal	crenata	clonal	obovata	clonal
versiformis	clonal	obovata	clonal	ruscifolia	mixed
viridis	seeder	hirsuta	seeder	ferruginea	seeder

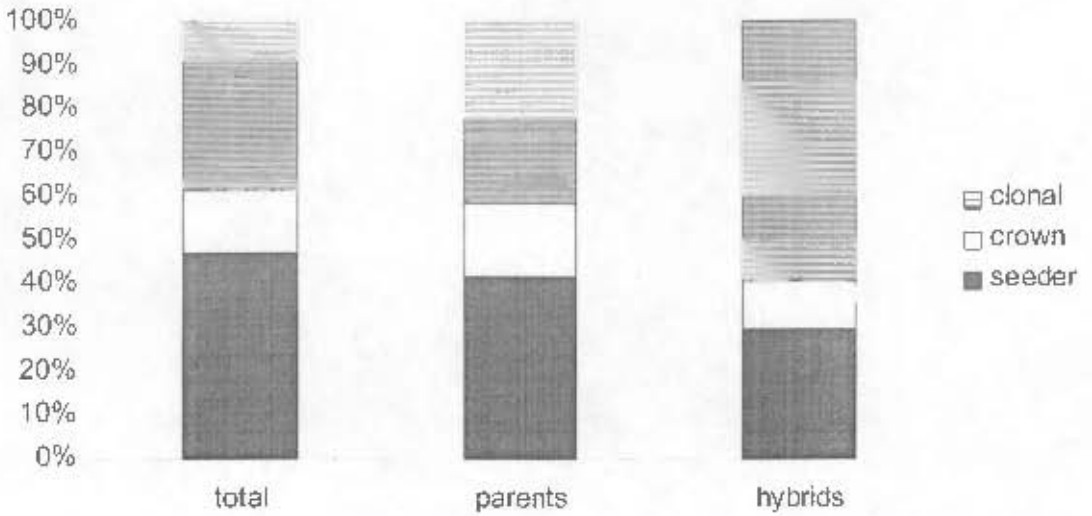

Fig. 4.7. Proportion of fire survival strategies found in all *Cliffortia* taxa, recorded hybrids and their putative parents (excluding mixed strategy species). Figures for hybrids and parents are from Table 4.7, and total figures from Table 4.6.

Evidence for other ecological shifts between hybrids and their parents is at present unavailable. This is either because one of the parents is missing, possibly having been replaced by the hybrid taxon, or because no ecological shift has been observed from both parents, the hybrid appearing to be sympatric with one or other of the parents. Even when no ecological shift is evident, some hybridization events can reveal novel or extreme character traits in the hybrids which are not present in the parents (Rieseberg & Ellstrand, 1993; Rieseberg *et al.*, 1999). These novelties might have an influence upon the ecology of the species, which is as yet undetermined or has not expressed itself. An example of this might be *C. monophylla*, a unifoliate species that grows in sympatry with its trifoliate parent, *C. filicaulis* (see above). Its putative other parent is also trifoliate, *C. hautamensis*, and so it appears as though the unifoliate state has arisen either as a result of hybridization or subsequent to it. However, no selective advantage has yet been shown for the unifoliate condition over the trifoliate one.

If there is no ecological shift, then the sympatry of parent and hybrid can only be a temporary condition and hence the hybrid is probably of recent origin. Consequently, the hybrid has three possible fates: a) that it will not persist, or if it does that it will either b) replace its sympatric parent, or c) shift its ecology with time. However, although most hybridization events cannot yet be shown to have played an important role in speciation, there is convincing molecular evidence that it has been pivotal in the generation of species richness in *Cliffortia*. It is therefore presumably only a matter of

time before more examples of ecological shifts can be proposed and the role of hybridization in speciation can be better demonstrated.

Neoteny as a speciation mechanism

The seedlings and resprouting leaves of many species have been found to have very different morphology to the mature foliage. This has led to several instances of apparent neoteny. In cases where closely related sister pairs exist with highly dimorphic foliage, one species can have both the juvenile and mature leaves, while the other only expresses the 'juvenile' foliage throughout the plant at all ages. Examples of this include: *C. strobilifera* and *C. longifolia*, *C. ferruginea* and *C. berberidifolia*, *C. aculeata* and *C. nivenioides*. In each example the latter only expresses leaves similar to the juvenile state of the former. This speciation process could be controlled by a single gene, loss of which causes failure of the mature leaves to develop. As all three species pairs are still more or less sympatric, if there was a selective advantage gained by the parental species in having the modified mature foliage then the neotenous progeny would presumably only persist if it was associated with a habitat shift as well. For *C. strobilifera* and *C. longifolia*, this appears to have taken place, *C. strobilifera* found in streams and rivers on neutral to acid soils, while *C. longifolia* grows on more alkaline soils often at the base of limestone hills (see distribution and localities in Fellingham, 1994). For the other two species a shift in ecology is less apparent but may still be there. In these cases the 'neotenous' species is a narrow endemic, and only known from a single locality. This might indicate that either the loss of mature foliage has specialised the species so much that it is highly restricted to its new habitat type, or that it is a newly arisen species and its propensity to persist or spread has not been given the opportunity to reveal itself.

Other cases of neoteny are not so apparent, but the presence of petioles in the seedlings of several species and the reversion of the leaves to a petiolate state in certain lineages may be evidence of a past neotenous speciation event that preceded diversification. Certainly, these observations illustrate that a more comprehensive study of seedling leaf-form will help us to understand better the evolutionary patterns by which the leaf morphology has developed.

Recognition of hybrid species:

Species that arise through allopolyploidy and homoploid hybrid speciation and are not only sexually fertile but occupy a different ecological niche to the parental species are recognised as distinct species by most systematists. Even, the Biological Species

Concept, which denies hybridization as an evolutionary important event, makes exceptions for these reproductively isolated new lineages, as "once speciation is complete, they behave like any other good species" (Mayr, 1992). However, asexual reproduction of a hybrid lineage, either vegetatively or through apomixis, makes determination of the boundary between hybrids and species with a hybrid origin much more difficult.

Many rosaceous taxa are known to reproduce apomictically, e.g. *Amelanchier* (Campbell *et al.*, 1997) and *Rubus* (Nybom, 1988; Amsellem *et al.*, 2001). In addition, *C. ruscifolia* is remarkable for the common observation that male flowers are extremely rare (Esterhuysen, 1947; Linder & Midgley, 1996). For example, on the Cape Peninsula, male flowers have only been collected twice (out of 32 collections) and they were both in 1933. (Levyns, who made both collections, also commented that "far more male plants have been seen this year than previously".) Despite this *C. ruscifolia* is one of the most widespread and abundant of *Cliffortia* species in the CFR and has frequently been observed recruiting from seed after a fire (pers. obs.). This must therefore be one of the strongest cases of circumstantial evidence for agamospermy.

Along with agamospermy is the propensity for many *Cliffortia* species to spread clonally through their root system. These two characteristics of *Cliffortia* mean that many species can spread widely from their point of origin without the need for sexual reproduction. Speciation by asexual reproduction is generally regarded as a special case (Mayden, 1997), which normally creates cryptic species varying only slightly from their sexual progenitors (Gornall, 1999). However, hybrids can exhibit extreme traits, unique combinations, or even novelties (Rieseberg & Ellstrand, 1993; Rieseberg *et al.*, 1999). Therefore, a chance hybrid that is able to spread asexually and appears markedly different from both its parents, could appear similar to a species that had adapted to its environment through selection pressures over time.

Certain of the species included in the phylogeny may well be such hybrids. *C. integerrima* is known from a single population and no male flowers have been observed within that population. Its ability to produce fertile seed has not been tested but it is able to spread clonally by its root system. While recognition of asexually reproducing lineages is generally accepted in species definitions, if the clone is unable to spread far beyond its point of origin then it will be highly susceptible to local extinction events. In the case of *C. integerrima*, no such ability has been proven and its

status as a species is highly questionable, as it may not be able to persist for long in the environment.

Even if a hybrid is able to produce viable seed, either asexually or sexually, whether it will persist in the environment will depend greatly upon its ability to occupy a different ecological niche to both of its parents. Therefore, it would be prudent to only recognise hybrids as species when an ecological shift can also be demonstrated. However, detecting these shifts can be difficult from field observations alone. Therefore, it is easier to use a surrogate definition and examine the hybrid for a unique trait in a morphological character that differs from both its parents (i.e. that its character states are not all intermediate). This character may have arisen as a transgressive trait or through selection subsequent to the hybridization event, but either origin might give the hybrid the ability to adapt to a different environment from both its parents.

Following these two criteria, examination of the hybrids in Table 4.7 would suggest that *C. cymbifolia*, *C. sp. cf. eriocephalina*, *C. sp. cf. glauca*1, *C. ×homunculi*, *C. integerrima*, *C. intermedia*, *C. sp. cf. juniperina*, *C. sp. cf. verrucosa*, should not be given specific status recognition as there is no evidence that they have spread from their point of origin other than by clonal growth. However, *C. integerrima* and *C. intermedia* are for the present retained as they are currently recognised as species and there is not sufficient evidence against their being so. Similarly, it may be difficult to maintain *C. sericea* as distinct from *C. lanata* and *C. polygonifolia* and *C. reticulata* as distinct from *C. odorata* and *C. pilifera*, as although these hybrids are both capable of reproduction via seed they do not show a definite ecological shift from one or other parent and only have characters that are intermediate between the two parental species.

Conclusions:

Speciation within *Cliffortia* has not been shaped by a single dominant selection pressure. Adaptation has been driven by a wide variety of ecological factors including substrate, altitude, water dependence and fire survival. As yet particular morphological traits have not been associated with adaptation to particular new ecologies but plenty of opportunity exists for investigating these further. For example, the pattern of leaf morphology evolution from a trifoliate leaf to a simple unifoliate leaf has occurred multiple times and an associated ecological shift can be sought.

While hybridization events may confound examination of morphological evolution they also provide exciting opportunities for investigating the role of hybridization in the

speciation process. In particular, hybrid species for which both parents are still extant can be sought, e.g. *C. meyeriana* or *C. versiformis* MS, and the original crosses artificially conducted to elucidate if particular characters are associated with the hybridization event or have evolved through selection subsequent to it. Furthermore, hybrid species that are still sympatric with one or other of their parents need to be more closely studied to identify the mechanisms that permit coexistence of the two species and prevent their assimilation into a single species, e.g. *C. monophylla* and *C. filicaulis*.

The prevalence of apparent hybrid speciation within *Cliffortia* also demands more rigorous testing of what constitutes a true speciation event. Fertility of male and female flowers and the possible presence of agamospermy needs to be determined for many hybrid species. Presumably, some of the species currently recognised will be best regarded as a solitary hybridization event, while other hybrids might be better given specific status as they are able to produce viable seed through agamospermy or even sexually.

5. Biogeography

Introduction:

Weimarck was one of the major proponents of biogeography for the Cape. His seminal work on the subject (Weimarck, 1941) was based upon his earlier comprehensive study of *Cliffortia*, as well as other Cape taxa. More recent work on Cape biogeography (Oliver *et al.*, 1983; Linder & Mann, 1998; Linder, 2001a) has supported many of Weimarck's findings, although it has also highlighted some differences (e.g. Langeberg and South-east centres, Oliver *et al.*, 1983). In addition, the use of more refined techniques has provided empirical support for what had previously been intuitive interpretation to identify the observed patterns (Oliver *et al.*, 1983) and to suggest explanations (Linder & Mann, 1998).

Several different elements can be identified using a variety of biogeographical analyses (Morrone & Crisci, 1995). These areas and the species associated with them can then be used to discuss possible explanations for the patterns observed.

Phytochoria:

Phytochoria are areas that have similar species composition. These areas can be defined objectively by using similarity matrices and clustering algorithms (Pielou, 1979). Weimarck (1941) proposed five phytogeographical centres for the CFR based upon observation of their common species compositions. These have been more or less retained by subsequent authors, with minor modifications of boundaries and the addition of one of his subcentres for the limestone and lowland areas around Bredasdorp (Oliver *et al.*, 1983). Indeed, the most recent conspectus of the flora (Goldblatt & Manning, 2000) has used the previous analyses to recognise those six centres, namely: North-western, South-western, Agulhas Plain, Karoo Mountain, Langeberg, and South-eastern.

Centres of diversity:

Centres of diversity can be easily identified by simply mapping the number of species per unit area. From a biological viewpoint, centres of diversity are of interest in terms of how and why are so many species present in a restricted area, especially compared to the less speciose areas around the centre. However, there is also much interest in identifying these areas for conservation purposes (Reid, 1998). The limited budgets

available to governments, scientists and conservation bodies means that preservation of species needs to be targeted (Roberts, 1988). One way this can be done most effectively is by conserving areas that contain the most species.

Centres of endemism:

Of more relevance to conservation than centres of diversity are areas that contain a high density of range-restricted species. Rare species are far more vulnerable to extinction than widespread species and therefore should be preferentially targeted in conservation planning (Rebelo & Tansley, 1993). These too can be located easily by mapping the range-restricted species, either using an arbitrary cut-off for maximum range size (e.g. Rebelo & Tansley, 1993; Linder & Mann, 1998) or weighting each species inversely to the size of its range (e.g. Linder, 2001b). However, centres of endemism will be strongly correlated with those found for the centres of diversity because the species used to calculate them will always be a subset of those used for the previous analysis. Therefore, an alternative option is to calculate the proportion of endemic species present rather than the absolute number (Kershaw *et al.*, 1995; Linder, 2001b).

Areas of endemism:

Areas of endemism is a related concept to phytochoria, but the areas found are not always congruent. A phytogeographic centre can be the result of overlap between species characteristic of two different regional centres of endemism, and so itself may only contain a few endemic species. On the other hand, areas of endemism are areas which are congruent to the distribution of range-restricted species. These areas are important for conservation as they have been the focus of diversification in the past and hence may have the greatest evolutionary potential for the future (Brooks *et al.*, 1992). An area of endemism could be defined for each individual species (Polunin, 1960); by definition each species is endemic to its own distribution range (Kruckeberg & Rabinowitz, 1985). However, most biogeographers regard the minimum requirement for an area of endemism that it contains at least two species of restricted range (Nelson & Platnick, 1981; Morrone & Crisci, 1995). To this basic requirement, Linder (2001a) adds further criteria for identifying areas of endemism:

1) The areas are narrower than the entire study area, so that several areas are located. If this was not done, the whole of the CFR could be identified as an area of endemism for *Cliffortia*, which although theoretically correct would not be very useful for discussing the biogeography of the species.

2) The areas of endemism must be mutually exclusive.

3) The area should be optimised so that the range of species to the area are maximally congruent.

Linder then implements a method using parsimony analysis for locating areas containing species with congruent distributions. By upweighting those species with narrower ranges areas of endemism can be objectively define (Linder, 2001a).

Phytogeographical species groups:

In contrast to locating areas that have similar species compositions, groups of species that share common distribution patterns can be detected. These can be located in the same way as phytochoria, using similarity matrices and clustering algorithms. When the distribution ranges of the species groups located are small and continuous, this will correspond to an area of endemism. However, many species have broader distributions, which share a common climatic or edaphic regime. In some cases they can share a common interval in their distributions, which may give an indication of a shared biogeographic history. Two explanations are possible for these shared intervals: 1) that they both had an identical long distance dispersal event, or 2) that the species involved were at one point more widespread, but the ranges have subsequently contracted. According to Weimarck (1941) the high degree of range restricted species in the CFR would indicate that the latter explanation was more likely, as vicariance is commoner than long distance dispersal.

Factors affecting biogeographic analysis:

Analyses of both phytogeographic and endemic regions are very dependent upon the scale of the units used in the analysis and the comprehensiveness of the coverage for the area being sampled (Nelson *et al.*, 1990; Linder, 2001b). A large unit area means that documentation of the taxa present will probably be accurate, but the resolution and hence informativeness is reduced. Decreasing the unit area, will increase the resolution but it also becomes more affected by undercollecting, and hence incomplete sampling of the taxa present, as well as there being a greater risk of erroneous placement of the collecting localities. More importantly, the resolution used in any analysis will affect the determination of which factors best account for the patterns of diversity observed (Willis & Whittaker, 2002). Therefore, it is vital to choose the appropriate scale for the questions being asked.

Assessments of biogeography can also be affected by the taxon level at which they are conducted (La Ferla *et al.*, 2002). In addition, the taxa that are used are rarely, if ever, directly comparable and will differ in numerous factors regarding their biological or evolutionary histories (Williams *et al.*, 1997; Avise & Johns, 1999; Berry, 2002). In most analyses, species are used as the indicator taxon for diversity and endemism, although at the continental scale genera or even families are often employed (e.g. Williams *et al.*, 1994; Gaston *et al.*, 1995; Williams *et al.*, 1997). Species are often chosen as the indicator taxon because they are regarded as being real in some way (Davis & Heywood, 1963; Rieseberg & Burke, 2001) and hence can be directly compared. However, some regard species as just another rank and therefore should not be compared (e.g. Mishler, 1999). In addition, a single species concept is not unanimously accepted (see Chapter 3). Hence, species delimitation is not always uniform across or even within genera. To circumvent this problem equal age lineages can be used to compare more inclusive groups than species by creating an ultrametric tree (Avise & Johns, 1999).

One reason for using more inclusive groups of species in biogeographic analysis is to prioritise for conservation taxa that are genetically or morphologically more diverse (Gaston *et al.*, 1995; Williams *et al.*, 1997). Species complexes can result in numerous small splits of species that are poorly separated from one another morphologically and are genetically very similar (especially in apomictic lineages). This can affect counts of species diversity, weighting them in favour of those complex lineages. Endemism rates are also affected, as the minor splits of species are often geographically restricted and peripheral to the parent species (e.g. *C. purpurea* and *C. verrucosa* from *C. ruscifolia*). Therefore it is often suggested that branch lengths should be taken into account when assessing priorities for conservation, and focussing upon conserving the greatest degree of morphological features (Faith, 1992) or genetic diversity (Crozier, 1992). Using an ultrametric tree and equal age lineages at particular depths of the tree would be one way to help conservation efforts identify groups for maximising phylogenetic diversity (Avise & Johns, 1999).

Methods:

A database was constructed by recording the collections in the herbaria BOL, GRA, K, NBG, NU, PEU and PRE. Only the records whose identity I had personally verified were included in the analysis. In addition to these, personal field observations of species

with unambiguous delimitation were added and indicated as such. The addition of these sightings was useful for filling in the gaps in the distributions of widespread species that are frequently under-collected, e.g. *C. erectisepala*, *C. ruscifolia* and *C. tuberculata*.

Each collection was located using 1:50,000 maps where available, or failing this 1:250,000. Wherever possible the localities were given a 6-figure code to indicate which $^1/_4$ degree square they occurred in (hereafter termed grid square). The first four digits of the code indicate the latitude south and the longitude east. The degree square is then subdivided into quarters, which are themselves subdivided into four, and coded as shown in Fig. 5.1a. When more accurate placement of the locality was possible, latitude and longitude values were also entered to the nearest minute, as well as a value expressing the degree of accuracy for its placement. The degree of accuracy was either '1' indicating that it was found within that minute square, '3' for within one minute square either side, or '5' for within two minute squares; when the locality could not be placed that accurately, a value of '0' was entered. These latitude and longitudes were used to add an additional figure to the code for the grid square to create a scale of coding to a 5 minute square accuracy ($^1/_{12}$ degree square and hereafter termed 5-min square) as shown in Fig. 5.1b.

a)

AA	AB	BA	BB
AC	AD	BC	BD
CA	CB	DA	DB
CC	CD	DC	DD

b)

1	2	3
4	5	6
7	8	9

Fig. 5.1. Coding (a) used to divide individual degree squares into grid squares, and the additional numerical figure (b) used to further subdivide each grid square into 5-minute squares.

Collecting density:

To investigate geographical variation in collection intensity the number of unique collections (i.e. duplicates in different herbaria for the same collection were omitted) for each grid square was divided by the number of species found in that grid square. This calculation makes several presumptions such as the amount of collecting in an area

should be directly proportional to the number of species that are present there and the density of each species within an area is equal. However, it does permit some degree of comparison as to how uneven collecting has been, especially between squares with comparable compositions of habitat diversity.

Phytochoria:

To establish biogeographic areas for *Cliffortia* a similarity matrix was constructed following that outlined by Linder & Mann (1998). Each grid square was scored for the presence or absence of each species or lineage. Grid squares with a single taxon were omitted from the analysis, as were squares outside the CFR (<31°S and >26°E). The Jaccard coefficient was used to calculate the similarity of grids to each other as this disregards shared absences, which can be the result of undersampling in certain areas. Clustering was carried out using the UPGMA algorithm as implemented in NTSYS 2.10q (Rohlf, 2000). All trees with ties were found and a strict consensus calculated from them.

Centres of diversity:

The database was used to extract the number of species present in each unit area. These were then plotted onto an outline of the CFR using a greyscale from light to dark grey proportional to the number of species. The effect of unit area size on the result was investigated by using four different unit areas: a degree square, a ½ degree square, a grid square and a 5-min square.

To examine the effect of using more inclusive species groups on the centres of diversity an ultrametric tree was created. On account of the difficulties created by reticulations, the chloroplast and nuclear trees were used separately in the analysis. A single tree for each was chosen by filtering all the trees to select one that created the shortest tree when the opposing datasets were optimised onto it. By doing this, the two trees chosen, despite the inevitable conflicts caused by reticulations, should have the greatest degree of congruence. The trees with their parsimony branch lengths were entered into TreeEdit (Rambaut & Charleston, 2001) and non-parametric rate smoothing (Sanderson, 1997) was applied to create an ultrametric tree with proportional branch lengths. Unfortunately, TreeEdit failed to cope with trees containing 133 terminals. This meant that for the chloroplast tree three taxa had to be pruned before entering the tree for smoothing, while in the nuclear tree 19 taxa had to be pruned. The taxa chosen were replicates of species or very closely related sister taxa such that one or other of the

terminal branches had a zero branch length. In this way the removal of the taxa would not adversely affect the shape of the smoothed tree.

Four lines were placed across the ultrametric trees, such that the distance between the ancestral node for *Cliffortia* and the terminals was divided into five equal portions. These lines were used to examine the effect of sampling at different depths within the tree upon lineage diversity within the CFR. For each line the number of lineages at that depth within each grid square was plotted using a greyscale similar to that used for species diversity. To compare this with the effect of using different ranks to estimate diversity, the diversity of subgenera and sections were also mapped in a similar fashion.

This replacement of species for lineages was also applied in the endemism analyses but using only the chloroplast tree, as the more robust of the two trees, and the fourth line, as it retained the highest number of items.

Centres of endemism:

The weighted and corrected weighted endemism methods used by Linder (2001b) were applied to the data. For the weighted endemism value, each species or lineage was given a weight equivalent to the inverse of the number of grid squares that it was present in (i.e. species present in one grid square were given a weight of one, two grid squares 0.5, three grid squares 0.333, etc.) and the values in each grid square were then summed. This was 'corrected' by dividing the result by the actual number of species or lineages present in each grid square. The resultant figures were then plotted onto the map in the same way as for the centres of diversity, except the range was subdivided into five bands. The weight3 method used below was also mapped to compare the effect of that weighting scheme upon the location of the centres of endemism.

Areas of endemism:

Following Linder (2001a), the species and lineages were weighted using the inverse method and a modification of the weight3 method. For the latter method the values of a and p, for the equation e^{-ax^p}, were chosen such that species in four grid squares were weighted 10 times more than those found in nine grid squares ($a = 0.0005$, $p = 3.858$). To allow the taxa to occur in four grids and retain a similar weighting to those found in a single grid square was important because a narrow endemic that occurred on the corner of a grid square might well be found in four separate grid squares yet be as restricted in its range as a species occurring in a single grid square (Linder, 2001a). The values obtained were rescaled so that taxa found in a single grid square received a value

of 1000, while those found in greater than 11 grid squares received a value of 0 and were excluded from the analysis.

The weighted datasets were entered into Paup* 4.0b10 (Swofford, 2001). Grid squares with only a single taxon with weight greater than 0 were excluded from the analysis, which resulted in two matrices, one containing 86 grid squares (plus one outgroup) and 105 species, and the other with 56 grid squares and 48 lineages. Searches were conducted by using 500 random addition sequences, saving five trees for each replicate. The resulting shortest trees were then used to carry out a heuristic search until a maximum of 20,000 trees were found. Areas of endemism were recognised in the resultant trees that contained two or more endemic species to the area circumscribed by a particular clade.

Phytogeographical species groups:

Species groups for *Cliffortia* were detected by using the same matrix as for the phytogeographic centres except that grid squares outside the CFR were included but species occurring in a single grid square were omitted. The rows and columns were transposed so that the species were now the objects and the grid squares the characters. Again, the Jaccard coefficient was used to calculate a similarity matrix, which was then subjected to the UPGMA algorithm using NTSYS. All ties were found and a consensus tree produced.

Results:

Collecting density:

Fig. 5.2 shows that collecting density has been highest on the Cape Peninsula, the South-western Mountains between Bains Kloof and Kogelberg, the central Cederberg, Laingsburg Witteberg, Swartberg Pass and Garcias Pass. Notable areas for undercollecting include the Klein and Groot Swartberg mountain ranges between the various passes, the mountains around Willowmore, the SE Langeberg beyond Garcias Pass and the coastal area between De Hoop Nature Reserve and Cape Agulhas. Considering the large number of species present (n = 15), the eastern Riviersonderend Mountains (3419BB) are also comparatively poorly collected and the number of species could be expected to rise upon more thorough searching.

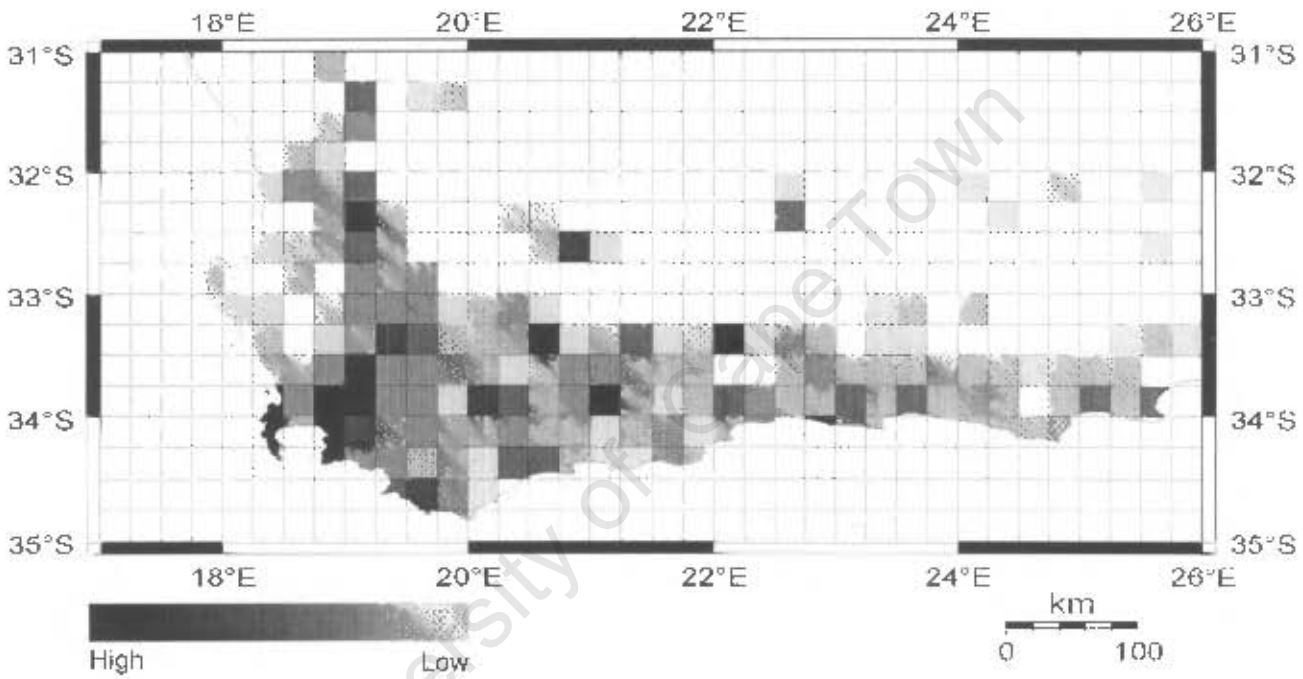

Fig. 5.2. Map to show the relative density of collecting in the CFR in proportion to the number of species present. Completely white squares have had no species collected and so are inapplicable for the comparison.

Phytochoria:

The clustering algorithm found 576 tied trees, thus a strict consensus tree was calculated to show the groups that were present in all the trees (Fig. 5.3). Phytochoria were recognised by selecting the largest clusters possible and these were mapped to show their distribution (Fig. 5.4). Clusters with fewer than 5 terminals were not recognised but if they formed continuous blocks that corresponded to a geographical feature, these were noted on the phenogram. Five phytochoria could be recognised: the North-western Mountains between the Robertson Langeberg and Nieuwoudtville, the South-western Mountains, including the Cape Flats and Overberg, the Agulhas coast between Gansbaai and Wilderness, the Southern Langeberg between Swellendam and Riversdale, and the South-eastern Mountains between George and Port Elizabeth. Within these larger clusters several smaller clusters that corresponded to geographical features could be recognised. In addition, some small clusters were present outside of the major phytochoria, i.e. the Klein & Groot Swartberg, Hantam & Roggeveld Mountains and the Western Klein Karoo Mountains.

Centres of diversity:

The concentration of species within South Africa and the Cape Floristic Region are shown in Fig. 5.5–6 using degree squares and grid squares respectively. The effect of increasing the size of the square used upon the resolution and accuracy of diversity within the CFR is shown in Fig. 5.7. The degree square map shows that this scale is far too coarse for any meaningful recognition of centres within the CFR. The South-western Mountains clearly show the greatest concentration with a gradual decline to both the north and east. The ½ degree square map shows exactly the same pattern though with slightly more refinement. Only when the grid square map (thus a ¼ degree map) is examined can areas outside of the South-western Mountains such as the Cape Peninsula, Agulhas Plain, Cederberg, Witteberg and Anysberg, Langeberg at Swellendam and Riversdale and the Klein and Groot Swartberg be located as centres of diversity. However, at this level the effects of collecting concentration also become evident. For example, the Groot Swartberg west of Meiringspoort (3322AD) and the Langeberg between Garcias and Cloetes Passes (3321DC) show considerably fewer species than the grid squares on either side of them, yet they contain very similar ranges of habitat diversity. The inadequacy of collecting in some areas becomes even more apparent for the 5-min square map, as well as the overcollecting of certain areas such as around Cape Town (3318CD9), Jonkershoek (3318DD9) and Franschhoek (3319CC5),

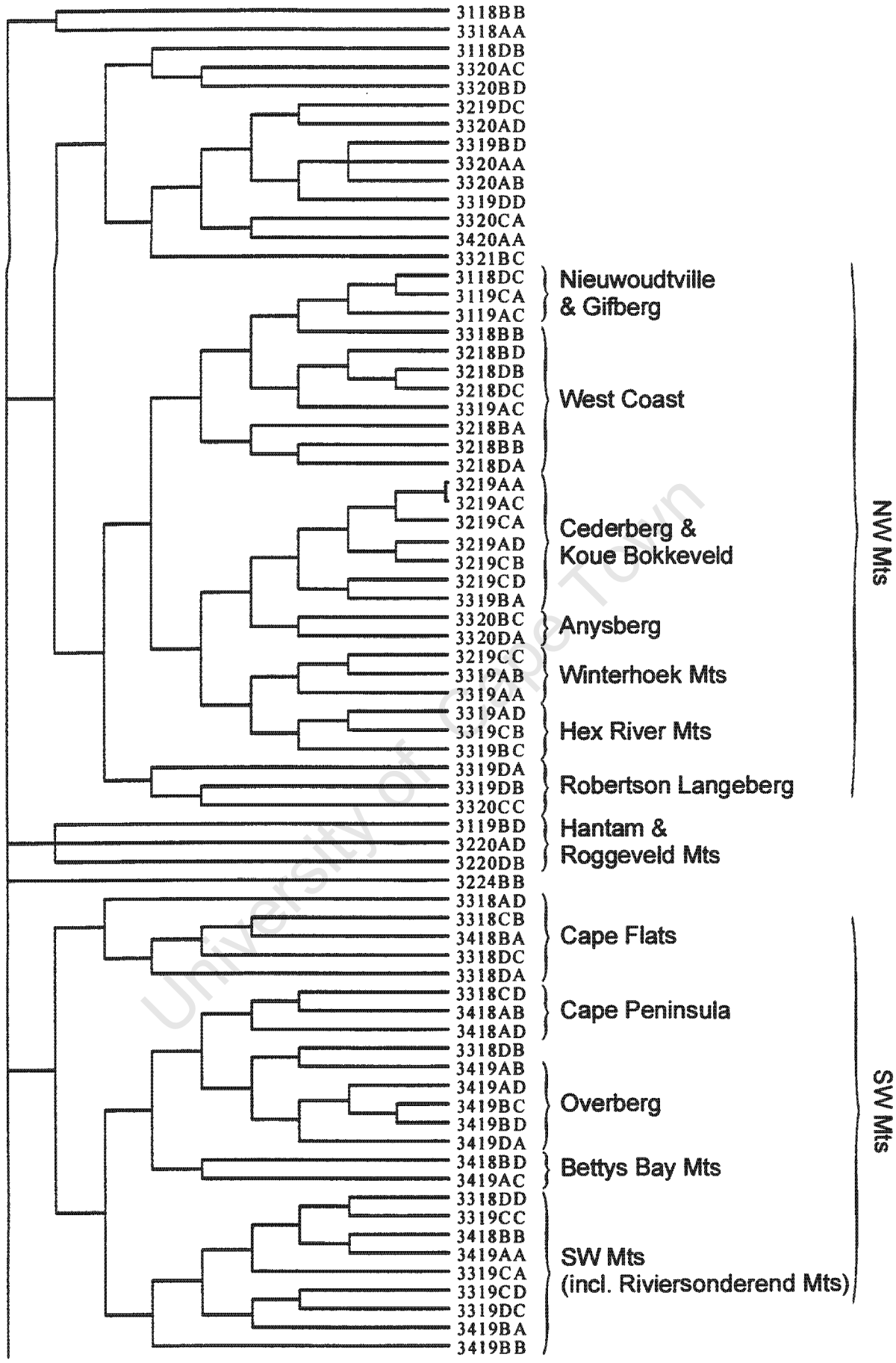

Fig. 5.3. Strict consensus tree of the trees obtained by the UPGMA clustering algorithm upon a Jaccard similarity matrix of taxa present in each grid square. Phytogeographically coherent groups of grids are indicated on the right hand side.

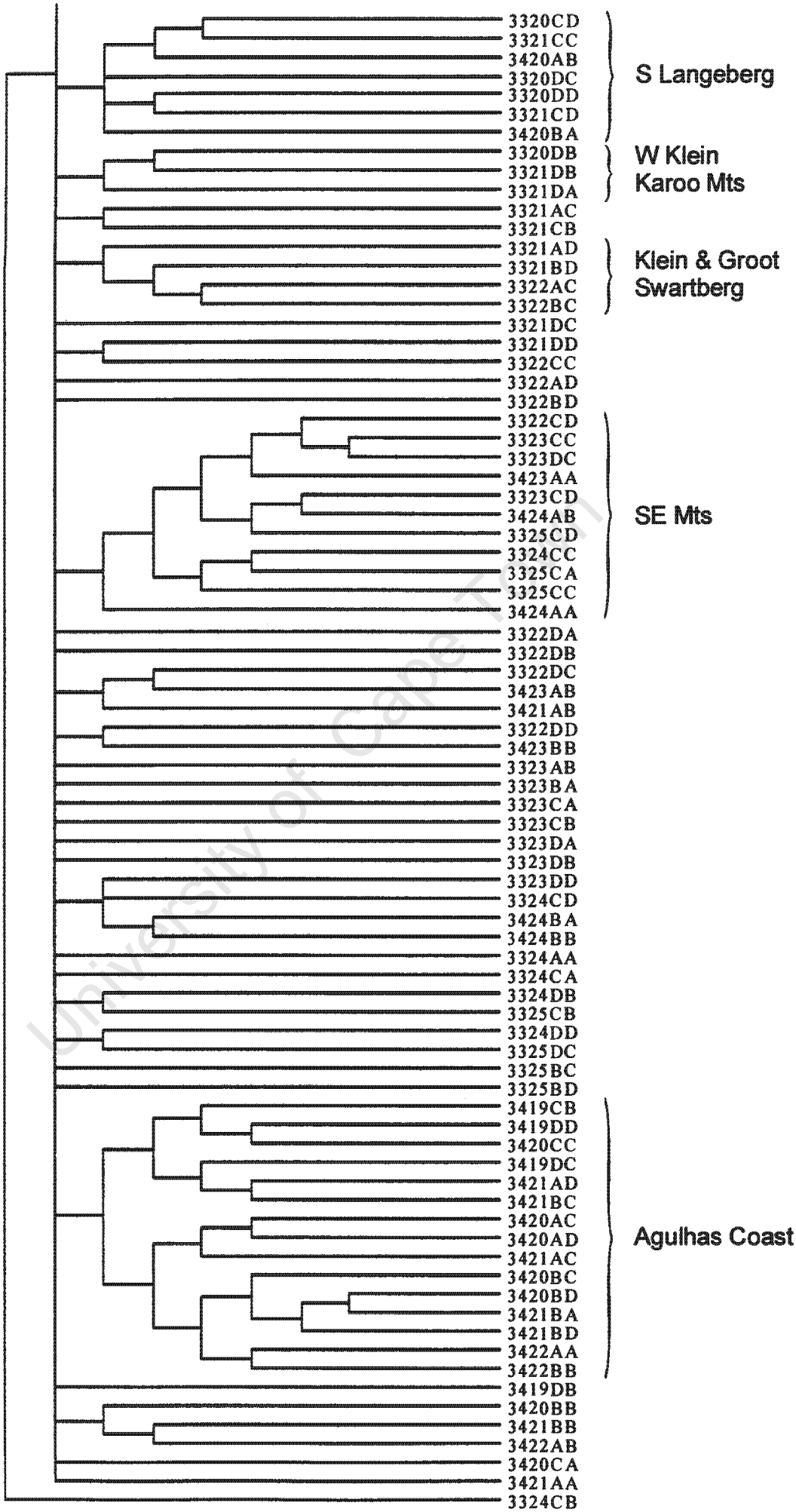

Fig. 5.3 cont.

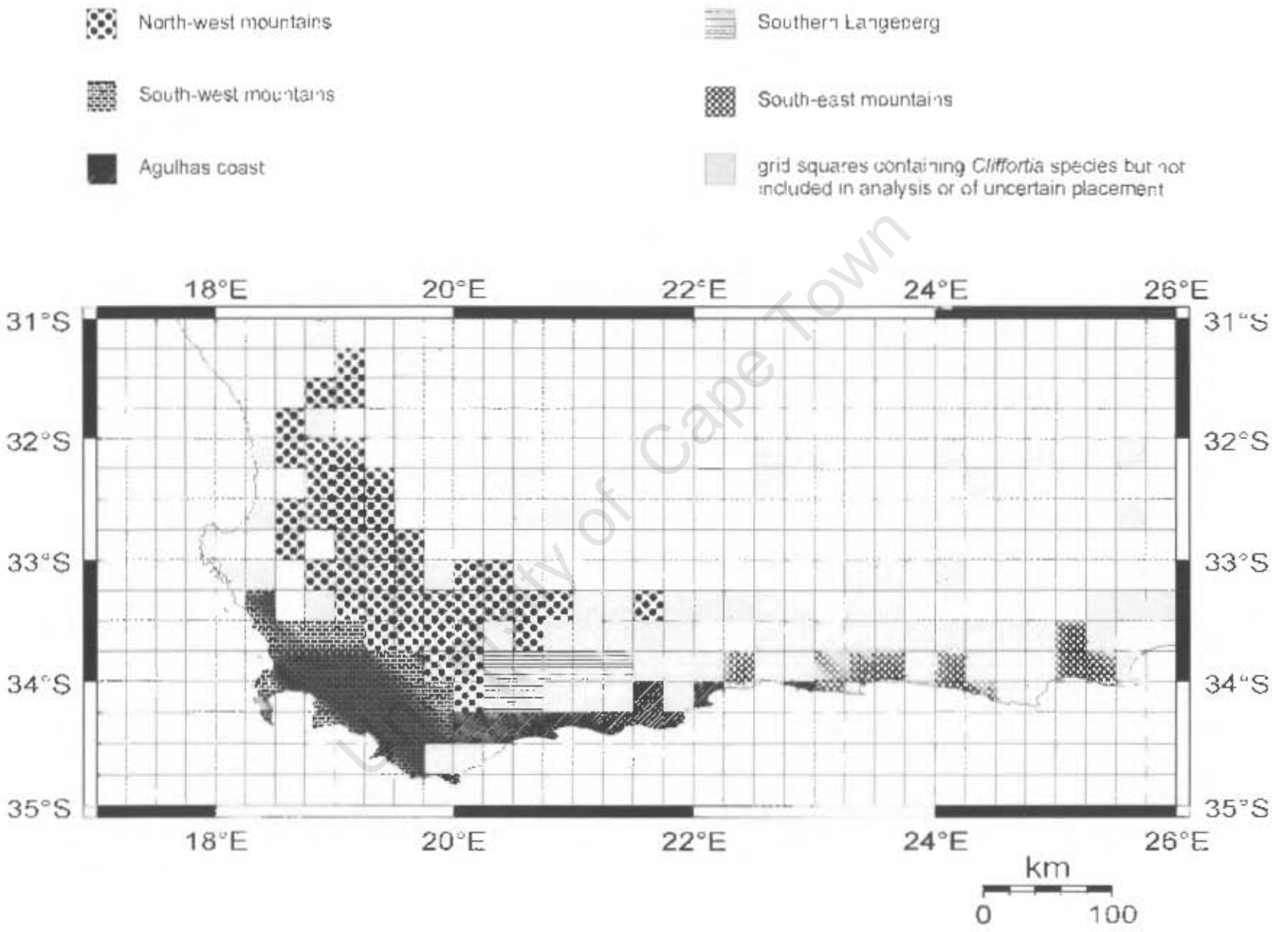

Fig. 5.4. Map of the Cape Floristic Region showing the placement of grid squares in to phytochoria based upon the clustering analysis shown in Fig. 5.3.

which have the highest concentration of species. However, this map highlights the way that the mountain ranges emerge as having the highest diversity. Indeed, *Cliffortia* is generally absent from low-lying areas except for the Cape Flats, Agulhas Plain and the Knysna coastal strip. The accuracy of this map will be expected to increase with proximity to Cape Town as the thoroughness of collecting becomes more complete. With this in mind, the location of the grid square 3418BB as containing the highest diversity of species can be more refined, and the mountains between Sir Lowry's Pass, Stellenbosch and Franschhoek might be better regarded as the true centre of diversity within *Cliffortia*. Second to this are four localities of comparable diversity: the Cape Peninsula, the mountains around du Toits Kloof and Bains Kloof, the Winterhoek Mountains near Tulbagh and the western end of the Riviersonderend Mountains. Outside of the CFR only the NE Drakensberg and possibly the Mpumalanga Highlands can be identified as centres of diversity (Fig. 5.5).

Examination of the maps of diversity of lineages, in both the chloroplast and nuclear trees, gives very similar results (Fig. 5.8 9). Indeed only when the lineages from the greatest depth within the tree are used does the pattern start to become less distinct. This can be supported by calculating the Spearman Rank-order Correlation Coefficient for these data (Table 5.1) and comparing them with the species diversity, as well as the sectional and subgeneric maps (Fig. 5.10).

Table 5.1. Spearman rank-order correlation coefficients for taxon diversity at varying ranks compared with lineage diversity at varying depths of the chloroplast (cp) and nuclear (5s) ultrametric trees.

	chloroplast				nuclear			
	1	2	3	4	1	2	3	4
Species	0.8437	0.9558	0.9871	0.9960	0.7839	0.9529	0.9919	0.9979
Sections	0.8662	0.9612	0.9769	0.9755	0.8079	0.9468	0.9734	0.9755
Subgenera	0.8150	0.7341	0.7328	0.7203	0.8364	0.7762	0.7173	0.7226
Weimarck sections	0.7176	0.8487	0.8701	0.8750	0.6630	0.8037	0.8767	0.8752

Comparison of the correlation coefficients shows, unsurprisingly, that the strongest correlation with the species rank is found at the shallowest depth (level 4). This is found to be true regardless of whether the chloroplast or nuclear tree is used. For the sectional level, the pattern is slightly different, with the second shallowest (level 3) level in the chloroplast giving the highest coefficient, although all the first three levels in both chloroplast and nuclear trees are strongly correlated. Most interesting however, is that at the subgeneric rank, although the correlation is at its weakest across all levels, the

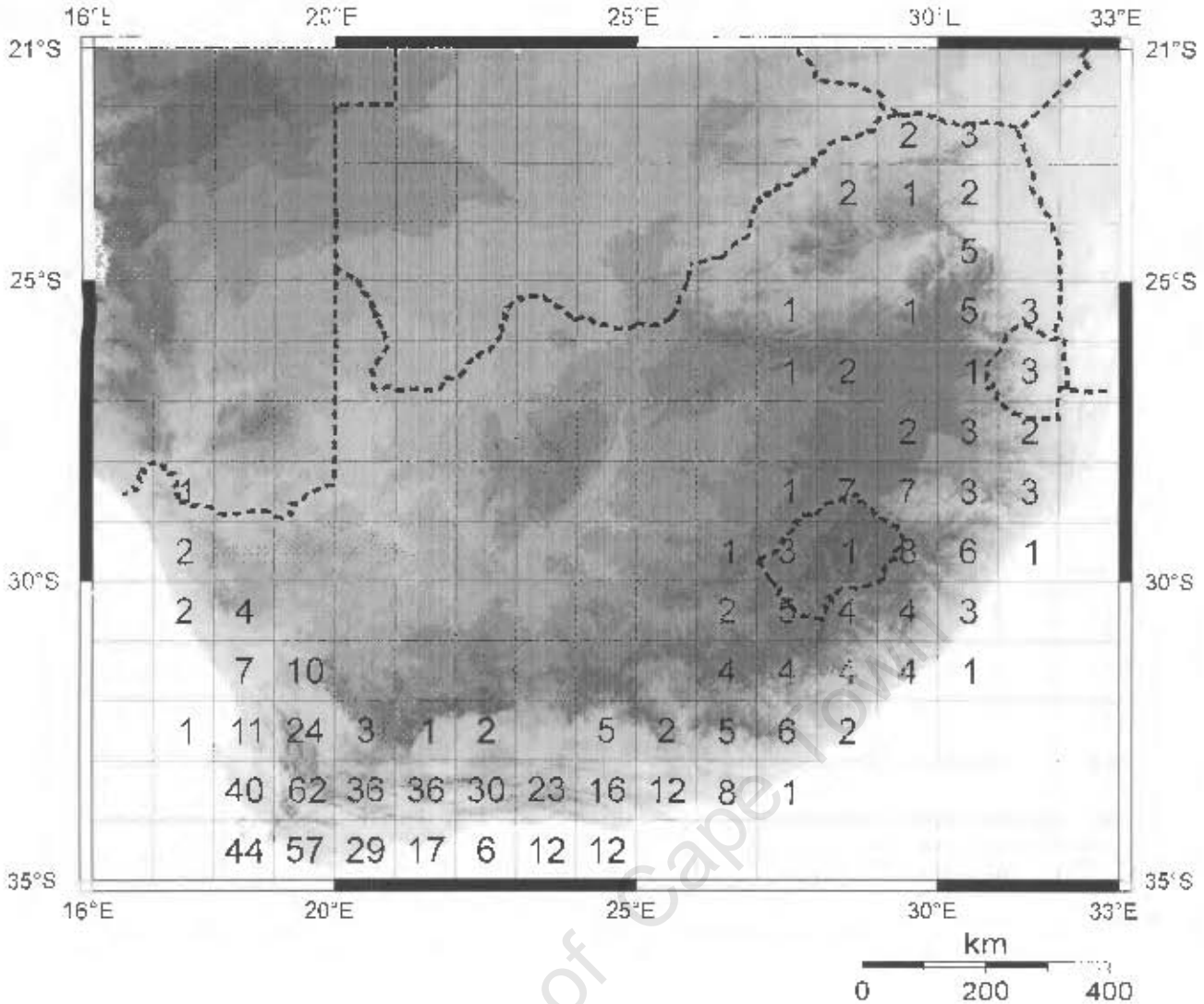

Fig. 5.5. Map to show the number of *Cliffortia* species present in each degree square within South Africa.

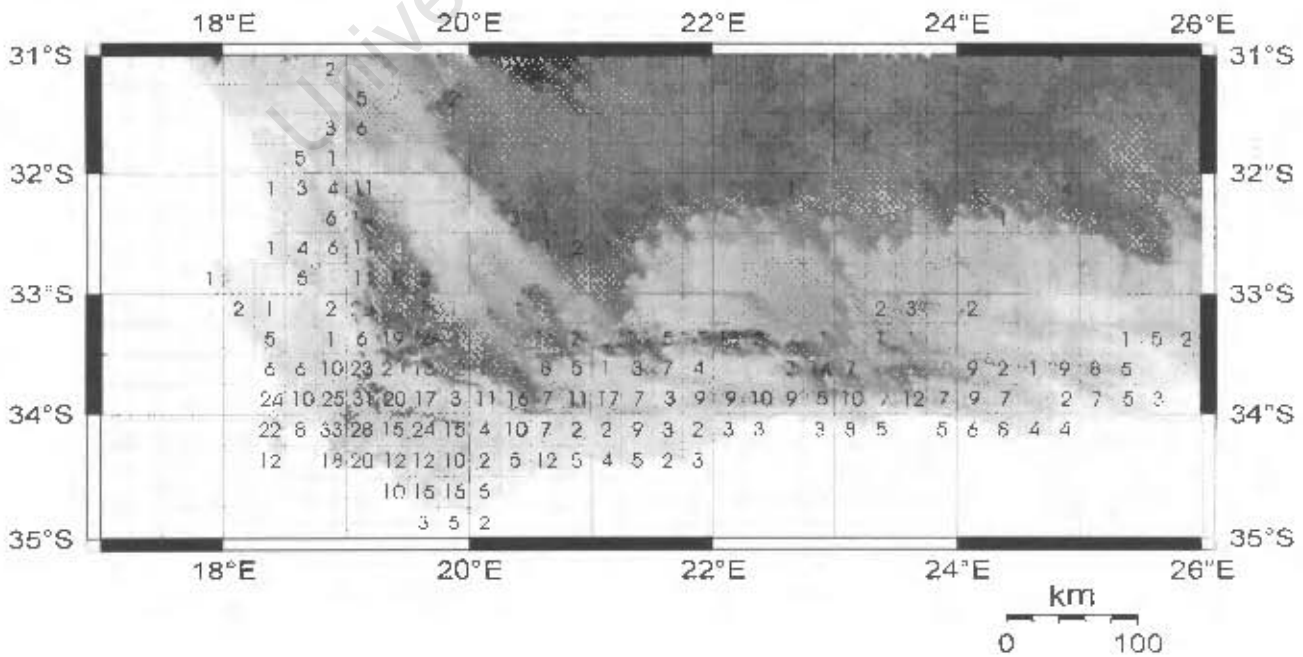

Fig. 5.6. Map to show the number of *Cliffortia* species present in each grid square within the Cape Floristic Region.

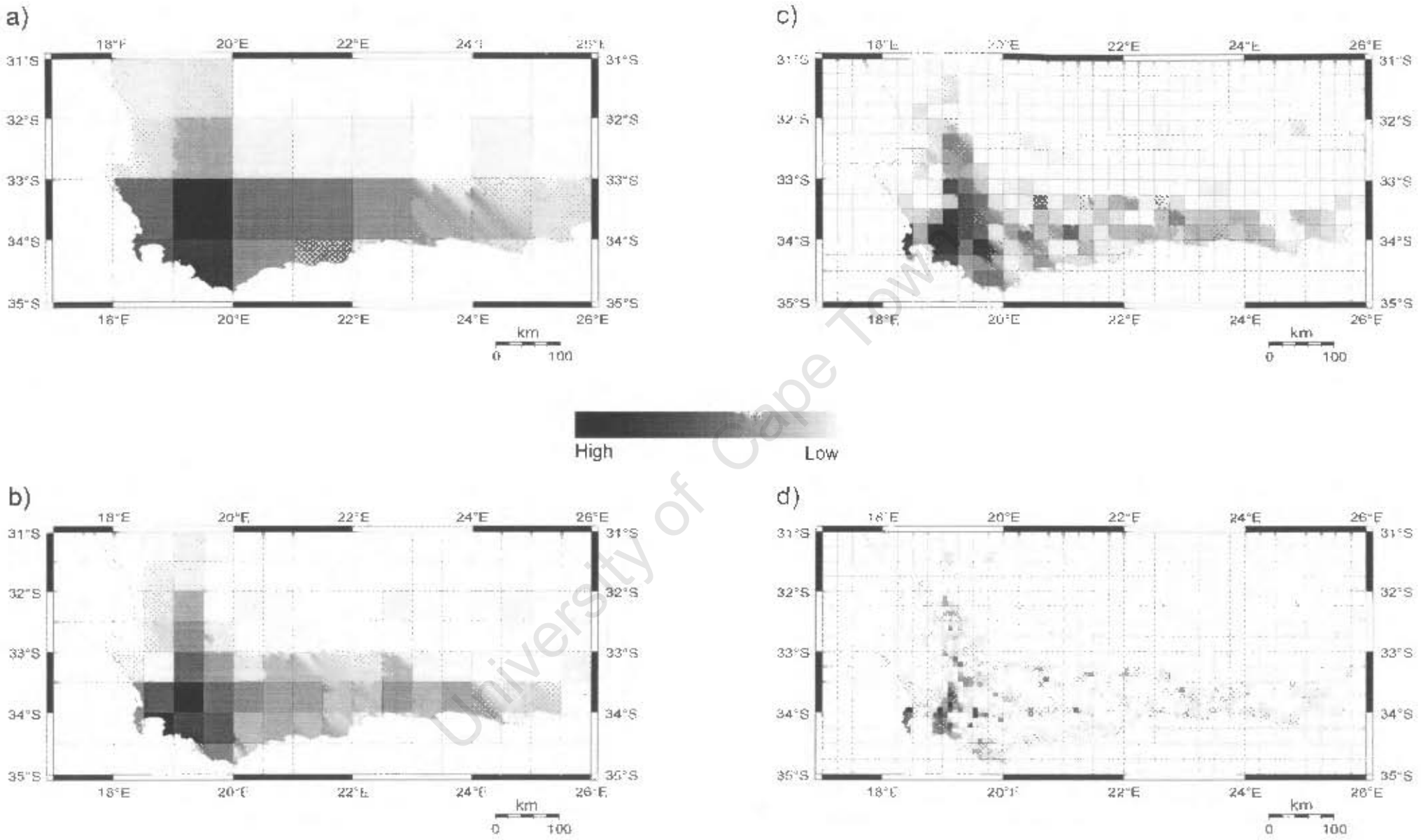

Fig. 5.7. Relative proportions of *Cliffortia* species within the Cape Floristic Region using different geographical unit sizes. a) degree square, b) ½ degree square, c) grid square, d) 5-min square.

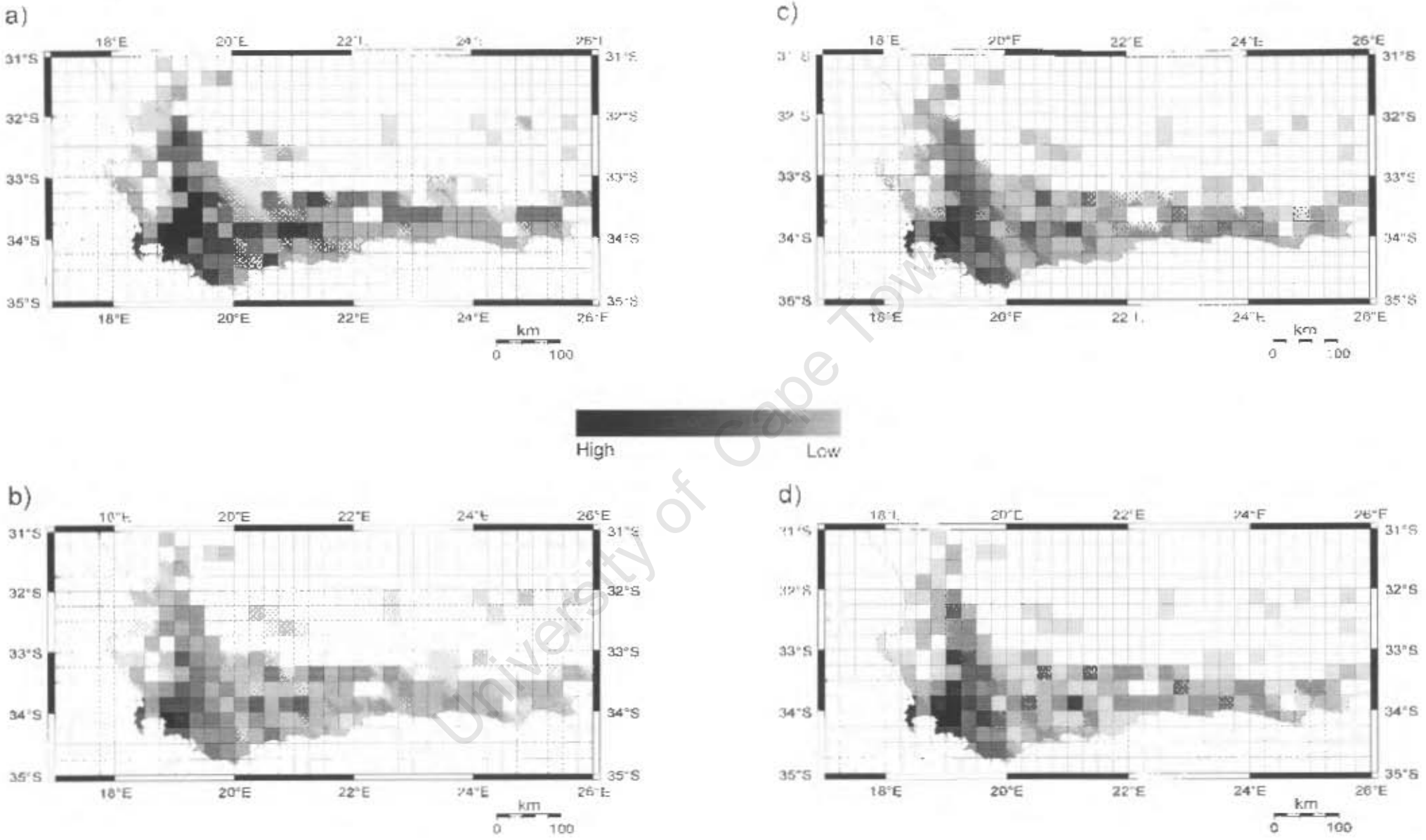

Fig. 5.8. Relative proportions of *Cliffortia* lineages within the Cape Floristic Region at different depths within the ultrametric chloroplast tree (see following page). a) level 1, b) level 2, c) level 3, d) level 4.

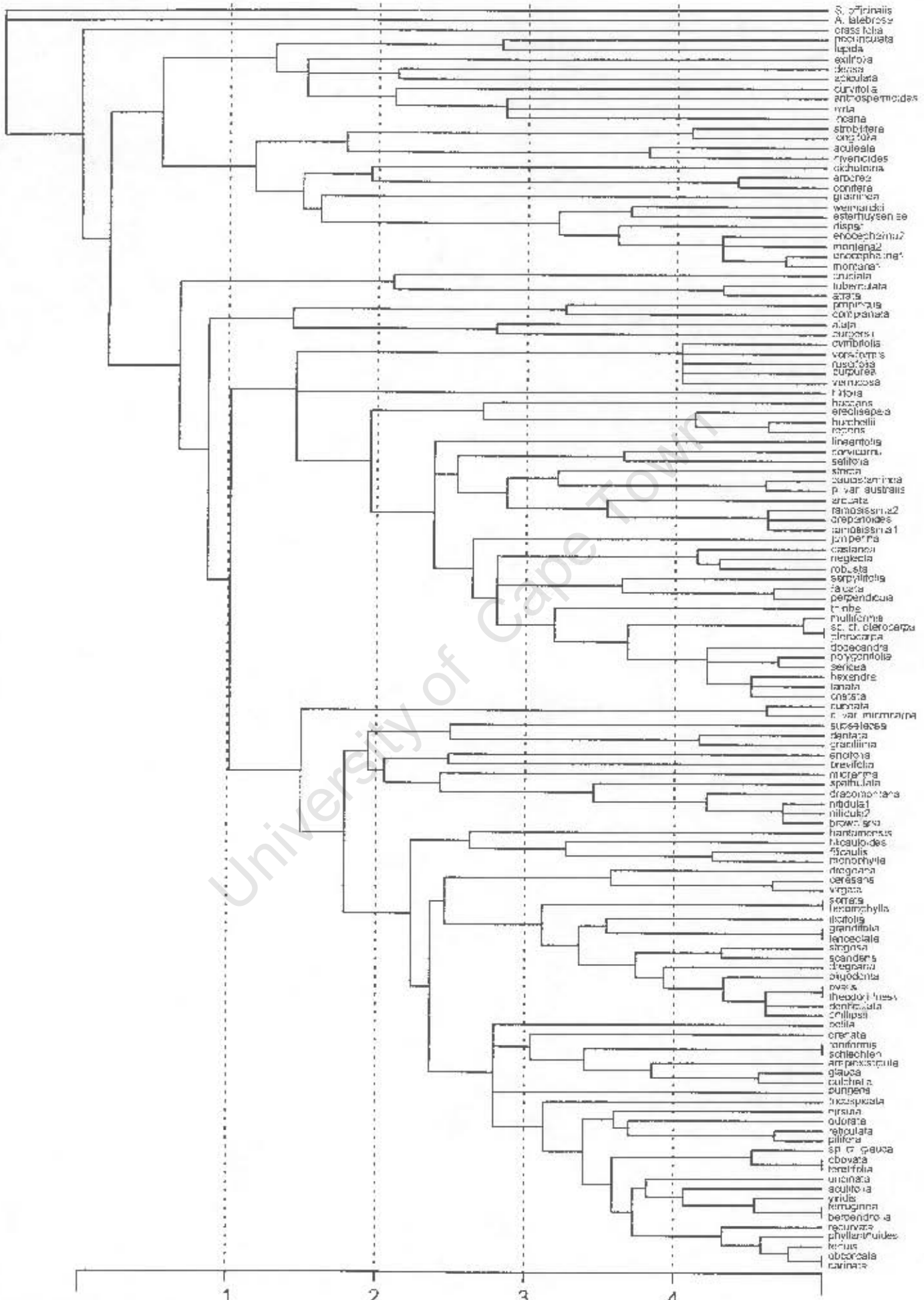

Fig. 5.8 cont.

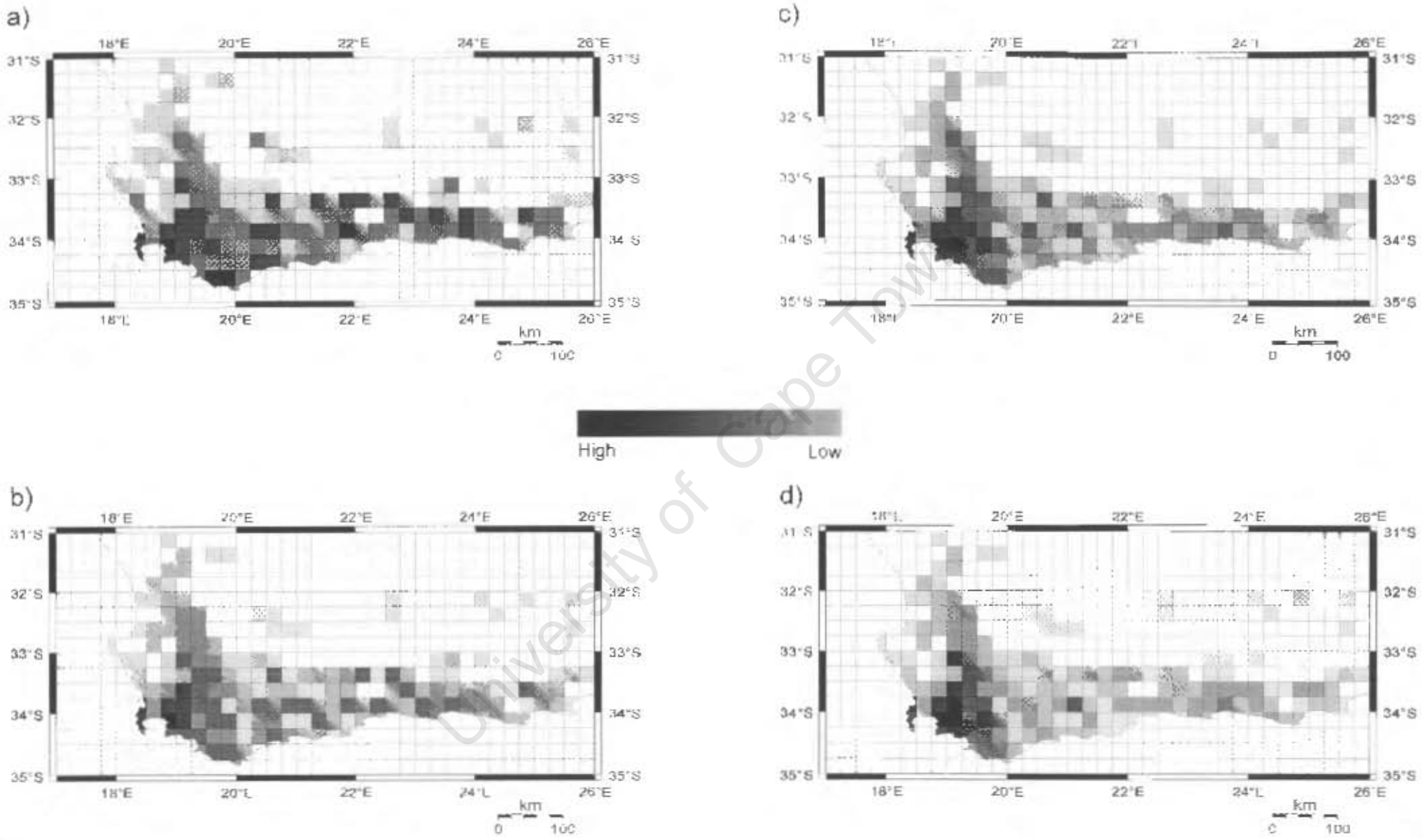

Fig. 5.9. Relative proportions of *Cliffortia* lineages within the Cape Floristic Region at different depths within the ultrametric nuclear tree (see following page). a) level 1, b) level 2, c) level 3, d) level 4.

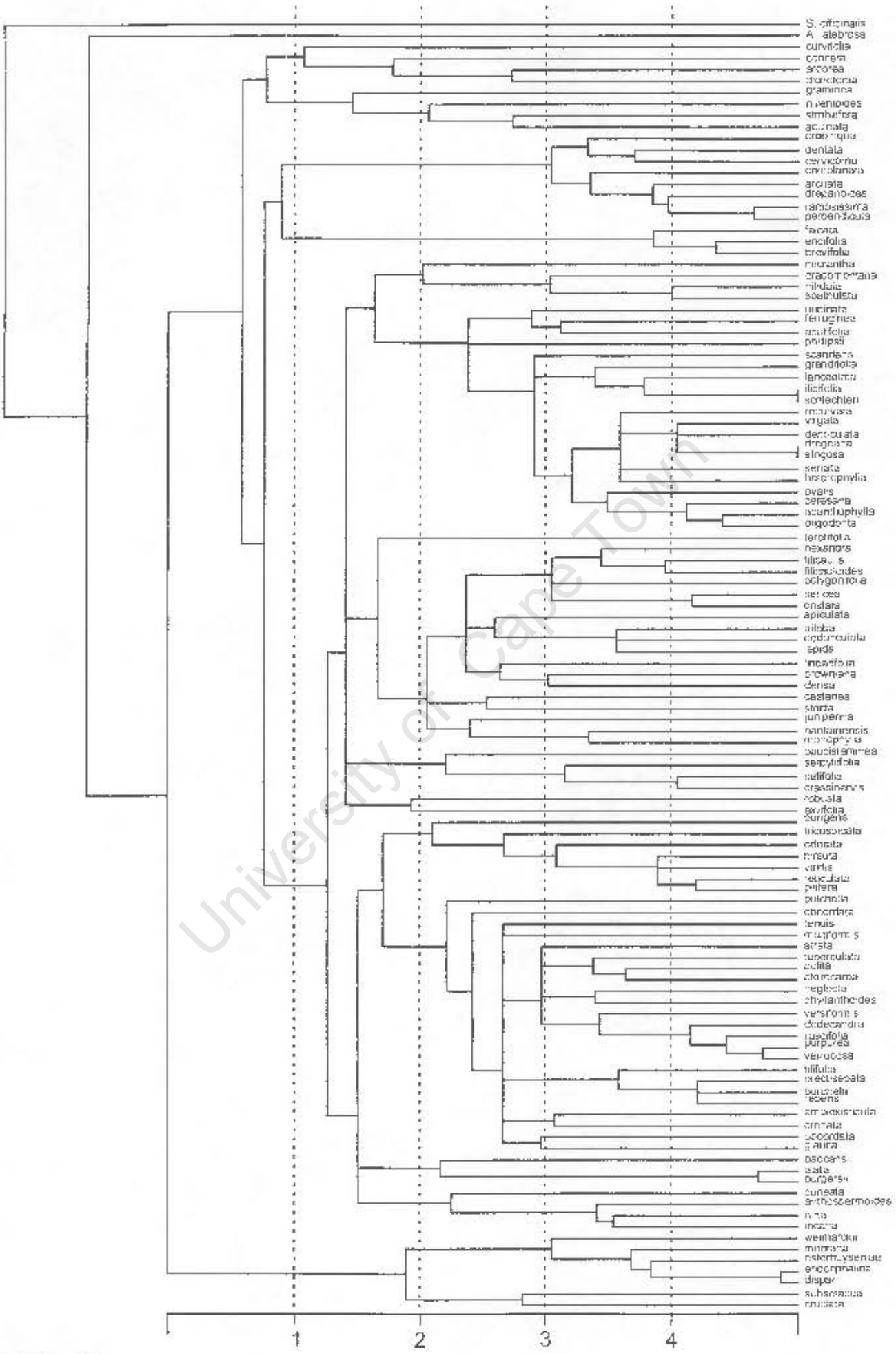

Fig. 5.9 cont.

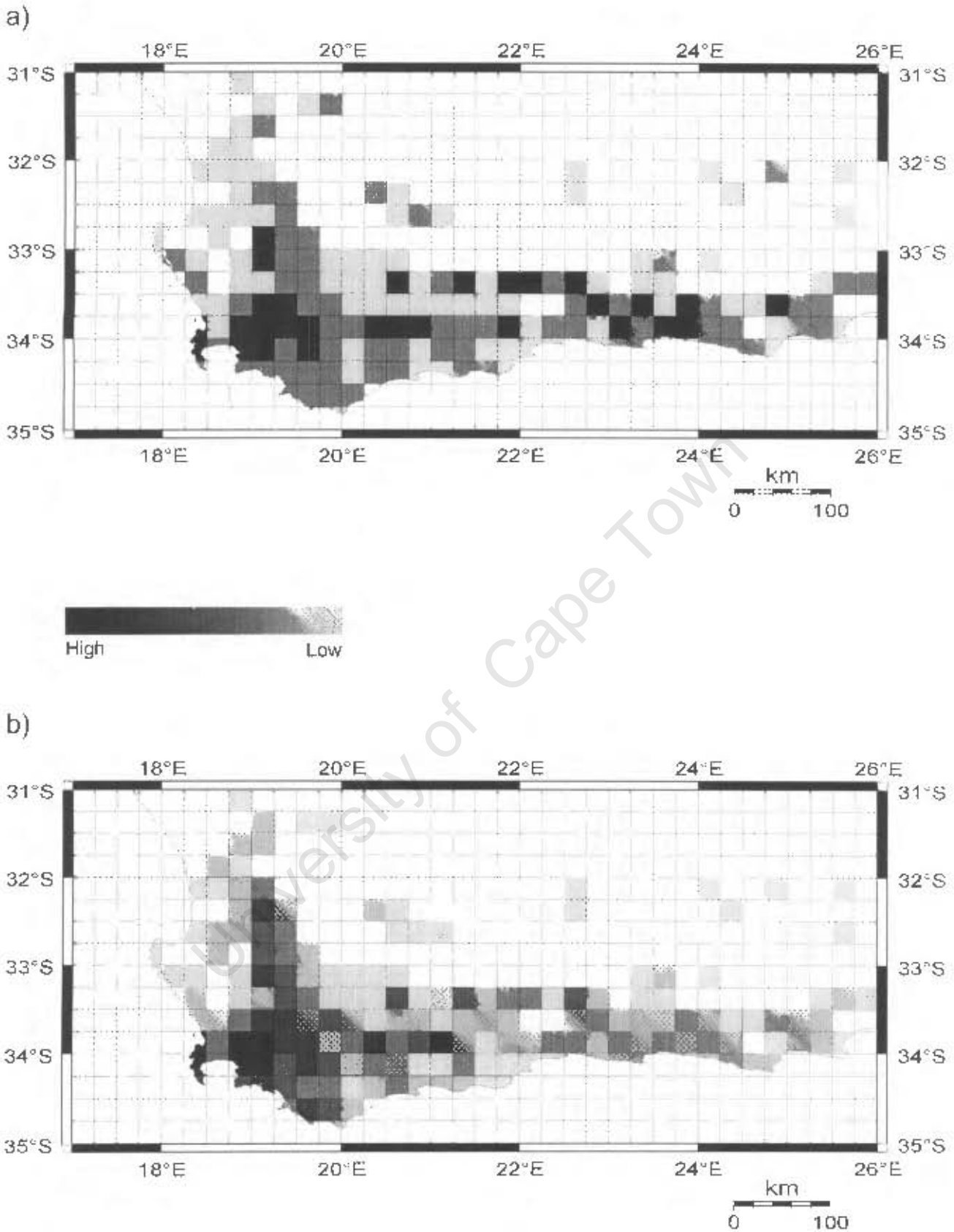

Fig. 5.10. Maps to show the relative density of (a) *Cliffortia* subgenera and (b) sections within the Cape Floristic Region.

strongest correlation is at the deepest level within the tree (level 1). At all levels, Weimarck's sectional classification gives a much poorer estimate of true diversity than the sections proposed here.

Centres of endemism:

The density of range-restricted species under the different weighting schemes is shown in Fig. 5.11. The inverse and weight3 weighting produce very similar results with a concentration of endemic species in the South-western Mountains, although the inverse weighting highlights the area between Franschhoek and Bains Kloof most strongly. Outside of this area the Cape Peninsula, Agulhas Plain, Nieuwoudtville, Cederberg, Langeberg, Swartberg and Graaff reinet Mountains all have small centres of endemism. On the other hand the pattern in the corrected inverse weighting scheme is harder to detect. High proportions of endemic species are found in du Toits and Bains Kloof Mountains, Kleinmond Mountains, De Hoop Nature Reserve, Blesberg in the Eastern Swartberg, Nieuwoudtville and Graaff reinet Mountains.

Using the more inclusive lineages the pattern is very similar (Fig. 5.12). The greatest difference is in the weight3 weighting, which highlights the greatest concentration of endemic lineages as being slightly further north between Groot Winterhoek Mountains, Hex River Mountains and du Toits Kloof Mountains. Furthermore, the corrected inverse weighting shows even less pattern, with most areas showing a uniform degree of endemism except for high proportions in De Hoop Nature Reserve, Blesberg, Nieuwoudtville and Graaff reinet Mountains.

Areas of endemism:

Over 20,000 trees were found in the parsimony analysis using species as characters, but only 5993 trees were found when using lineages. As a result, the consensus tree was more resolved when lineages were employed (Fig. 5.15), although a greater number of distinct endemic areas could be identified when using species (Fig. 5.13). However, the areas retrieved were similar, although there were slight variations in size (see Fig. 5.14 & 5.16). Seven areas of endemism could be recognised in common to both analyses: North-western Mountains, South-western Mountains, Cape Peninsula and Cape Flats, Agulhas Plain, Southern Langeberg, Klein and Groot Swartberg, and Drakensberg (Table 5.2), but there were also some differences.

The Anysberg formed a clade with the Hex River valley on the species tree, but to the Hantam and Roggeveld Mountains on the lineages tree. The South-western Mountains

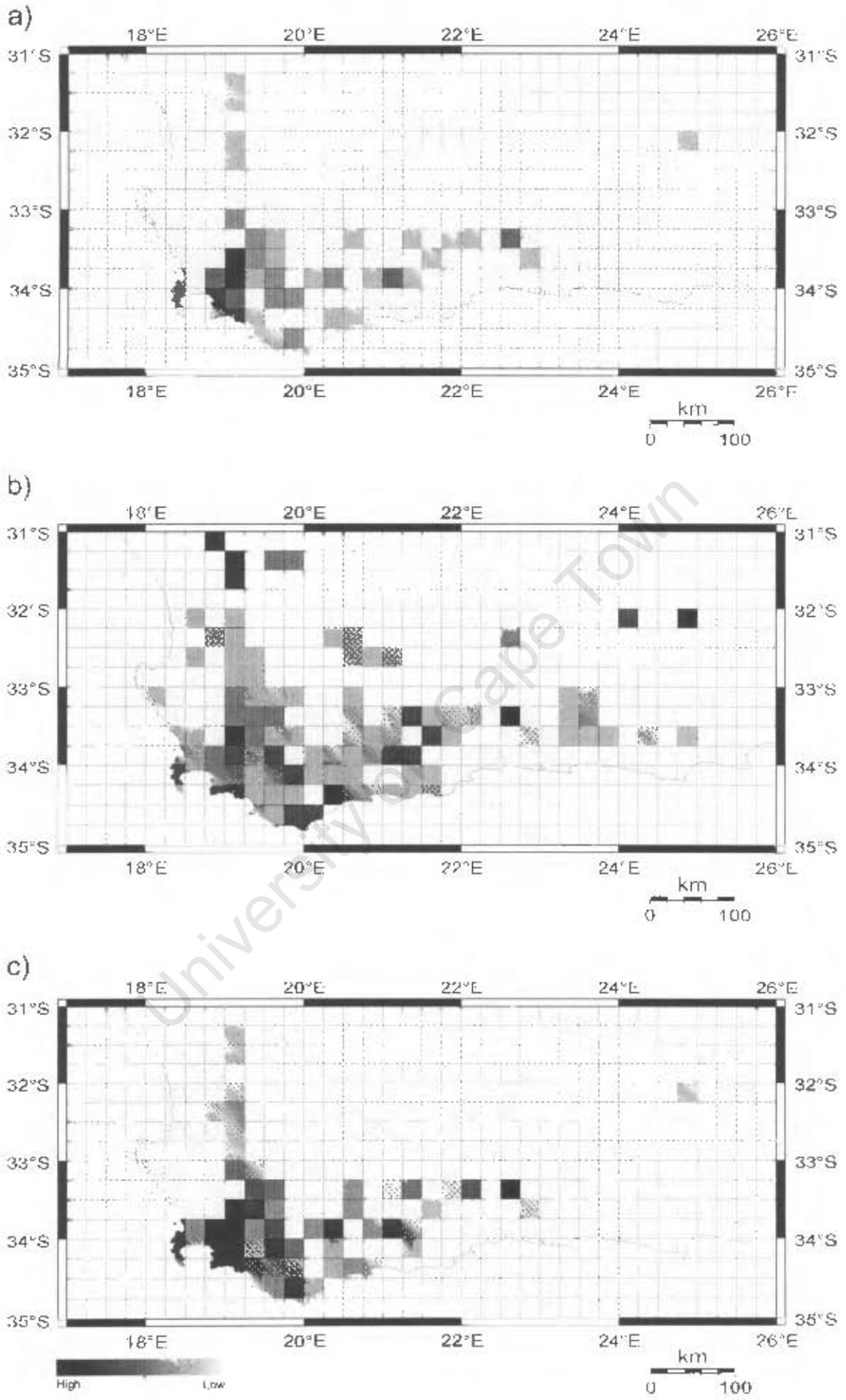

Fig. 5.11. Comparison of different weighting schemes to detect centres of range-restricted species: a) inverse, b) corrected inverse, c) weight3.

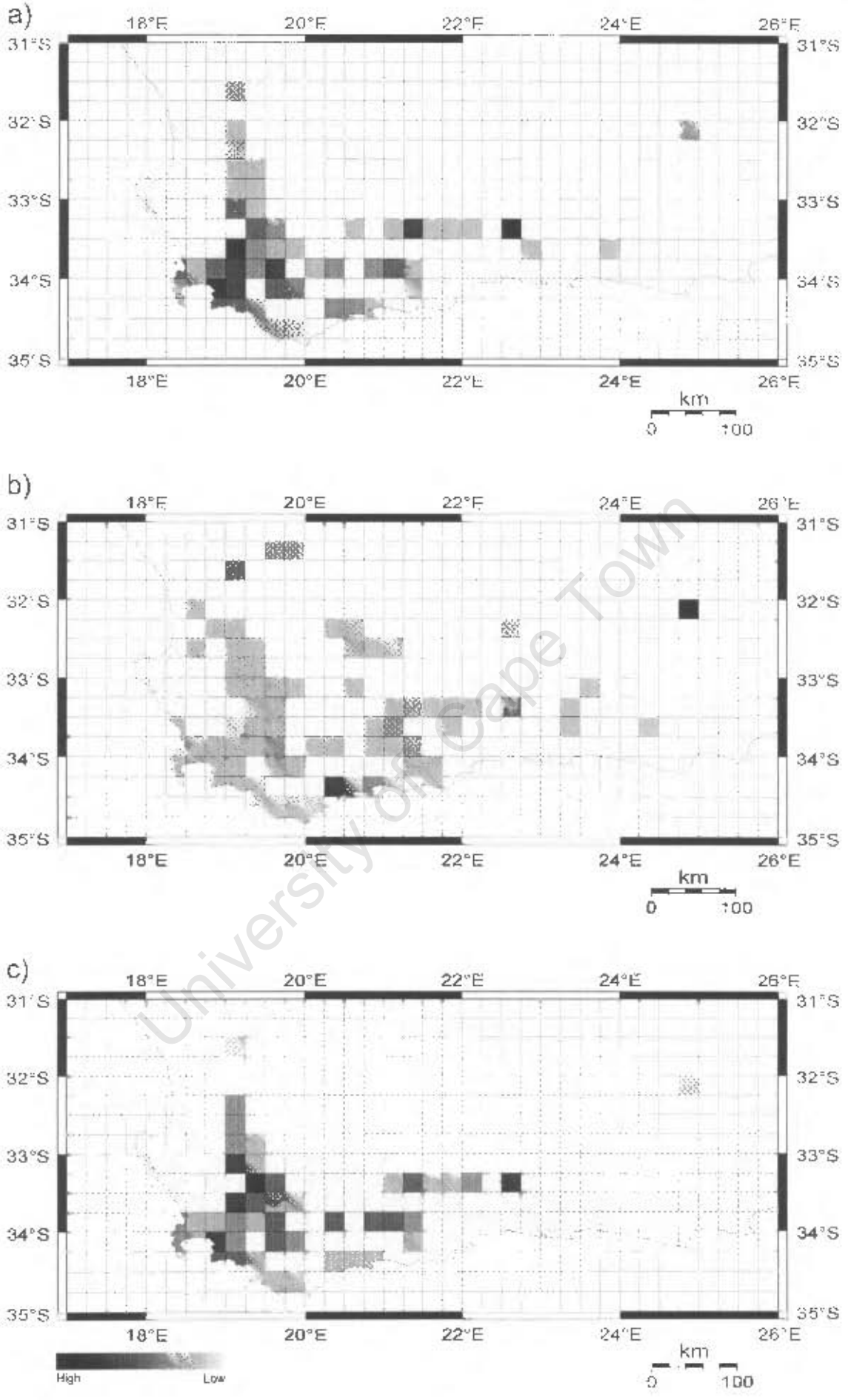

Fig. 5.12. Comparison of different weighting schemes to detect centres of range-restricted lineages based upon the chloroplast tree level 4 (Fig. 5.8): a) inverse, b) corrected inverse, c) weight3.

formed a separate unit from the Bettys Bay Mountains in the species analysis and the Riviersonderend Mountains were a sister group, while in the lineage analysis all three areas were intermixed in the same clade.

The most surprising failing of the lineage analysis was that the Swartberg clade collapsed in the strict consensus, as it contains several endemic and near-endemic species (Table 5.2). This is possibly due to *C. robusta*, which is predominantly a Swartberg endemic but is also known from Mannetjiesberg in the Kammanassie Mountains. That range forms part of the South-eastern Mountains group, which conversely failed to resolve in the species analysis. The Robertson Langeberg clade resolved in both analyses but it lacks a single endemic species and so could not be recognised as an area of endemism. This is presumably because several range-restricted species overlap here, and this indicates a problem with this analytical technique.

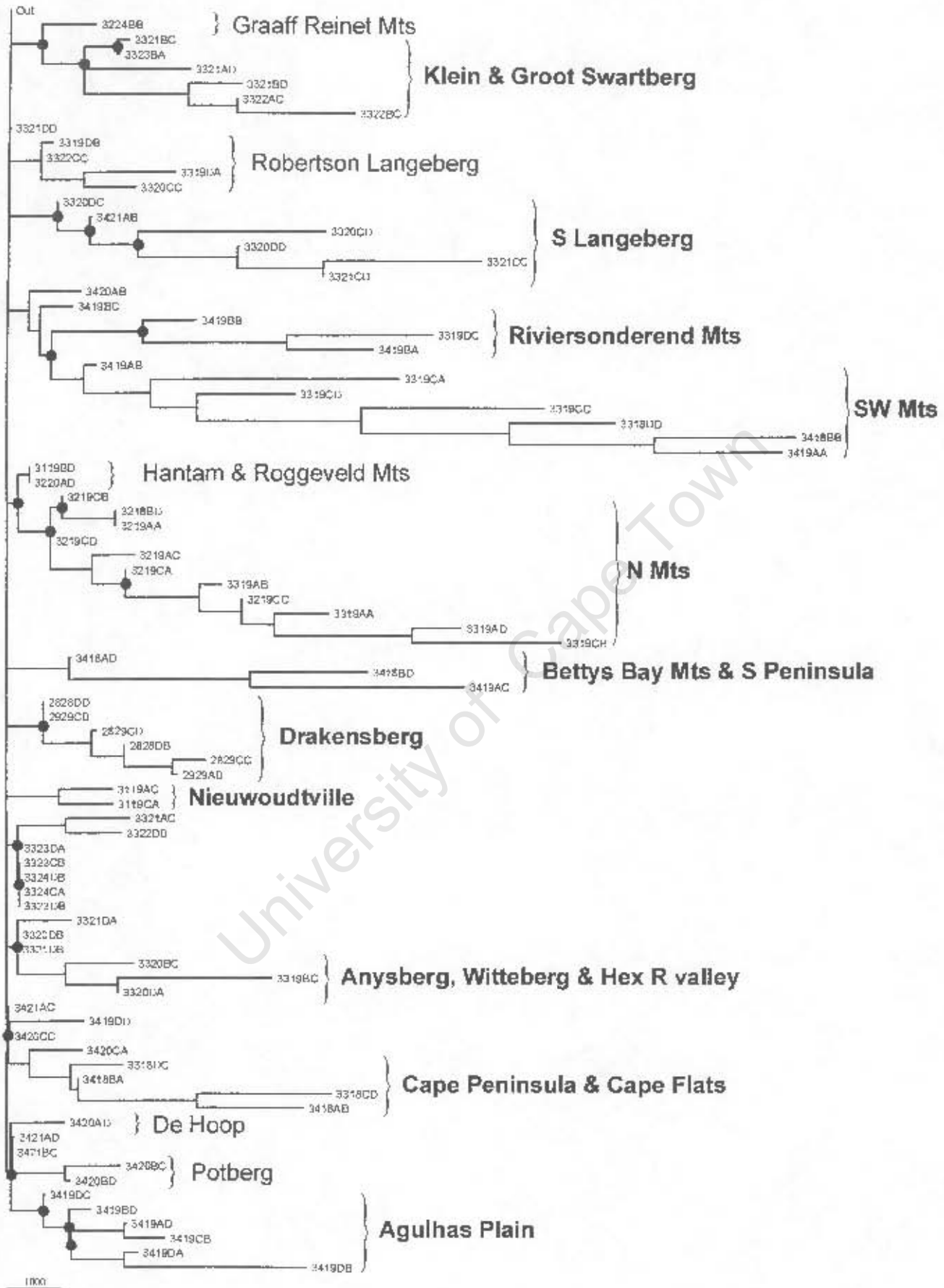

Fig. 5.13. Phylogram of an arbitrarily chosen tree from the parsimony analysis of endemic areas using species as characters. Dots indicate nodes that collapse in the strict consensus. Areas in bold contain at least two endemic species.

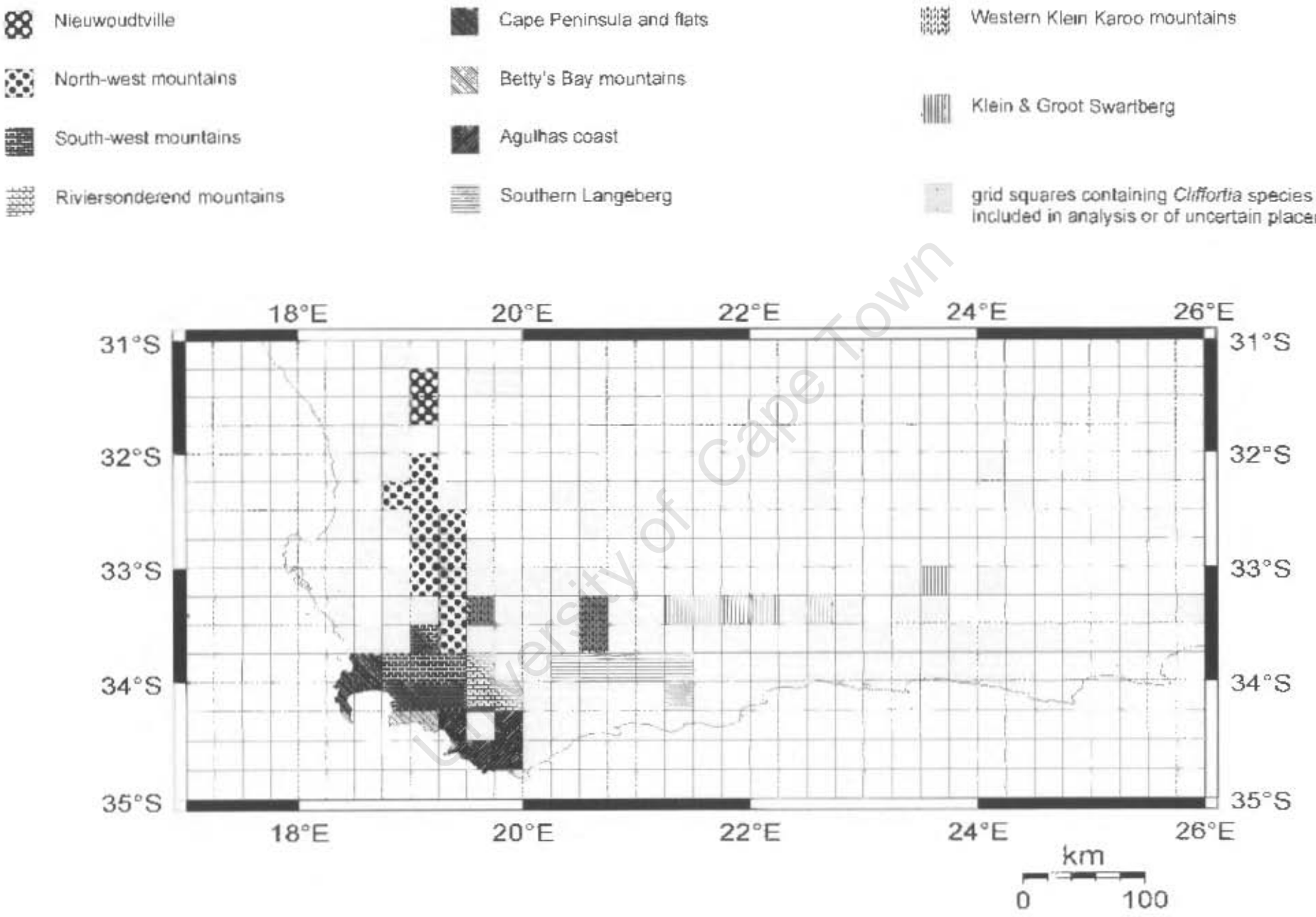

Fig. 5.14. Map of the Cape Floristic Region showing the delimitation of endemic areas using species as characters in the parsimony analysis (Fig. 5.13).

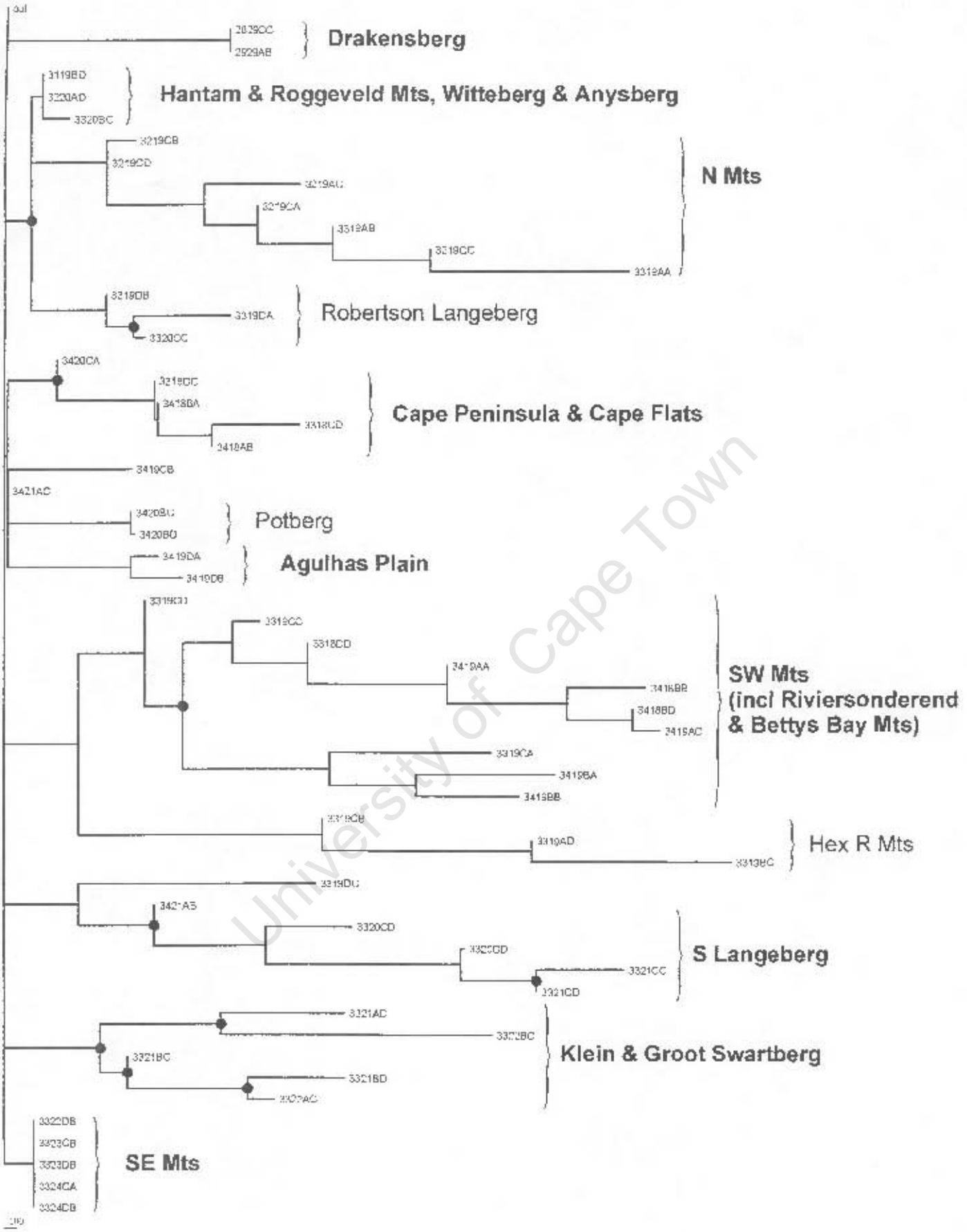

Fig. 5.15. Phylogram of an arbitrarily chosen tree from the parsimony analysis of endemic areas using lineages from the chloroplast tree level 4 (Fig. 5.8) as characters. Dots indicate nodes that collapse in the strict consensus. Areas in bold contain at least two endemic species.

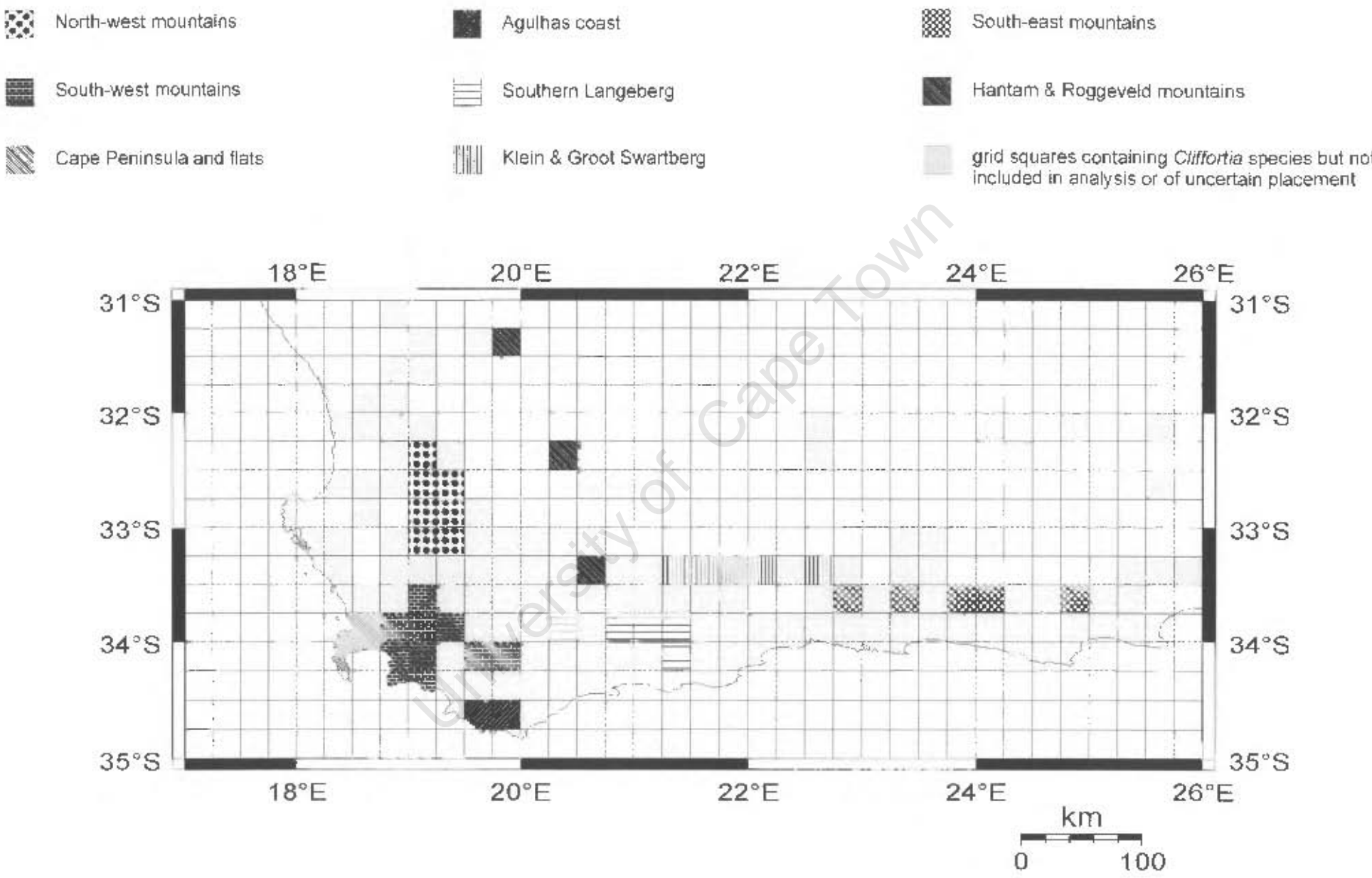

Fig. 5.16. Map of the Cape Floristic Region showing the delimitation of endemic areas using lineages as characters in the parsimony analysis (Fig. 5.15).

Table 5.2. Areas identified in both analyses with species endemic to them. Near-endemics, with only one or two localities that occur outside of the designated area, are given in brackets.

Endemic Area		
Lineages	Species	Endemic Species
Drakensberg	Drakensberg	(*C. browniana*), *C. dracomontana*, *C. filicauloides*, *C. spathulata*
	Graaff Reinet Mts	*C. bolusii*
Hantam-Roggeveld Mts		*C. arborea*
SE Mts		*C. dispar*, *C. drepanoides*, *C. ×homunculi*, (*C. polita*)
Klein & Groot Swartberg	Klein & Groot Swartberg	*C. aculeata*, *C. crassinervis*, (*C. montana*), *C. nivenioides*, (*C. robusta*), *C. setifolia*, *C. verrucosa*
S Langeberg	S Langeberg	*C. alata*, *C. densa*, *C. glauca*, (*C. gracillima*), *C. grandifolia*, *C. lanceolata*, *C. reniformis*
Potberg	Potberg	*C. incana*
	De Hoop	*C. burgersii*
Agulhas Plain	Agulhas Plain	*C. anthospermoides*, *C. berberidifolia*, *C. curvifolia*, *C. multiformis*, (*C. phyllanthoides*), *C. schlechteri*, (*C. perpendicula*), *C. tenuis*
South-western Mts	Betty's Bay Mts	*C apiculata*, *C. geniculata*, *C heterophylla*, *C. recurvata*, *C. serrata*, *C. versiformis*, *C. viridis*
	Riviersonderend Mts	*C. crenulata*, *C. cruciata*, *C. meyeriana*, *C. scandens*
	South-western Mts	(*C. dentata*), *C. denticulata*, *C. gracilis*, *C. oligodonta*, *C. ovalis*, (*C. pedunculata*), *C. pilifera*, *C. pubescens*, *C. rigida*, *C. strigosa*, *C. subdura*
		C. complanata, *C. cuneata*, *C. hirsuta*, *C. phillipsii*
Cape Peninsula & Flats	Cape Peninsula & Flats	*C. carinata*, (*C. ericifolia*), *C. hirta*, *C. integerrima*, *C. intermedia*, *C. theodori-friesii*
Hex R Mts	Northern Mts	*C. esterhuyseniae*
Northern Mts		*C. acanthophylla*, *C. hexandra*, *C. pilosula*, *C. reticulata*, *C. triloba*, *C. uncinata*
		C. ceresana, (*C. virgata*), *C. weimarckii*
Anysberg	Anysberg	*C. conifera*
	Nieuwoudtville	*C. acutifolia*, *C. dichotoma*, *C. purpurea*

By assigning species to the various endemic areas, 69 of the species are accounted for with a further 11 near endemics (Table 5.2). This supports Linder's (2001a) finding that using grid squares is a good proxy for the various ecological factors that define endemic areas.

Phytogeographical species groups:

The clustering algorithm found 228 ties between trees, but there was still considerable resolution in the strict consensus tree (Fig. 5.17). Fifteen distinct phytogeographical

groups could be identified, most of which corresponded closely to Weimarck's groupings.

The Northern Mountains group form a large group of species that can be subdivided into two smaller groups that correspond to an arid versus mesic divide; the arid species being found in the north and along the eastern edge and onto the north-western reaches of the Klein Karoo Mountains.

The Betty's Bay Mountains group contains all endemic species except for *C. dodecandra* and *C. subsetacea*, which also occur on the southern Cape Peninsula and around the coast as far as Brandfontein. The South-western Mountains group somewhat surprisingly includes *C. marginata*, but this is presumably an artefact of the resolution, the species truly being found on the low clayish soils of the Cape Flats between Somerset West and Stellenbosch. The Cape Ubiquists and the Southern group are both widespread species, the latter, however, not being found far from the southern coastal belt. The presence of *C. strobilifera* here rather than in the Extra-Cape group is unexpected as it is found all the way to the Soutpansberg. However, none of the Extra-Cape group is found to the west of Swellendam, while *C. strobilifera* is found up the West Coast as far as the Kamiesberg. Apart from this, only *C. eriocephalina* (mountains around Graaff Reinet and Amatola Mountains) and *C. odorata* (Pondoland) extend outside the CFR. Within the Cape Ubiquists, a subgroup can be recognised that contains *C. eriocephalina*, *C. neglecta* and *C. tuberculata*, which are all widespread species but are more or less restricted to high mountain peaks. The Riviersonderend Mountains form a separate group but clearly associated with the above four groups of species.

Resolution affects the next grouping, as the Cape Peninsula endemics, *C. theodori-friesii* and *C. intermedia* are included within a broader Cape Flats and Agulhas Plain group. This is attributable to the Cape Flats endemic *C. hirta* and near endemic *C. ericifolia*, which occur in the same grid squares. Sister to this Peninsula group is an interesting group that contains all the widespread species that can tolerate more alkaline conditions. The other species that can grow in neutral to alkaline soils are more restricted in their distributions and so have less reliable associations.

The Langeberg group forms an association with the western Klein Karoo Mountains, while the Swartberg group are more closely associated with the South-eastern Mountains. It is in this latter group that *C. ramosissima* is placed. It has a very unusual distribution, from the low clay hills around Elim to Blouberg in the Soutpansberg, but is particularly common in the south-eastern CFR, which may explain its association here.

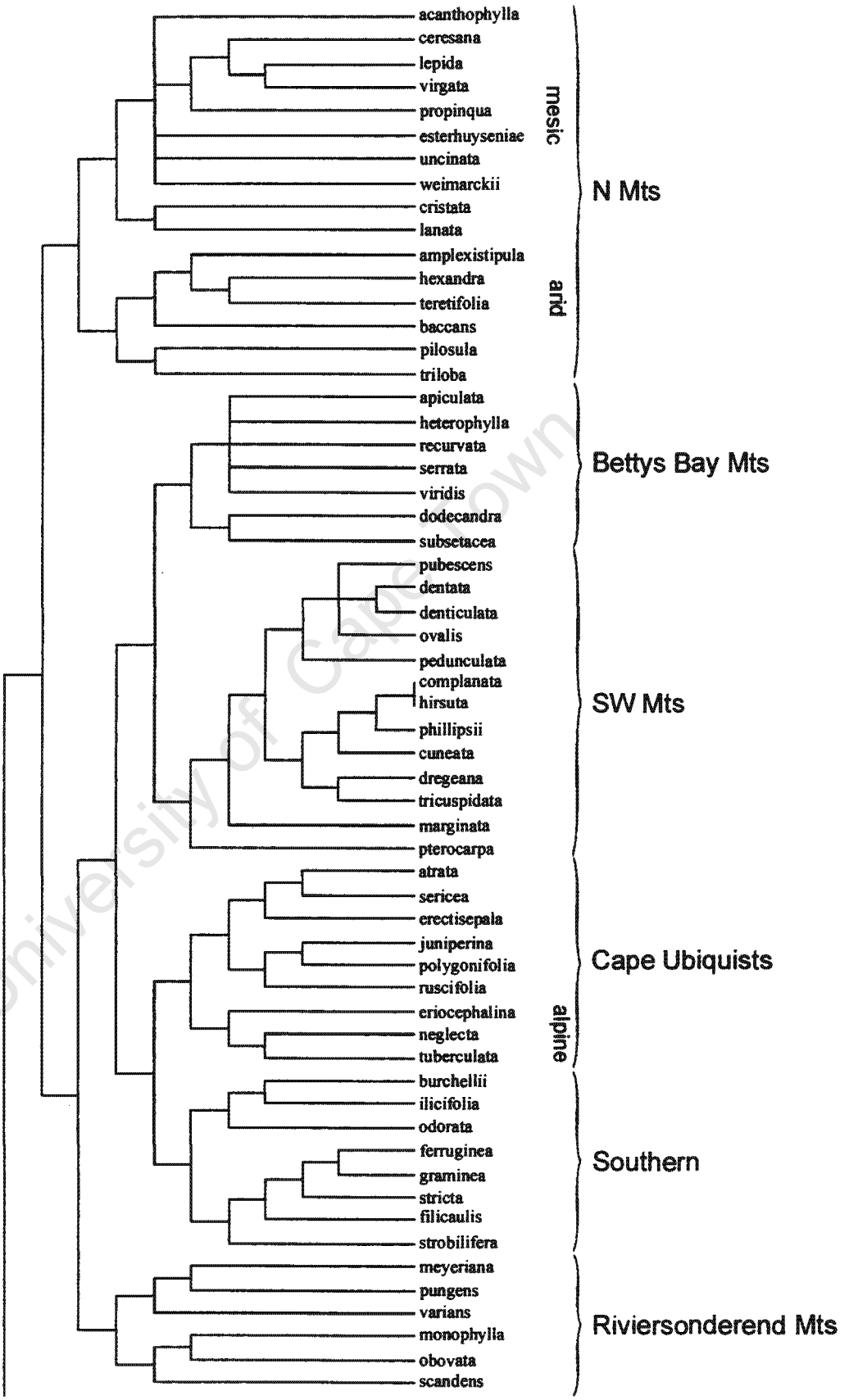

Fig. 5.17. Strict consensus tree from the UPGMA clustering algorithm on the Jaccard similarity matrix retrieved from using species as items and grid squares as objects. Species found in only a single grid square are excluded.

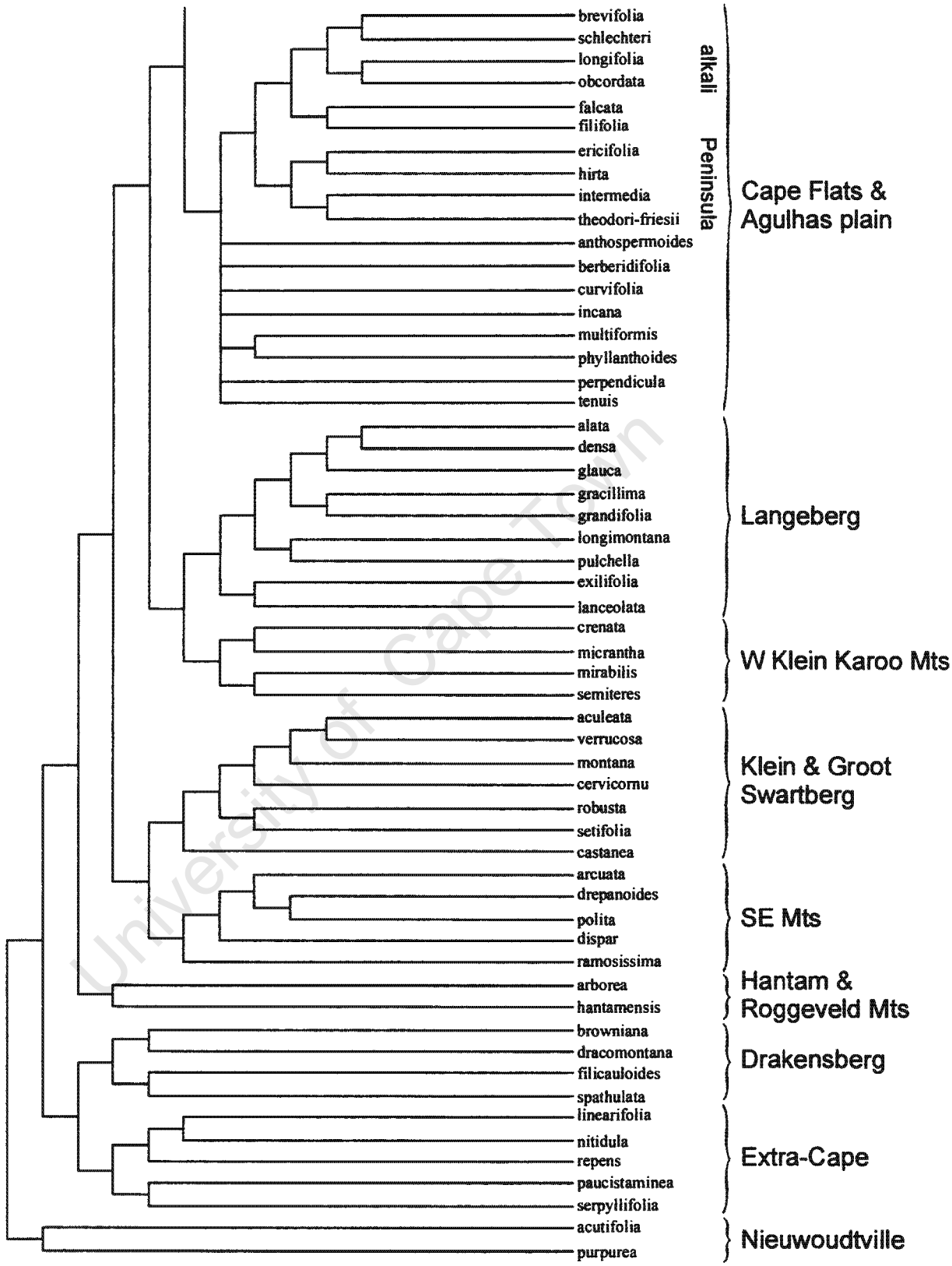

Fig. 5.17 cont.

The Bokkeveld Escarpment at Nieuwoudtville forms a small isolated group but otherwise the remaining groups all contain species that have all or the greater part of their distribution outside the CFR. *C. linearifolia*, *C. paucistaminea* and *C. serpyllifolia* of the Extra-Cape group and *C. hantamensis* of the Hantam-Roggeveld group all extend their range within the CFR for some distance. While the Drakensberg group are all endemic to the area except for *C. browniana*, which also occurs in the Mpumalanga Highlands.

Discussion:

Phytochoria:

The identification of five large phytochoria for *Cliffortia* in the CFR corresponds to similar findings by Weimarck (1941). However, there are notable differences. Weimarck only recognised the Agulhas coast as a subcentre, while here and in other studies (Oliver *et al.*, 1983; Linder & Mann, 1998) it is classified as one of the main phytochoria. On the other hand, Weimarck identified the Karoo Mountain Centre, while this was not corroborated by either Oliver (1983) or this study. Although the Klein and Groot Swartberg clustered together in this study but were not recognised as phytochoria because of the arbitrary cut-off of only recognising clusters with at least five terminals. In addition, the grid square 3321BC (Gamkaskloof) is situated between the Klein and Groot Swartberg yet clustered with the North-western Mountains and hence recognition of the Swartberg cluster would have created a discontinuous area. However, the Gamkaskloof area is very poorly collected (2 collections, 3 sight records, Fig. 5.2) and further collections may corroborate its placement with the Swartberg mountain chain. If this is confirmed the Swartberg should be recognised as a sixth phytochorion.

Centres of Diversity:

The effect of scale and collecting density upon recognition of centres of diversity is evident from this study. By identifying areas that are more diverse in *Cliffortia* species than their surrounding squares, both the degree square (Fig. 5.7a) and ½ degree square (Fig. 5.7b) maps locate just a single centre in the South-western Mountains, with a gradual decrease in species richness to the north and east, but for the grid square map (Fig. 5.7c) around eight centres can be recognised. However, the problems of collecting density also start to become apparent, and this is emphasised by the large number of centres that could be recognised for the 5-min square map (Fig. 5.7d). This highlights

the need for examining the effects of scale upon the results in every study conducted, so that neither species rich subcentres are missed, nor numerous small centres are created that actually belong to a single centre caused by undercollecting in the intervening areas. A trade-off between resolution and accuracy is needed in every biogeographic study, and this demonstrates that for *Cliffortia* it was optimal to use the grid square scale for the data available.

The use of higher taxa as surrogates for identifying centres of diversity has been debated (La Ferla *et al.*, 2002). Weimarck's sectional classification, which has been shown to not be a true reflection of the phylogeny (see Chapter 3), performed the worst when estimating species richness, but the new sectional classification based upon the phylogeny correlated well (Table 5.1). Hence, higher taxa can be used as surrogates for *Cliffortia* providing that they are phylogenetically based.

In this study, there is no need to use higher taxa as surrogates for species diversity, as documentation of species distribution has already been done. However, the more inclusive groups are useful for identifying centres that have greater genetic diversity and hence increased priority for conservation (Williams *et al.*, 1991). For *Cliffortia*, no strong difference is found between the centres located using species and those located using higher taxa or lineages. This implies that areas rich in species contain elements from across the range of lineages present within *Cliffortia* and that conserving areas rich in species will protect the majority of lineages too.

However, subgenus Arborea represents a lineage that does not correspond to the general pattern of diversity found within *Cliffortia*. It contains three (or possibly four) species that are found in scattered isolated populations in the Cape mountains on the edge of the Karoo and along the Great Escarpment. The basal position of this lineage and the scattered distribution of its species along the margins of the CFR might indicate that this may be an ancient lineage that might once have been more widespread and all that now remains are a few relic populations. This theory was proposed for *C. arborea* (the only species known at the time) by both Marloth (1906) and Weimarck (1934). However, all the species in the subgenus are allopatric and, growing on the edge of the CFR, they occur in areas that are often depauperate in other *Cliffortia* species. Therefore, the habitats that contain these species will not necessarily be identified in assessments of diversity, and may require special consideration in prioritising conservation areas.

The general patterns follow those found in many other fynbos plants, e.g. *Aspalathus*, Bruniaceae, Ericaceae, *Muraltia*, Penaeaceae, Proteaceae and Restionaceae (Oliver *et*

al., 1983). A notable exception is the almost complete absence of species on the West Coast Flats. This paucity of species can even be recognised on the quarter degree square level, where only three species are recorded. This area was recognised as a phytogeographical centre by Oliver et al. (1983) and a subcentre by Weimarck (1941). This highlights the problem in choosing single taxa to identify priority areas for conservation (e.g. Proteaceae, Rebelo & Siegfried, 1990; Rebelo & Siegfried, 1992; Rebelo & Tansley, 1993; Reid, 1998). Van Jaarsveld et al. (1998) have shown that different taxa rarely overlap in their distributional patterns, especially between higher taxa, and that the use of indicator taxa as surrogates for conservation planning are flawed. In this example, if *Cliffortia* had been used to identify species rich areas in the CFR then the West Coast Flats would have been excluded.

Endemism:

The patterns shown by the centres of endemism using both the inverse and the weight3 weighting reflect those detected by the centres of diversity. This is not surprising considering that the score for each grid square is directly affected by the number of species present. However, adjusting the figures for the number of species using the corrected inverse weighting produces unexpected results. This is accentuated when using the more inclusive lineages, with four squares receiving disproportionately high figures: De Hoop Nature Reserve (3420AD), Blesberg (3322BC), Nieuwoudtville (3119CA) and Graaff reinet Mountains (3224BB). These squares each have in common a single endemic species (i.e. only known from that grid square, namely: *C. burgersii*, *C. nivenioides*, *C. dichotoma* MS, *C. bolusii* MS respectively), yet comparatively few species otherwise. Using inverse weighting, endemic species in a single grid square receive twice the weighting of those in two, and this appears to cause an imbalance in using this method for locating centres of endemism. Therefore it might be more appropriate to correct for species diversity on the weight3 weighting scheme, as this places less emphasis on species found in only a single grid square.

The high correlation between using lineages or species in estimating centres of diversity is possibly because sister species are often allopatric or parapatric. This is especially so in species complexes where discrete morphological differences are rare and therefore variation is often associated with geographical patterns. Hence, although species complexes may increase the total number of species, in a subunit of geographical area such as a grid square, there is unlikely to be an increase in species richness. However, when estimating centres and areas of endemism, the degree of division into species

within species complexes can affect the results quite strongly because division of species into smaller units will also decrease the range sizes of the species involved, thereby increasing their weighting in any analysis. Therefore, using lineages rather than species will put a greater emphasis upon those species that are phylogenetically more distant as well as narrowly endemic, i.e. palaeoendemics. For example, the corrected inverse weighting highlights the du Toits Kloof and Bains Kloof Mountains (3319CA) for the species map but it is not distinguished on the lineage map. These mountains have four species that are endemic to them, *C. gracilis*, *C. pilifera*, *C. strigosa* and *C. subdura*, but all are added to more widespread lineages for the lineage map. On the other hand the four species mentioned above, *C. burgersii*, *C. nivenioides*, *C. dichotoma* MS, *C. bolusii* MS, are not part of more inclusive lineages at the level chosen for the lineage map and so their respective squares remain highlighted. Therefore, the latter four species could be regarded as palaeoendemics and the emphasis placed upon their grid squares being an important reflection of their status for conservation.

Using either species or lineages identifies seven areas of endemism in common (Table 5.2, Fig. 5.14 & 5.16). Although the analysis using species identifies slightly larger areas, this can be accounted for partly because of the greater number of grid squares retained in the analysis. These common areas of endemism correlate closely with the phytochoria that were identified. Exceptions include the recognition of the Cape Peninsula endemic area as distinct from the South-western Mountains, the presence of the Swartberg endemic area, which was doubtfully not recognised as a distinct phytochorion (see above), and the South-eastern Mountains only being resolved as an area of endemism in the lineage analysis. In addition, each analysis identified the Anysberg (3320BC) as an area of endemism but associated it with different areas. *C. conifera* and *C. arborea* identify an area of endemism between the Anysberg and the Hantam and Roggeveld Mountains when using lineages, while *C. conifera*, *C. mirabilis* and *C. semiteres* identify an area of endemism between the Anysberg and the Hex River valley when using species. As both *C. mirabilis* and *C. semiteres* are poorly understood species, and are possibly hybrids with multiple origins, the emphasis placed upon them in identifying common biogeographic areas is doubtful. However, by using lineages an area of endemism is created that does not have any endemic species with overlapping ranges and contains just a single endemic lineage, *C. arborea–C. conifera*. The only species that occurs on both the Anysberg and Roggeveld Mountains is the widespread *C. ramosissima* (Fig. 5.18), which was excluded from the analysis. Unfortunately, the analysis appears to have been confounded by the presence of *C. hantamensis* on the

Witteberg, which occurs in the same grid square as the Anysberg. Therefore, the uncertainty in these results means that neither of these endemic areas involving the Anysberg should be recognised until more information upon the status of the species involved and their distributions are gained.

Geographic species groups and intervals:

The species groups detected again correspond closely to the phytochoria and endemic areas, showing the cohesiveness of these units. It is also to be expected that the species endemic to areas of endemism resolve as species groups because the areas of endemism have been optimised to find species with highly congruent distributions. Therefore, it is of more interest to identify species groups that do not correspond to one of the areas of endemism already located. Five such groups can be recognised: Hantam and Roggeveld Mountains, Western Klein Karoo Mountains, Southern, Cape Ubiquists and Extra-Cape. The first two represent possible alternative areas of endemism. However, *C. hantamensis* is not endemic to the Hantam and Roggeveld Mountains. While the area defined by the species of the Western Klein Karoo Mountains overlaps with other areas of endemism and hence this is excluded by the criteria for defining areas of endemism (see introduction). The other three groups represent the widespread species, most of which were excluded from the analysis of areas of endemism.

The Cape Ubiquists are found throughout the CFR, with only the occasional outlier beyond its bounds, e.g. *C. eriocephalina* in the Graaff reinet Mountains, *C. ruscifolia* in the Kamiesberg. However, several of the species are found almost exclusively in the western CFR, e.g. *C. atrata*, *C. polygonifolia* and *C. sericea*. This is in contrast to the Southern group, which are generally widespread species found between the Cape Peninsula and Port Elizabeth, but only, *C. odorata* extends for any distance up the West Coast as far as Tulbagh. The other group of widespread species are whose centres of distribution are outside of the CFR. They are clearly associated with the Drakensberg group but are not endemic to any particular region. *C. serpyllifolia* extends as far west as Swellendam, while *C. nitidula* is found in the Afromontane heathlands as far as Mount Kenya.

Within *Cliffortia* there are a few species whose distribution patterns are unique, the most remarkable of which is probably *C. ramosissima* (Fig. 5.18). It groups with the South-eastern Mountains but may better be placed within the Extra-Cape group, as it has the greater part of its distribution outside the CFR. The two collections of this species, which were analysed for molecular data, came from the low clay hills south of

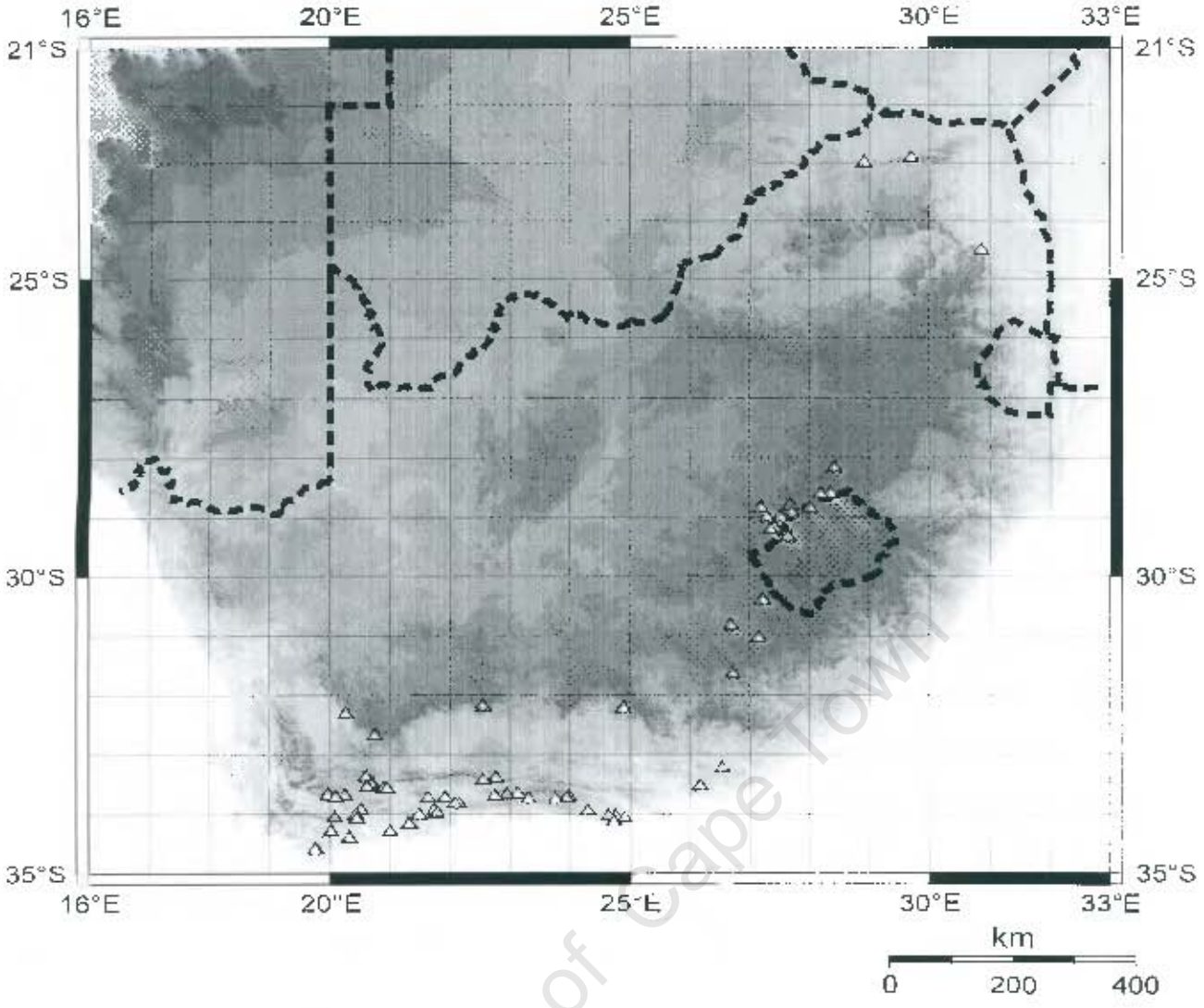

Fig. 5.18. Distribution of *C. ramosissima* within South Africa.

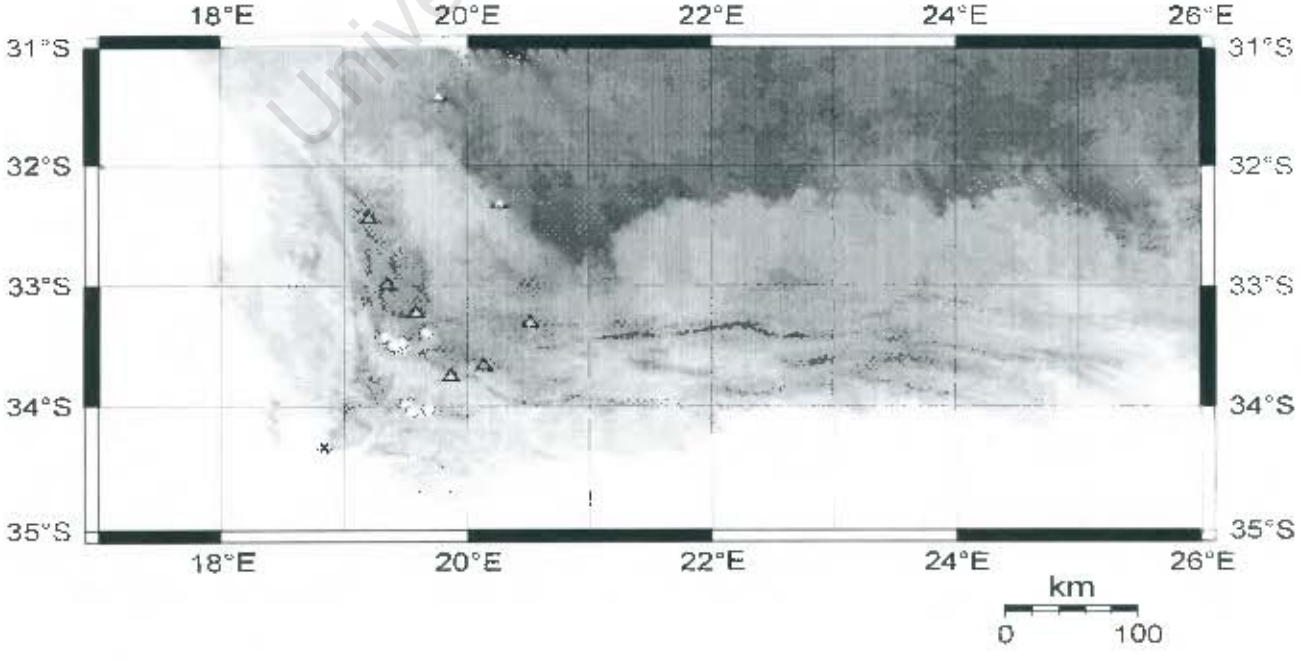

Fig. 5.19. Distribution of *C. hantamensis* (triangles) and *C. pungens* (circles) within the CFR. Circle with a cross indicates hybrid between *C. pungens* and *C. ruscifolia* (=*C. cymbifolia*).

Swellendam and the Komsberg on the Great Escarpment and give no evidence that more than one entity is involved. Certainly morphologically, there is nothing to distinguish the plants around Elim from those in the Soutpansberg. However, while the other Extra-Cape taxa follow the hills and mountains along the eastern flanks of the Drakensberg escarpment, *C. ramosissima* is found on the high inland mountains of the eastern Free State. This pattern is unique within *Cliffortia* and it will be interesting to examine other genera for species with a similar distribution. To some extent it fits Hilliard & Burtt's (1987) phytogeographical group 2b (11 species, e.g. *Lithospermum papillosum*), but is only found in the eastern Free State and western Lesotho. *Disa porrecta* also has a similar distribution but does not extend far into the Western Cape (Linder & Kurzweil, 1999). Within the CFR itself, it is the only species with a clear Knysna interval (Weimarck, 1941), present on both the Agulhas Plain and around Humansdorp, but only on the lower slopes of the Klein Karoo Mountains in between.

Another species, which shows an unusual distribution, is *C. pungens* (Fig. 5.19). The Breede River is regarded as a phytogeographical barrier separating the South-western Mountains from the Northern Mountains and Langeberg (Oliver *et al.*, 1983). *C. pungens*, however, grows both on the Hex River Mountains and the western Riviersonderend Mountains but not in the intervening mountains to the west. This could be attributed to a single chance dispersal event between the two discontinuous mountain blocks, but the distribution is made all the more remarkable by the presence of a hybrid with *C. ruscifolia* (see *C. cymbifolia*, Table 2.10) on the low sandy flats around Pringle Bay, some 70 km away across a large mountain block. Presumably therefore, *C. pungens* was at one time much more widespread and has retreated to the higher reaches of the mountains either side of the Breede River valley, where it is still common. However, its absence from other apparently suitable sites between Pringle Bay and Ceres is still mysterious.

C. hantamensis also has a very disjunctive distribution suggesting that it too was more widespread and common (Fig. 5.19). Its distribution relates well with Weimarck's Doorn River interval. He suggested two possible migration routes from the CFR to the Hantam and Roggeveld Mountains, either northwards across the Doorn River valley or a more easterly route across the Koedoesberg (Weimarck, 1941). The distribution of *C. hantamensis* does not discount either of these possibilities and it would make an interesting phylogeographic study to examine these isolated populations.

Other species with distinct intervals in their distribution include the closely related *C. brevifolia* and *C. ericifolia*, both growing on the recent sands of the Cape Flats and Agulhas Plain (the latter species being only recently confirmed from there also). However, unlike the other species thus far mentioned, this interval has been found in several other species, e.g. *Ischyrolepis sabulosa* (Linder, 2001c), *Leucospermum hypophyllocarpodendron* subsp. *hypophyllocarpodendron* (Rourke, 1972; Rebelo, 1995) and *Muraltia mitior* (Levyns, 1954), and a cause has been proposed. It is attributed to the fact during the Pleistocene when the sea retreated it would have exposed the coastal shelf along what is now the rocky coastline between Somerset West and Hermanus. This would have provided a suitable habitat for migration of those species between the two now disjunct areas (Rourke, 1972; Taylor, 1978).

Two different species have very similar intervals, for which a shared biogeographic history might be investigated: *C. ferruginea* and *C. odorata*. *C. ferruginea* grows on the Cape Peninsula and along the coast as far as Cape Agulhas, there is then an interval until Knysna, when it is again common until Port Elizabeth. *C. odorata* also grows on the Cape Peninsula, though extends its distribution northwards along the West Coast as far as Tulbagh, and has an interval as far as George, when it too is common until Port Elizabeth before having an outlying population around Port St John's in the Pondoland Centre. However, their similar distributions are made more remarkable by each having an intervening population inland on the Langeberg: *C. ferruginea* between Tradouw and Garcias Passes and *C. odorata* between Montagu and Robertson. Population level studies on both these two species would help to clarify the origins of these congruent disjunct populations.

Relationships of *Cliffortia* species outside the CFR:

Of the species that occur in the Drakensberg only the species of section Dracomontanae appear to have diversified there. *C. filicauloides* is endemic to the area but its nearest close relative is *C. filicaulis* that grows as far east as the Knysna district. A similar pattern is found in the near endemic *C. repens*. Its closest relative is *C. burchellii*, which grows along the south slopes of the southern mountains from Genadendal to Uitenhage. Interestingly, the chloroplast regions sequenced are almost identical suggesting that their separation is only comparatively recent. The other species occurring in the Drakensberg are widespread and also occur in the CFR.

It appears therefore that several lineages have succeeded in spreading from the CFR into the mountains and grasslands of the rest of South Africa. Indeed, none of the subgenera

are endemic to the CFR and of subgenus *Cliffortia*, six different sections, as well as *C. linearifolia*, have representatives that occur or extend their range well to the north and east of Port Elizabeth. However, all the species that have their centres of distribution in Kwazulu-Natal and northwards are placed in derived positions within their clades, thus indicating that the clades themselves originated within the CFR.

6. Hybridization

The most remarkable finding of this study has been the revelation of the reticulate origins of the majority of species within *Cliffortia* (Table 6.1). Although putative hybrids had been suggested for occasional collections previously (Weimarck, 1934; Weimarck, 1946), the possibility that any of the species arose as the result of previous hybridization events had not been proposed. Even more so, the degree of reticulation shows that it is not just an exceptional occurrence but appears to be a fundamental cause for the diversity now found within *Cliffortia*. The implications of this finding are great, since hybridization has previously been neglected in most of the literature on the Cape Flora. Here, three particular aspects of the effect of hybridization on the systematics of *Cliffortia* and the Cape Flora in general will be highlighted.

Table 6.1. Summary of the degree of incongruence observed between taxa. Figures based upon Table 2.9 and Fig. 2.13.

Hybrids described by Weimarck:	8 (7.1%)*
Taxa with incongruence:	90 (61.6%)**
Taxa with strong incongruence:	55 (37.7%)**
Taxa showing incongruence across different sections:	28 (19.2%)**
Clades with incongruent positions:	9

* Percentage of total number of species accepted by Weimarck.
** Percentage of total number of taxa analysed for incongruence.

Phylogeny reconstruction and classification

Hybrids cause three main problems for the reconstruction of a species-level phylogeny: (1) how to identify the hybrids, either before or after the analysis, (2) how to reconstruct a phylogeny that contains reticulations, (3) how to extract a useable classification from a reticulating phylogeny. While hybrids have been detected in many other studies, they are usually regarded as exceptional cases in otherwise neatly resolved phylogenies. This study highlights the difficulty in circumventing these problems when the number of reticulations appears to be very high.

Identifying hybrids:

In the process of creating any species-level phylogeny, consideration of species delimitation is needed as they represent the terminal taxa in the tree. However, if reproductive isolation is not complete between the species then hybrids can form. As *a priori* detection of hybrids is often difficult (Rieseberg & Ellstrand, 1993), wherever hybrids form there is the danger of selecting a specimen that is of hybrid origin rather

than representative of the species as a whole. Furthermore, introgression and chloroplast capture can result in certain populations appearing 'typical', yet containing genetic elements, in particular the chloroplast, from an alternative species (Rieseberg & Wendel, 1993). The only way to detect this is for widespread sampling of each species across its entire range. While sequencing of multiple accessions of each species for both the nuclear and chloroplast genome would be desirable, in the consideration of a large Cape genus like *Cliffortia* this is not yet feasible in terms of time, money and computational limitations (Rieseberg & Soltis, 1991). However, it is worth noting that the numerous incongruencies within the datasets presented here are unlikely to be purely a result of unfortunate sampling, as only in one case (*C. nitidula* subsp. *pilosa*) when multiple sampling has been employed have the two samples been placed in widely different positions on either tree. On the other hand, considering the frequency of hybridization, greater sampling of the species is likely to reveal more cases of apparent introgression, such as has been found in *Alnus* (King & Ferris, 2000), *Armeria* (Fuertes Aguilar *et al.*, 1999), *Phlox* (Ferguson & Jansen, 2002) and *Senecio* (Comes & Abbott, 2001).

The frequency of incongruence between nuclear and chloroplast genomes in *Cliffortia* highlights the danger of reconstructing phylogenies using only the plastid genome (Rieseberg & Soltis, 1991). Although the nuclear genome has recently been much maligned for the fear of paralogous copies (Mayol & Rossello, 2001), this study emphasises that ignoring it completely casts doubt upon the integrity of any phylogenetic hypothesis produced, even if multiple sampling of taxa is employed (Rieseberg & Soltis, 1991). While not every taxon will be as subject to hybridization as *Cliffortia*, with its unusually extensive reticulations, many studies are showing the presence of one or two putative hybrids amongst otherwise highly congruent datasets (Bakker *et al.*, 1998; Mort *et al.*, 2002; Yoo *et al.*, 2002). Without evidence from the nuclear genome, conflicts between the chloroplast phylogeny and morphology are too easily attributed to causes other than hybridization, e.g. incomplete taxon sampling (Bakker *et al.*, 1999a; Bellstedt *et al.*, 2001), incorrect morphological phylogeny reconstruction (Bellstedt *et al.*, 2001), lineage sorting coupled with selection (Caujapé-Castells *et al.*, 1999) and convergence of morphological characters (Ohsako & Ohnishi, 2000).

In addition to this, both *Cliffortia* and other taxa would benefit from multiple regions of the nuclear genome being sampled, as has been done with cotton (Cronn *et al.*, 2002).

Concerted evolution of any part of the nuclear genome is usually unidirectional (Hillis *et al.*, 1991; Wendel *et al.*, 1995; Fuertes Aguilar *et al.*, 1998). Hence, on average only half of the hybrids will be evident as incongruencies between the uniparentally inherited plastid genome and the biparental nuclear regions; the remainder would resolve in the same place on both trees. Therefore, greater sampling of the nuclear genome will also increase the number of putative hybrids that can be detected.

Reconstructing reticulations:

Although a hybrid between two sister species will not affect a phylogenetic analysis adversely (McDade, 1995), hybrids between distantly related taxa can result in strong conflict and collapse of the tree if the datasets are combined (Bremer & Wanntorp, 1979). It is therefore important that datasets showing strong incongruence are not combined prior to analysis (de Queiroz, 1993; Huelsenbeck *et al.*, 1996). Unfortunately, detection of incongruence between datasets is not straightforward as no reliable test has yet been proposed (Yoder *et al.*, 2001; Dowton & Austin, 2002). However, in the case of *Cliffortia* the incongruence between the nuclear and chloroplast trees was so strong that the incompatibility of the two datasets was unequivocal.

Two methods were used here to combine the data from both gene trees. Firstly, reconstruction of a reticulate tree by comparison of the two individual gene trees. And secondly, selective combination of all the data to produce a compromise between a consensus and a total evidence tree. The first method was computationally difficult because of the number of incongruent placements between the two trees. Therefore, optimal placement of the taxa and reticulations cannot be assured. Furthermore, an indicator of the strength of each reticulation was needed to assess whether each incongruence observed was an accurate portrayal of differing phylogenetic histories or due to erroneous reconstruction. The second method relies firstly upon identification of nodes in common (i.e. consensus) and then add nodes that do not strongly conflict when using total evidence. Any evidence of reticulations is lost by this method, but taxa that do not have reticulations should be more confidently placed because of the greater amount of data included in the analysis.

While reticulate phylogenies have been reconstructed for a few taxa, e.g. peonies (Sang *et al.*, 1995; Sang *et al.*, 1997), the number of taxa involved has been relatively few, so that the reconstruction was unambiguous. Total evidence has also been used for phylogenies with reticulations but the data for conflicting taxa have been removed prior to combination of datasets, e.g. Macaronesian Crassulaceae (Mort *et al.*, 2002). The

complexity of the reticulations present within *Cliffortia* has not been documented for any other species-level phylogeny. However, similar situations will arise in population level studies and one possible solution to the difficulties posed by reticulate phylogenies is to use the various distance-based methods employed by these studies (Posada & Crandall, 2001).

Classifying hybrid species and reticulate phylogenies:

The way we classify both species and higher taxa continues to require further thought. Although attempts have been made to draw attention to the problems posed by hybrids in the past, they have been repeatedly dismissed as artefacts of over-zealous splitting of species (van Welzen, 1998) or inconsequential as they only occur between closely related taxa (McDade, 1995). However, this study has revealed numerous reticulations between morphologically and functionally diverse lineages indicating that the problems caused by hybridization for classification are possible, although certainly not universal. Hence, a classification system is still needed that can accommodate these natural patterns and still be as widely applicable by as many end-users as possible. Here a two-level Linnaean classification has been constructed, so that those that insist upon only monophyletic taxa can recognise only the subgeneric rank as legitimate, while those who need a classification that can be used to communicate concepts regarding the functional diversity within *Cliffortia* can recognise the sections and series as well.

Hybridization and the origins of the Cape Flora

Processes influencing the speciation and radiation of *Cliffortia* have been shown to be varied (Chapter 4). No single morphological or ecological feature of *Cliffortia* was demonstrated to explain the immense diversity in growth form and adaptation to a wide range of environments. Therefore speciation within the genus appears to have been driven by a variety of environmental factors such as substrate, fire tolerance, water dependence and altitude. Speciation events have been both allopatric and sympatric across strong ecological gradients. Furthermore, the distribution of species within the CFR is similar to other Cape taxa, e.g. Proteaceae, Restionaceae, *Aspalathus*, and *Muraltia* (Oliver *et al.*, 1983), implying that the processes that have influenced diversification affect a wide range of taxa. However, common to all the lineages within *Cliffortia* is the evidence of hybridization and hence this needs to be investigated further to understand the evolution of the genus. In particular, it needs to be determined whether the prevalence of hybridization across the genus is a phenomenon restricted to

Cliffortia, because of certain attributes of the genus, or a more general feature of the Cape Flora that will be applicable to all speciose genera.

Is hybridization unique to *Cliffortia*?

Ellstrand *et al.* (1996) showed that the distribution of natural hybrids was not random amongst taxa, and concentrated in groups with particular characteristics. In three of the five floras examined by Ellstrand et al., Rosaceae featured as one of the five families with the most hybrids recorded. In addition, the presence of hybrids in several genera of Rosaceae have been confirmed by molecular techniques, e.g. *Amelanchier* (Campbell *et al.*, 1997), *Geum* (Smedmark & Eriksson, 2002) and *Rubus* (Alice *et al.*, 2001). Within the British Flora, Ellstrand et al. identified that genera susceptible to hybridization were characteristically perennials, outcrossing and capable of clonal reproduction, either vegetatively or by agamospermy. Species of *Cliffortia* are all perennial and many are capable of vegetative spread. The unisexual flowers will increase the probability of outcrossing, while the rarity of male flowers in several species means that agamospermy is suspected to be common.

It therefore appears, with the benefit of hindsight, that *Cliffortia*, an outcrossing member of the Rosaceae capable of clonal spread through vegetative growth and agamospermy, should be susceptible to hybridization. In particular, hybrids within the other genera of the Sanguisorbeae have already been reported, e.g. the *Bencomia* alliance (Francisco-Ortega *et al.*, 2000) and even an intergeneric hybrid between *Acaena* and *Margyricarpus* (Crawford *et al.*, 1993). As a result, the role of hybridization as a speciation and diversification force within the Cape Flora, could be seen as exceptional to *Cliffortia* and extrapolation to other Cape taxa is dubious. However, this presumption may be rather premature.

The occurrence of natural hybrids in other Cape taxa is rarely documented in monographs, e.g. *Erica* (Oliver, 1991), *Freesia* (Goldblatt, 1982), *Geissorhiza* (Goldblatt, 1985), *Leucadendron* (Williams, 1972), *Microloma* (Bruyns & Linder, 1991) and *Romulea* (de Vos, 1972). Usually, comments are restricted to intermediate taxa that cannot with certainty be placed into one of the two putative parental taxa. However, prior to this study a similar situation existed for *Cliffortia* with only eight putative hybrids being proposed by Weimarck (Table 6.1). None of these eight were tested in the current study, hence all the hybrids found were previously unknown or recognised as species. Therefore, the previous documentation, or absence thereof, should not be used as an indicator of whether hybridization is prevalent within a genus

or not. For example, a recent molecular study of eastern North American *Phlox* also revealed evidence for a high degree of reticulation in the evolution of the species, yet "it is very rare to come across an herbarium specimen that appears to be a hybrid plant" (Ferguson & Jansen, 2002). Furthermore, artificial crosses of sunflowers have shown hybrids within a few generations to express genotypes closer to one parent than would be expected, thus their intermediate nature is rapidly lost and makes their detection especially difficult (Rieseberg & Linder, 1999).

A better indicator of whether a genus is susceptible to hybridization would be to compare the attributes listed by Ellstrand *et al.* (1996) with those known for Cape taxa. An examination of the families subject to frequent hybridization in other floras suggest that several important Cape taxa might also be worthy of investigation for evidence of hybrids (Table 6.2). In addition, particular attention should be paid to those Cape taxa that express the three criteria that allow a genus to engage in frequent spontaneous hybridization, namely perennial, outcrossing species with the ability for clonal reproduction. Bearing this in mind, the geophytic genera, especially of the Hyacinthaceae and Iridaceae, which produce numerous offsets, would be good candidates to investigate for evidence of speciation via hybridization. This is supported by the readiness of these taxa to form hybrids in cultivation (Goldblatt, 1971), e.g. *Watsonia* (Horn, 1962), *Lachenalia* (Duncan, 1988) and between different sections in *Gladiolus* (Takatsu *et al.*, 2001). Already, natural hybrids have been suggested for incongruence observed in *Pelargonium* from molecular analysis (Bakker *et al.*, 1998), supported by the ease with which artificial hybrids, some fertile, have been created within and between sections (Bakker *et al.*, 1999b). It remains to be seen whether the increasing number of molecular studies of Cape taxa reveal more hybrid relationships.

Table 6.2. List of families mentioned by Ellstrand et al. (1996) for other floras, as containing high numbers of hybrids, that are also present in the Cape Flora and the largest genera contained therein (figures from Goldblatt & Manning, 2000).

Family	Largest genus in Cape Flora and number of species
Asteraceae	*Senecio* (110)
Boraginaceae	*Lobostemon* (28)
Campanulaceae	*Wahlenbergia* (60)
Cyperaceae	*Ficinia* (57)
Euphorbiaceae	*Euphorbia* (45)
Fabaceae	*Aspalathus* (272)
Lamiaceae	*Salvia* (18)
Orchidaceae	*Disa* (92)
Poaceae	*Pentaschistis* (43)
Rosaceae	*Cliffortia* (114)
Rubiaceae	*Anthospermum* (13)
Scrophulariaceae	*Selago* (101)

Hybridization as a process for diversification:

If hybridization is shown to be a factor affecting diversification within more than one Cape taxon then there is a need to address the question of whether there is a reason for this. Is this particular to the Cape Flora, mediterranean climate floras or to floras that have experienced a recent radiation? Natural hybridization has frequently been dismissed, especially by zoologists, as being of little long-term evolutionary importance (Arnold, 1997). The dismissal of hybrids as an important cause of speciation centres around two main arguments. Firstly, it is thought that hybrids are always less fit than their parents (Mayr, 1963; Schemske, 2000). Secondly, they are only thought to occur in disturbed habitats, which can usually be attributed to the result of human activities (Anderson, 1948; Schemske, 2000). Both arguments are based upon a predisposition to the Biological Species Concept, in which the reproductive isolation of species is inherent and therefore the acceptance of hybridization as evolutionarily important would be inadmissible (Arnold *et al.*, 2001).

However, it has been shown that the fitness of hybrids is not always inferior to their parents, but covers a wide variation from sterility and inviability to distinct hybrid vigour (Arnold & Hodges, 1995; Burke *et al.*, 1998; Arnold *et al.*, 2001; Schweitzer *et al.*, 2002). In addition, many hybrids demonstrate transgressive characters or novelties, allowing them to colonize intermediate or new environments so that both they and their parents can persist (Rieseberg & Ellstrand, 1993; Rieseberg *et al.*, 1999). Even if the original cross is less fit than the parents, subsequent backcrossing and introgression can allow the flow of traits between species, which could promote speciation within the

parents (Rieseberg & Wendel, 1993; Arnold *et al.*, 1999). Therefore, hybridization is increasingly being seen as a potentially important process in the evolution of many organisms (Arnold, 1997; Bachmann, 2000).

The second argument against hybridization as a speciation process has received less attention but is highly relevant to the present study. While hybrids have been shown to colonize intermediate (Kentner & Mesler, 2000) and new (Welch & Rieseberg, 2002) habitats, the number of cases in habitats unaffected by man are still few. The converse is also true in that numerous reports are coming to light of hybrid swarms forming in man-disturbed habitats, especially between native and introduced species (Abbott, 1992; Ellstrand & Schierenbeck, 2000). In his seminal paper on the subject, Anderson (1948) suggests that the degree of habitat heterogeneity required for hybrid swarms to establish only occurs where man has greatly altered natural conditions. Hence, clear-cut hybrids are seldom encountered or are found only under peculiar circumstances. However, he goes on to make the proviso that 'hybridized habitats' would have existed in pre-human times when new areas were opened up to colonization. This statement is supported by recent findings that hybridization has been a major factor in the adaptive radiation of genera on volcanic islands, e.g. *Argyranthemum* (Francisco-Ortega *et al.*, 1996), *Sonchus* (Kim *et al.*, 1996) and *Teline* (Percy & Cronk, 2002) in Macaronesia and the silversword alliance in Hawaii (Baldwin, 1997).

A similar scenario would also have occurred in the Cape at the onset of the mediterranean-climate. With the increasing summer aridity, the forests retreated opening up large tracts of land, leaving the more sclerophyllous taxa, which extended their distributions into the newly vacated areas (Linder *et al.*, 1992). The role of hybridization in the adaptation and radiation of these surviving taxa into the CFR would presumably have been similar to that found in the island taxa. However, if hybridization was only significant in the early radiation of the flora into new lands, then new hybrids would not expect to be found after the immediate colonization. In *Cliffortia* this is not the case as many recent hybrids are still to be found. Therefore, the reticulate nature of the phylogeny should not be solely attributed to the initial radiation within the genus, but instead the current hybrid populations can be used to infer the processes that gave rise to the pattern of reticulation now observed (Arnold, 1992).

While disturbance in many habitats can be regarded as primarily man induced, for fynbos, and most other mediterranean-climate vegetation, fire creates a frequent and regular disturbance regime (Cowling, 1987; van Wilgen, 1987; Manders & Cunliffe,

1987). Fire has previously been regarded as a cause of vicariance by local extinction of intervening populations of species (Cowling, 1987) and of increasing generation turn-over in non-sprouting lineages (Ojeda, 1998; Wisheu *et al.*, 2000; Cowling & Lombard, 2002). However, here it is suggested that fire can also have a major role in providing new disturbed habitats for the establishment of hybrids and that these hybrids can be an important factor in subsequent speciation, either through allopolyploidization, homoploid hybrid speciation or introgression of foreign traits.

This theory would predict that the rate of speciation would increase in lineages that were susceptible to hybridization, such as those with the ability for clonal reproduction and hence persistence over successive disturbance events. Indeed, three of the sections in *Cliffortia*, Bifoliolae, Multifidae and Multinerviae, support this hypothesis (Fig. 4.6). This is in direct contradiction to the highly plausible and frequently invoked theory that seeder lineages are more vulnerable to local extinctions and therefore speciation (Cowling, 1987; Cowling & Holmes, 1992a; Schutte *et al.*, 1995; McDonald *et al.*, 1995; Trinder-Smith *et al.*, 1996; Ojeda, 1998). Hence, this prediction should certainly not be taken as a hard and fast rule. Indeed, sect. Filicaulae and Simplices are both exclusively seeder lineages that have diversified. However, the theory does provide a process by which increased speciation levels in resprouting lineages can be explained.

In the past, the high diversity within the CFR has been attributed to increased speciation across steep ecological gradients caused by limited gene flow between populations (Linder, 1985), frequent vicariance events caused by fire (Cowling, 1987), increased generation turnover amongst fire-prone lineages (Ojeda, 1998; Wisheu *et al.*, 2000; Cowling & Lombard, 2002) and reduced extinction due to a stable climate (Dynesius & Jansson, 2001). The intention here is not to supplant these general hypotheses and suggest that the immense diversity found within the fynbos, and other fire-prone mediterranean floras, is solely due to hybridization caused by regular disturbance. However, hybridization should be seen as a factor that can augment other speciation processes, especially in resprouting lineages. Hence, the presence and distribution of hybrids across a wide range of taxa within these climatic zones should be sought all the more readily and not dismissed as obstacles in the way of producing neat phylogenies and classifications.

Conservation

Many of the species that grow in fynbos are under increasing threat of extinction. The primary threats include destruction of habitat for farming, building and water-supply (Macdonald, 1989), invasion of natural habitat by alien plants such as *Hakea*, *Acacia* and *Pinus* (Richardson *et al.*, 1992), and inappropriate fire regimes caused by purposeful and accidental ignition of fynbos (van Wilgen *et al.*, 1992). Habitat destruction is concentrated in the lowland areas (Rebelo, 1992), including the species rich Cape Flats (Jarman, 1986) and Agulhas Plain (Lombard *et al.*, 1997; Heydenrych *et al.*, 1999), but the other two threats affect the mountainous regions too. While much of the mountainous area is already conserved as water catchment zones (Rebelo, 1992), in the lowlands a series of small fragmented reserves is divided by vast areas of agricultural or urban land (Rebelo & Siegfried, 1990; Lombard *et al.*, 1997).

The high degree of endemism within the Cape Flora means that many of the species are naturally rare, sometimes confined to a single valley or mountain peak. These species are particularly vulnerable to extinction (Kruckeberg & Rabinowitz, 1985; Tansley & Brown, 2000; Goldblatt & Manning, 2002), especially due to inappropriate or absent conservation management procedures, e.g. *Sorocephalus tenuifolius* (Tansley, 1988). To this end much work has been done within the CFR to construct a potential network of reserves to protect the maximum number of rare and threatened species (Rebelo & Siegfried, 1990; Rebelo & Siegfried, 1992; Rebelo & Tansley, 1993; Lombard *et al.*, 1997; Cowling & Pressey, 2001). Modelling for much of this work has utilised either the Proteaceae, because it is the most comprehensively documented of the Cape taxa, or the Red Data Book of threatened plants (Hall & Veldhuis, 1985; Hilton-Taylor, 1996), as they list the species with the highest priority for conservation.

This study has shown that the distribution of species and endemic areas for *Cliffortia* are very similar to what has been found in other taxa. In addition, Rebelo & Tansley (1993) have shown that there is a strong relationship between Proteaceae, Red Data Book and total species richness. As a result, these taxa may be used as surrogates for *Cliffortia* in designing conservation strategies that will preserve all the species of *Cliffortia* currently threatened. Although no test of this has been conducted, at present only a few species are known that do not occur inside any existing reserve: *C. acocksii*, *C. bolusii* MS, *C. cruciata* MS, *C. curvifolia* (possibly now included within the new Cape Agulhas National Park) and *C. marginata*. It would therefore only require that at

least one population of each of these remaining five species be targeted for all species to be included within a protected area.

However, not all threatened species are of equal concern and several methods have been proposed to prioritise for conservation species to maximise cladistic diversity (Vane-Wright *et al.*, 1991; Williams *et al.*, 1991), genetic diversity (Crozier, 1992), feature diversity (Faith, 1992), functional diversity (Linder & Midgley, 1994), or evolutionary potential (Linder, 1995). Thus in the case of the five species mentioned above, although *C. acocksii* and *C. marginata* are probably the most threatened as they occur on the clayish soils of the Cape Flats around Somerset West and Stellenbosch, they are also the least taxonomically distinct species having very close sister species which are not threatened, i.e. *C. filifolia* and *C. tenuis*. Thus it is recommended that species like *C. bolusii* MS, *C. cruciata* MS and *C. curvifolia* should be targeted preferentially when designing reserves to maximise genetic diversity.

This is relevant for hybrid species, especially those narrow endemics whose parents are more widespread. For example, *C. intermedia* may be confined to the Cape Peninsula, but the parental species *C. ruscifolia* and *C. ilicifolia* are found almost throughout the CFR. Therefore, in terms of preserving genetic diversity these recent hybrid species could be regarded as practically expendable. Indeed, such argumentation resulted in specific exclusion of hybrids from the American Endangered Species Act. This caused severe problems with conservationists when endangered taxa were shown to have hybridized or have hybrid origins (O'Brien & Mayr, 1991; Rhymer & Simberloff, 1996). However, while genetic diversity might not be lost if these hybrid species disappear, the evolutionary potential of the species involved might be harmed. This is important because if we are to preserve the processes that maintain genetic diversity and promote diversification then the evolutionary potential of species needs to be explicitly considered in conservation planning (Brooks *et al.*, 1992; Linder, 1995; Cowling & Pressey, 2001).

Although the threats mentioned above are by far the most serious affecting the Cape Flora today, the role of hybridization has been noted as a conservation issue of growing importance (Abbott, 1992; Ellstrand, 1992; Rhymer & Simberloff, 1996; Levin *et al.*, 1996; Ellstrand & Schierenbeck, 2000). While it has not yet become a conservation issue here for plants, it may be just a matter of time as it is already a concern for a few amphibians, birds and mammals (Rebelo, 1992), e.g. African wild cat and domestic cat (Stuart & Stuart, 1991; Wiseman *et al.*, 2000), mallard and yellowbilled duck (Anon,

1994), and has been recognised as a major problem in another mediterranean climate flora, California (Rhymer & Simberloff, 1996). The threat from hybridization has been most widely reported between introduced and native related species, but concern has also been expressed that it can cause loss of biodiversity between two sympatric native species (Ellstrand, 1992; Rhymer & Simberloff, 1996).

For example, *C. strigosa* is a highly localised endemic species, known from only a small area in one mountain range. It is a distinct species (no other member of section Multinerviae is so hairy) but putative hybrids have still been found with *C. dregeana* (C.M. Whitehouse 86). There are two effects of heterospecific hybridization that could affect the long-term survival of this and other rare species that hybridize with a more common species: outbreeding depression and genetic assimilation (Ellstrand, 1992; Rhymer & Simberloff, 1996; Arnold, 1997). Outbreeding depression can result from either a reduction in the number of viable offspring produced or the hybrid progeny demonstrating lower levels of overall fitness (Ellstrand, 1992). This will adversely affect the rare taxon by increasing the number of lost opportunities for recruitment (Levin *et al.*, 1996). Genetic assimilation on the other hand involves the loss of the rare taxon's genotype or phenotype through asymmetric gene flow from the common taxon. Within *Cliffortia* this would be accentuated in those species that regularly fail to produce male flowers, which makes gene flow almost unidirectional.

How real the threat from heterospecific hybridization is between species of *Cliffortia* remains to be seen. It could be that species such as *C. cruciata* MS demonstrate that genetic assimilation has already occurred in that its chloroplast genome is representative of a more widespread taxon. However, this should be regarded as just part of the natural evolution of the genus and has probably occurred frequently even before man started to change the environment. Thus the problem of heterospecific hybridization should only be a conservation concern for species where it can be shown that hybrids have formed as a direct result of human intervention.

One such area of human intervention is in the introduction of species not native to an area. However, the problems for conservation associated here are not the same as between rare and widespread native taxa for, except in large-scale farming, the introduced species will be the rare taxon, at least initially. Rather the danger comes from the possibility that the hybrid will show increased fitness relative to its parental taxa. This leads to two concerns about hybrid taxa: firstly, that the 'new' hybrid species might outcompete the native species and thus replace it, and secondly that the 'new' species

can become invasive and start to change the environment in such a way that other species are also detrimentally affected (Ellstrand & Schierenbeck, 2000).

Within the fynbos the primary effect of introduced plant species is their invasion of natural vegetation (Richardson *et al.*, 1992). Hybridization between these introduced plants and the native flora is not a problem because there are not close enough relatives for gene flow to be successful. This can be attributed in a large part to the high endemicity of the Cape Flora such that very few genera occur both in the CFR and other similar habitats elsewhere. The only example of hybridization between a native and an introduced species involving a Cape taxon is for *Carpobrotus* in North America (Gallagher *et al.*, 1997).

However, horticulture and in particular flower farming practices are changing distribution patterns of species within the CFR itself. Desirable species for the cut-flower and garden industry are being grown more commonly, on larger scales and throughout the CFR. In addition, this often takes place amongst naturally occurring stands of fynbos. While the horticultural potential of *Cliffortia* may have to wait a while until it is fully exploited, the risk of previously allopatric species being brought into close contact is a real one for many fynbos species, especially *Protea*. While sympatric species may have developed isolating mechanisms, there will have been no selective pressure to produce them in allopatric species, e.g. Darwin's finches (Grant & Grant, 2002). Therefore, hybridization between previously allopatric species is more likely than between sympatric species (Grant, 1981; Ellstrand, 1992). Although no cases have yet been found for the formation of invasive hybrids within fynbos, there are reports of hybrid taxa between cultivated and native species, e.g. *Erica nana* x *patersonia* (Hitchcock, 2002). As yet it is not known whether these will pose a threat to the conservation of the natural populations. However, concern is already being raised regarding the problems caused by horticulture in the Canary Islands through hybridisation between rare endemic species and those introduced from neighbouring islands (Levin *et al.*, 1996; Francisco-Ortega *et al.*, 2000).

However, even if this proves to be the case over the coming years as commercial flower farming increases in the area, the question still needs to be asked whether this is detrimental (Arnold, 1997). While the genetic identity of the species native to the area may not be preserved, it is possible that its evolutionary potential may be increased through genetic enrichment (Arnold *et al.*, 1999). In a habitat that is increasingly more fragmented, and with the threat of global climate change, there is great concern about

the ability of species to adapt to the environment (Brooks *et al.*, 1992; Cowling & Pressey, 2001). Corridors and ecological gradients along which the species used to migrate have been lost and the rate of change appears to be too fast for natural evolutionary processes to allow for adaptation (Rebelo & Siegfried, 1990). However, if hybridization has been a major factor in the past evolution of Cape taxa, then the introduction of new genes via hybridization that enable species to adapt to the changing climate *in situ* could be seen as just a continuation of that process. This is clearly a controversial suggestion, but introduction of foreign 'subspecies' so that hybridization will occur has already been deliberately implemented amongst some highly endangered mammal and bird species in America (O'Brien & Mayr, 1991; Fergus, 1991; Rhymer & Simberloff, 1996). The question of whether we should be concerned about possible 'contamination' of wild populations from cultivated plants is worthy of debate, especially in the light of the evolutionary processes that created the amazing diversity of the Cape Flora in the first place.

7. Synthesis

This study has produced a phylogenetic hypothesis for the large Cape genus *Cliffortia* (Rosaceae) using macromolecular and morphological data. The sampling of taxa has been high, with 91.5% of the currently accepted species (n = 117), as well as several new taxa, being sequenced for at least one DNA region. Of the 143 taxa (of which 16 are newly recognised and awaiting formal description and publication), 126 are included in the molecular analyses.

Two molecular phylogenies were produced, one from the nuclear genome and one from the chloroplast. The lack of congruence between the two trees indicates that there has been extensive recent and past hybridization within the genus. In some instances these hypotheses of hybridity are supported by the presence of additive sequences in the nuclear genome, suggesting that there are different copies of the gene region from alternative parents, or by the intermediate nature of the morphology of the accessions. Surprisingly, for most incongruent accessions of species, no hybrid hypothesis had been suggested prior to comparison of the nuclear and chloroplast trees.

While hybridization is generally considered only to be common between closely related species (McDade, 1995), within *Cliffortia* reticulations can also occur across widely separated parts of the tree. Indeed, the lack of molecular characters to resolve closely related species means that most of the strongly supported cases of incongruence are for species that are placed in different sections in the two trees. As a result of this, and because of the conservative criteria used to record incongruence, the levels of hybridization reported are probably lower than they should be. Nevertheless, 90 (61.6%) of the 146 accessions included in the analysis show some evidence of incongruence, 55 (37.7%) of which show strong incongruence (>80% jackknife support for alternate positions in the two genome trees). Furthermore, because of concerted evolution, greater sampling of the nuclear genome will most likely reveal even greater incongruence between the different gene regions. Therefore, the importance of the role of hybridization in the evolution of *Cliffortia* appears irrefutable.

Both the nuclear and chloroplast trees are inconsistent with the subgeneric and sectional classification proposed by Weimarck. Therefore, a new classification is proposed here. However, the presence of reticulations means that a monophyletic model can only recognise four monophyletic clades. In the Linnaean classification, these four clades are attributed the rank of subgenus: Arborea, Cliffortia, Eriocephalina and Graminea.

However, subgenus Cliffortia contains the majority of the variation present within the genus, and to have no means of classifying the species into more convenient supraspecific taxa would be unhelpful in further discussions of the genus. Therefore, the recognition of paraphyletic taxa was regarded as inevitable and 17 sections (15 of which belong to subgenus Cliffortia) were created. In addition, monotypic sections were deemed unwarranted and were therefore avoided for subgenus Cliffortia by the creation of a group of species labelled *incertae sedis*.

All morphological characters were homoplastic to some degree and reconstruction of ancestral conditions for the deeper nodes of the tree was difficult. However, leaf form was relatively conservative and an ancestral trifoliate form is hypothesised. The presence of a petiole, as hypothesised by Dahlgren (1971), in the ancestral leaf was not supported, but ontogenetic arguments based upon seedling leaves might suggest that it was ancestral. Flower and inflorescence structure was also relatively conservative. Serotinous inflorescences were exclusive to subgenus Arborea, while the presence of four sepals was a diagnostic character in four of the sections of subgenus Cliffortia.

The optimisation of habitats onto the phylogeny of *Cliffortia* suggests that the ancestral forms grew on well-drained TMS in seasonally dry areas. More derived forms then diversified onto different substrates, such as lowland shales and marine sands or limestone, and into areas that are wet all year or seasonally waterlogged. However, as no clock can be attached to the phylogeny, as there is no suitable calibration point, attaching these shifts in ecology to particular past climatic events is not possible. Only a single clade, series Hirta, gives any indication of diversification following a shift in substrate preference, but four different clades show diversification following adaptation to increased water supply: subgenus Graminea, section Simplices, and series Ericifolia and Pedunculata.

The role of fire in the diversification of *Cliffortia* is more complex. Three different growth forms in response to fire are present: crown resprouters, clonal resprouters and seeders. While a seeder life history is optimised as the ancestral condition, confidence in this finding is low and could easily change with increased resolution at the base of the tree. Resprouting is generally considered the ancestral condition in angiosperms and the presence of clonal growth in other rosaceous taxa and across the breadth of the phylogeny appears to support this hypothesis for *Cliffortia* too. Transition between the different growth forms appears to have occurred many times, except between the crown and clonal resprouting forms. It is therefore hypothesised that a crown resprouter only

originates from a seeder ancestor. This is supported by the facultative nature of crown resprouters, which are sometimes killed by intense fires or only survive in more protected areas.

The presence of clonal vegetative reproduction through underground roots is associated with increased evidence for past hybridization events. This can be attributed to the greater chance of survival for hybrids that are able to resprout after fires and to spread from their point of origin without the need of sexual reproduction. It is also possible that it may be the result of reversal to a plesiomorphic state (Funk, 1985), as some hybrids have both parental species lacking the ability to spread clonally. The persistence of hybrids in the environment through clonal growth is probably an important factor in the contribution of hybridization to the evolution of *Cliffortia*. Resprouting hybrids are able to overlap generations and therefore backcross with their parents. This increases the chance of transfer of alleles between species through introgression or the possibility of unreduced gametes forming fertile allopolyploids. This is supported by the diversification within predominantly clonally resprouting lineages such as sections Bifoliolae, Multifidae and Multinerviae. This is in conflict with the frequently cited hypothesis that seeders have increased rates of speciation compared to resprouters (Wells, 1969; Cowling & Holmes, 1992a; Schutte *et al.*, 1995; Ojeda, 1998; Cowling & Pressey, 2001).

The patterns of distribution of *Cliffortia* species and areas of endemism within the CFR is very similar to that found for many other Cape taxa, especially those that grow predominantly in fynbos, e.g. *Aspalathus*, *Erica*, *Protea* and Restionaceae (Oliver *et al.*, 1983). Therefore, many of the factors that have influenced diversification of *Cliffortia* are probably also applicable to these taxa. In addition, conservation planning based upon targeting those taxa will also help to conserve the majority of *Cliffortia* species.

In conclusion, the diversification of *Cliffortia* has been affected by many different elements: geographical isolation, specialisation for different fire-strategies, adaptation to different soil types or different rainfall regimes. No single factor has played an over-riding role in speciation events. Moreover, the rate of speciation has been strongly influenced by frequent hybridization events, which will have allowed transfer of genetic information between species. Much work remains to be done upon confirming the extent of hybridization and its influence upon speciation within *Cliffortia*, especially in the ability of hybrids to adapt to new habitats. Furthermore, the presence of hybrids in

other Cape taxa should also be sought to determine if the importance of this phenomenon is unique to *Cliffortia* or a more general feature of the Cape flora.

Future research on *Cliffortia*

The analyses carried out here provide a useful framework for our understanding of the evolution of *Cliffortia*, but much future research is needed to confirm many of the hypotheses that have been proposed.

- Greater support is needed for the phylogeny to confirm the placement of the branches and reticulations. While additional chloroplast regions can be sequenced, especially to resolve the nodes deeper within the tree, the three regions currently sequenced provide a relatively robust tree. Hence, there is a greater priority for more data from the nuclear genome to complement the solitary dataset already obtained. As well as providing support for the current nuclear tree, further nuclear sequences will presumably reveal more hybrids and reticulations. Faster evolving gene regions should also be sought to help clarify relationships between closely related species.
- Population-level molecular studies are needed to confirm putative hybrids and their parental taxa or populations. Several hybrid swarms have been detected, e.g. between *C. denticulata*, *C. dregeana* and *C. ovalis* above Jonkershoek, and between *C. ilicifolia*, *C. integerrima*, *C. intermedia*, *C. ruscifolia* and *C. theodori-friesii* on the Cape Peninsula, and the degree of interbreeding and population structure of these complex taxa need to be discerned.
- Cytological studies of the species would be desirable to reveal ploidy levels. This would allow identification of instances of autopolyploidy within species and in particular whether hybridization has occurred through allopolyploidization or homoploid hybridization.
- Experimental evidence is needed to confirm the presence of apomixis, examine the extent of its distribution amongst the species and confirm whether pollen is needed to initiate seed production. It is especially important to ascertain this for recent hybrids as their ability to spread and influence future generations will be heavily dependent upon their ability to persist through successive burning events.

- The extent of gene flow within a species is vital to our understanding of species concepts within *Cliffortia* and the distance across which hybrids can form. Information on flowering times of species and the occurrence and frequency of male and female flowers is needed to comprehend pollination biology of the species. Identification of environmental factors that trigger male flowering in species where it is rare will help indicate the causes that promote sexual over asexual reproduction and possibly also trends from monoecy to dioecy.

- Study of seedlings, and in particular the ontogeny and morphology of seedling leaves will increase our understanding of the evolution of the various leaf-types.

- Further phenological studies, such as carried out by Fellingham (1999), across a broader range of species will also help to increase our understanding of the range of growth forms that exist within *Cliffortia*.

- Better characterisation of the ecological requirements of sympatric sister species may reveal the ecological differentiation between sympatric species. This could reveal factors that may have influenced speciation. This is especially relevant for new hybrids, as the ability of the hybrid to persist will depend upon whether it can occupy a different ecological niche to both its parents.

- Morphometric studies of species complexes are needed to identify morphological characters important for identification of difficult species. Widespread species can be examined for clinal variation in morphology and associated with climatic trends to help examine the factors that can influence speciation.

- More comprehensive coverage of the distributional ranges of *Cliffortia* species are needed to fill in the gaps in distribution. This will allow greater resolution in the detection of biogeographic patterns without the errors being introduced from undercollecting.

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Appendix 1. Outline of the proposed classification for *Cliffortia*.

Characters in bold type are useful diagnostic and easily observable characters for the taxa either within the section or the genus as a whole. For each supraspecific taxon, the subordinate taxon that contains the type for that name is indicated by underlining. These type indications are provisional and will only be officially designated in the published revision. Exceptions to this are the type species of the genus, which had already been chosen, and the originally monotypic subgenera Arborea, Hermaphroditicae and Monographidium.

Cliffortia

L., Sp. Pl.: 1038 (1753) & Gen. Pl., ed. 5: 460 (1754); Weimarck, Monogr. Gen. Cliffortia: 1–229 (1934) & in Bot. Not. 1948: 167–203 (1948). Type species, chosen by M.L. Green, Prop. Brit. Bot.: 192 (1929): *C. polygonifolia* L.

Morilandia Neck., Elem. Bot. 2: 98 (1790). Type species unknown
Monographidium Presl, Epim. Bot.: 202 (1849). Type species: *C. obcordata* L.f.

Low semi-herbaceous trailing shrubs to small trees, able to resprout after fire, in which case sometimes clonally spreading by roots underground, or only surviving fire as seed. Young stems glabrous to densely hairy, forming brachyblasts or not. Sheaths hairy or glabrous both adaxially and abaxially, usually with a fringe of hairs on the adaxial side at the point of insertion of the leaflets; stipules when present clear and membranous, brown and scarious, or green and leaf-like; petiole present or absent. Leaves glabrous to hairy; uni-, bi- or trifoliate, if trifoliate then leaflets always single-nerved to the base, if bifoliate always multi-nerved, and if unifoliate then either single- or multi-nerved; leaflet shape very variable, from needle-shaped to subcircular, apex rounded to sharply acuminate or pungent, margins entire to markedly toothed, flat or inrolled beneath, midrib prominent or not, whole leaf curved upwards and towards the stem or downwards and away from the stem. Monoecious but sometimes only one sex present, inflorescence usually with flowers solitary in the axil of ordinary vegetative leaves, sometimes female, or rarely male, flowers clustered into a distinct inflorescence, which is sometimes serotinous. Bracteoles 2–3, glabrous or hairy, shorter or longer than immature receptacle, usually sessile, rarely on a distinct elongate peduncle; sepals 3 or 4, glabrous or hairy; male broadly ovate, rarely narrowly elliptic; female from linear to broadly ovate or triangular, erect to recurved, rarely absent or vestigial; stamens few to numerous, filaments red or whitish green, anthers reddish brown or yellow, rarely with hairs on the connective; receptacle ribbed or entire, glabrous or with short hairs; carpels 1 or rarely 2 or more, ovule solitary, pendulous, one ovule per locule; stigma short and hidden or long and protruding, strap-like to much-branched and feathery, red to greenish white, one stigma per carpel. Achene broadly ellipsoid to narrowly cylindrical or irregular, sometimes slightly curved, ribbed or winged, glabrous or with a few short hairs, sometimes tuberculate, occasionally with a membranous outer layer, very pale brown to black, sometimes reddish.

Subgen. **Arborea** (*Weim.*) *C. Whitehouse* subgen. et stat. nov.

Syn. *Cliffortia* L. sect. *Arboreae* Weim., Monogr. Gen. Cliffortia: 91 (1934); E.G.H. Oliv. & Fellingham in Bothalia 24: 153–162 (1994)

Tall erect densely branching shrub or **small tree**. Young stems with dense curled hairs, forming brachyblasts. Sheath hairy abaxially, glabrous adaxially except for fringe of hairs at apex; stipules present or often absent, membranous and free; petiole absent. Leaves unifoliate or trifoliate, single-nerved, hairy above, usually densely whitish hairy beneath; leaflet margins inrolled beneath, midrib prominent. **Female flowers clustered in a serotinous cone-like structure**, male flowers scattered at base of ordinary vegetative leaves. Bracteoles hairy all over; sepals 3 or 4, female erect, narrowly oblong to linear; carpel single; stigma red and strap-like, prominent out of cone-like structure. **Achenes irregular, 3–4 ribbed**, entire and glabrous, dark brown to **black**.

Species: *C. arborea* Marloth, *C. conifera* E.G.H. Oliv. & A.C. Fellingham, *C. dichotoma* A.C. Fellingham sp. nov.

Subgen. **Eriocephalina** *C. Whitehouse* subgen. nov.

Low to medium erect or decumbent densely branched shrubs. Young stems densely long-spreading or curly hairy, forming brachyblasts. **Sheath hairy abaxially, densely hairy adaxially**; stipules present or absent, membranous and free with a ciliate margin; petiole absent. Leaves unifoliate or trifoliate, single-nerved, **often greyish hairy above and usually densely hairy beneath**; leaflets elliptic to needle-shaped,

usually contracted at base to form a pseudopetiole, margins entire, inrolled beneath. Flowers solitary in the axil of ordinary vegetative leaves. Bracteoles hairy all over, longer than receptacle; sepals 3, hairy on abaxially, female erect, narrowly oblong to linear; receptacle glabrous, distinctly ribbed; carpel single; stigma pinkish white to red, usually prominent above leaves. Achenes ellipsoid, ribbed, glabrous, beige to brown.

Species: *C. dispar* Weim., *C. eriocephalina* Cham., *C. esterhuyseniae* Weim., *C. montana* Weim., *C. weimarckii* C. Whitehouse sp. nov.

Subgen. **Graminea** *C. Whitehouse* subgen. nov.

Medium to tall densely to sparsely branched shrub, resprouting after fire from a thick caudex, sometimes scrambling amongst surrounding vegetation. Young stems glabrous, with or without brachyblasts. Sheath glabrous abaxially, glabrous adaxially or sometimes with a short fringe of hairs at the apex; stipules present, often green and similar in texture to the lamina; petiole absent. Leaves unifoliate, **grass-like to long needle-shaped**, green above and beneath, usually glabrous, only rarely with a few hairs on the midrib beneath, single- or multinerved, apex long-acuminate to pungent. Flowers solitary in the axil of ordinary vegetative leaves. Bracteoles longer than receptacle; sepals 3, glabrous, **male often narrowly elliptic**, female erect; receptacle glabrous; carpel single. Achenes irregular to ellipsoid, ribbed, glabrous, brown. **Growing in permanently wet areas**.

Sect. **Graminae** *C. Whitehouse* sect. nov.

Leaves **multinerved**, margins toothed. **Male sepals fused, only splitting on underside and forming a hood over the stamens**.

Species: *C. graminea* L.f.

Sect. **Longifoliae** *C. Whitehouse* sect. nov.

Leaves **single-nerved**, margins entire. Male sepals free.

Series *Longifolia*

Tall robust erect shrubs. Leaves broad and grass-like.

Species: *C. longifolia* (Eckl. & Zeyh.) Weim., *C. strobilifera* L.

Series *Aculeata*

Low to medium scrambling shrubs. Leaves long needle-shaped or laterally flattened.

Species: *C. aculeata* Weim., *C. nivenioides* A.C. Fellingham.

Subgen. **Cliffortia**

Syn. *Cliffortia* L. subgen. *Digraphidium* Weim., Monogr. Gen. Cliffortia: 20 (1934)
Cliffortia L. subgen. *Monographidium* (Presl) Weim., Monogr. Gen. Cliffortia: 20 (1934)

Same as for genus except inflorescence never serotinous.

Sect. **Heteromorphae** *C. Whitehouse* sect. nov.

Low to medium densely to sparsely branched shrub, sometimes scrambling through surrounding vegetation. Young stems usually forming brachyblasts. Stipules present, free. Leaves trifoliate; leaflets single-nerved. Flowers solitary, in the axil of ordinary vegetative leaves or on a short to long peduncle. Bracteoles longer than immature receptacle although sometimes receptacle elongates very quickly; sepals 3; receptacle glabrous; carpel 1. Achene ribbed, glabrous, brown.

Series *Curvifolia*

Medium erect shrub, only surviving fire as seed. Stems forming brachyblasts. Sheath glabrous both abaxially and adaxially; stipules membranous, entire; petiole absent. Leaves glabrous, green with two pale stripes on either side of midrib; leaflets needle-shaped, **curved markedly upwards and in**. Flowers solitary, in the axil of ordinary vegetative leaves. Sepals glabrous; female recurved; **receptacle elongating markedly as it matures. Achene long, curved, cylindrical**.

Species: *C. curvifolia* Weim., *C. densa* Weim.

Series *Pedunculata*

Low or sometimes medium shrubs. Young stems usually glabrous, rarely with a few hairs on one side, rounded or often compressed and winged. Sheath glabrous both abaxially and adaxially, with or without a short fringe at the apex; stipules entire; **petiole present**. Leaves glabrous; leaflet margins entire or markedly toothed. Flowers solitary, **on a short to long peduncle in the axil of ordinary vegetative leaves**. Bracteoles glabrous, entire; sepals glabrous, spreading or rarely erect; receptacle smooth; stigma usually red, prominent above leaves. Achene covered by membranous outer layer.

Species: *C. lepida* Weim., *C. pedunculata* Schltr., *C. triloba* Weim.

Series *Hirta*

Medium erect densely branched shrubs. Young stems with **spreading dense hairs**, forming brachyblasts. Sheath usually hairy abaxially, glabrous or with scattered adpressed hairs and a short fringe at the apex adaxially; stipules membranous, usually ciliate; petiole absent. Leaves hairy beneath, although sometimes only on the midrib; leaflet **elliptic to needle-shaped**. Flowers solitary, in the axil of ordinary vegetative leaves. Female sepals erect, sometimes spreading; stigma red or sometimes pinkish. **Often growing at the boundary between alkaline and more acidic soils.**

Species: *C. anthospermoides* A.C. Fellingham sp. nov., *C. hirta* Burm.f., *C. incana* Weim.

Series *Apiculata*

Medium erect densely branched shrub, resprouting after fire. Young stems with spreading hairs. Sheath glabrous abaxially, with scattered adpressed hairs adaxially; stipules membranous, ciliate; **petiole absent**. Leaves glabrous; **leaflet elliptic to oblong, straight to slightly sickle-shaped, margins entire**. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteole glabrous but with a ciliate margin; sepals glabrous, female oblong to linear, erect; receptacle smooth; filaments red, anthers yellow; stigma red, prominent above leaves. **Achene covered by membranous layer**.

Species: *C. apiculata* Weim.

Series *Exilifolia*

Medium erect densely branched shrub, only surviving fire as seed. Young stems with dense upwardly adpressed hairs, forming closely overlapping brachyblasts. Sheath glabrous both abaxially and adaxially except for short fringe at apex; stipules membranous, entire; petiole absent. Leaves glabrous; leaflets **very narrowly needle-shaped** with a long acuminate apex, curved markedly upwards and in, margins entire to serrulate. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteole hairy; sepals glabrous, female erect; receptacle distinctly ribbed; filaments white, anthers yellow; stigma white to pinkish white. Achene not covered by membranous layer.

Species: *C. exilifolia* Weim.

Sect. **Petiolatae** *Weim.*, Monogr. Gen. Cliffortia: 28 (1934) pro parte

Medium erect densely branched shrubs. Young stems with upwardly adpressed to spreading short hairs, forming brachyblasts. Sheath glabrous adaxially with fringe of hairs at apex; stipules present, free, membranous, usually ciliate; **petiole present**. Leaves trifoliate, glabrous, green with two pale stripes on either side of midrib beneath; leaflets oblong to needle-shaped, **outer ones usually sickle-shaped and all curved upwards and towards the stem**, margins usually entire, midrib not prominent. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles usually ciliate on the margins; sepals 3, glabrous, female usually spreading; anthers yellow; bracteoles longer than immature receptacle; carpel single. Achene ribbed, brown to greyish brown, **covered with a membranous outer layer**.

Species: *C. arcuata* Weim., *C. drepanoides* Eckl. & Zeyh., *C. falcata* L.f., *C. perpendicula* C. Whitehouse sp. nov., *C. ramosissima* Schltr.

Sect. **Complanatae** *Weim.*, Monogr. Gen. Cliffortia: 20 (1934) pro parte

Low to medium densely branched shrubs, only surviving fire as seed or possibly facultatively resprouting from a caudex. Sheath hairy adaxially, sometimes markedly so, with a fringe at the apex; stipules present, membranous; petiole absent. Leaves unifoliate or trifoliate, single-nerved, glabrous; leaflets needle-shaped or variously toothed or lobed. Flowers solitary, in the axil of ordinary vegetative leaves. **Sepals 4**, rarely 3, glabrous; stamens few, 2–8, filaments and anthers usually reddish.

Series *Dentata*

Low decumbent almost herbaceous shrubs. Young stems with downwardly adpressed short hairs, not forming brachyblasts. Sheath hairy abaxially; stipules joined on the reverse side of the stem or free, margin ciliate. Leaves trifoliate; **leaflets obovate, lobed**. Bracteoles glabrous except for ciliate margins;

tips of male sepals long and attenuate, female sepals broadly ovate and recurved; bracteole longer than immature receptacle; receptacle glabrous, entire; **carpels 2**. Achene unribbed except for groove between carpels, entire, pale brown.

Species: *C. dentata* Willd., *C. gracilis* Harv., *C. gracillima* C. Whitehouse sp. nov.

Series *Complanata*

Low to medium densely branched erect shrubs, only surviving fire as seed or possibly facultatively resprouting from a caudex. Young stems with upwardly adpressed hairs, forming brachyblasts. Sheath glabrous abaxially, markedly hairy adaxially; stipules free, entire. Leaves trifoliate, glaucous; leaflets elliptic to obovate, margins entire or toothed. Bracteoles glabrous sometimes with shortly ciliate margins; sepals 4 or occasionally 3, female ovate, recurved; bracteole longer than immature receptacle, receptacle hairy, distinctly ribbed; stigma red; **carpels 2** or sometimes single. **Achene flattened and 2-winged, with a few short hairs in the lower half, red to reddish brown. Often growing in rock cracks.**

Species: *C. complanata* E. Mey. ex Harv., *C. propinqua* Eckl. & Zeyh.

Series *Cervicornu*

Low to medium densely branched erect shrubs, only surviving fire as seed. Young stems with spreading or curly hairs, forming brachyblasts. Sheath glabrous abaxially, markedly hairy adaxially; stipules free, entire. Leaves trifoliate, glabrous; **leaflets needle-shaped, deeply divided**, curved upwards and towards the stem. Male flower unknown. Bracteoles glabrous, entire; female sepals ovate, erect; bracteoles shorter than immature receptacle, receptacle distinctly ribbed, glabrous; carpels 1. Achene 4-ribbed, glabrous, brown.

Species: *C. cervicornu* Weim.

Series *Ericifolia*

Medium densely branched erect or sprawling shrubs. Young stems glabrous, forming brachyblasts. Sheath glabrous abaxially, markedly hairy adaxially; stipules free, margins ciliate. **Leaves unifoliate, needle-shaped, contracted at base to form a pseudopetiole, green above, whitish beneath, margins inrolled beneath**. Bracteoles glabrous sometimes with shortly ciliate margins; female sepals erect, soon falling; receptacle glabrous, ribbed; carpels 1; stigma red. Achene glabrous, 2–4-ribbed, brown.

Species: *C. brevifolia* Weim., *C. ericifolia* Eckl. & Zeyh.

Series *Subsetacea*

Low to medium densely branched erect shrubs, only surviving fire as seed. Young stems with upwardly adpressed or curly hairs, forming brachyblasts. Sheath glabrous abaxially, hairy adaxially; stipules free, entire. Leaves trifoliate, glabrous, green or with two pale stripes on either side of the midrib: leaflets linear to needle-shaped, curved upwards and towards the stem. Female sepals ovate, erect; stamens 6–8; bracteoles shorter than or as long as the immature receptacle, receptacle distinctly ribbed, glabrous; carpels 1. **Achene 4- or 8-ribbed**, glabrous, brown.

Species: *C. cruciata* C. Whitehouse sp. nov., *C. subsetacea* (Eckl. & Zeyh.) Diels ex Bolus & Wolley Dod

Sect. **Alatae** *Weim.*, Monogr. Gen. Cliffortia: 80 (1934) pro parte & in Bot. Not. 1948: 185 (1948) pro parte

Medium erect densely branched shrubs, clonally spreading by roots underground and able to resprout after fire. Young stems with short curly hairs, forming brachyblasts. Sheath hairy both abaxially and adaxially; stipules present or absent, free, ciliate; petiole absent. Leaves trifoliate, hairy beneath; leaflets needle-shaped, contracted at base to form a pseudopetiole, margins entire, inrolled beneath. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles hairy, shorter than immature receptacle; sepals 4, often hairy on abaxially, female broadly ovate, recurved; filaments red, anthers brownish red; receptacle cleary ribbed, hairy; carpels 1; stigma red. **Achene on a short recurved stipe, broadly 3–4-winged, tuberculate, hairy, reddish.**

Species: *C. alata* N.E. Br., *C. burgersii* E.G.H. Oliv. & A.C. Fellingham

Sect. **Filicaulae** *C. Whitehouse* sect. nov.

Low to medium, often sprawling or trailing, densely branched shrubs, only surviving fire as seed. Young stems with long spreading hairs, forming brachyblasts. Sheath hairy both abaxially and adaxially; stipules free, often **a similar texture to the leaf**, but sometimes membranous, ciliate; petiole present or absent.

Leaves unifoliate or trifoliate, hairy above and beneath; leaflets ovate, obovate or elliptic, sometimes toothed to lobed, apex acute to rounded, margins flat. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles hairy, longer than the immature receptacle; **sepals 4, sometimes fused towards the base**, hairy on abaxially, female erect; receptacle smooth, glabrous; carpels 1 or 2; stigma red to pinkish. Achene ellipsoid, unribbed, pale whitish brown.

Species: *C. filicaulis* Schldl., *C. filicauloides* Weim., *C. hantamensis* Diels, *C. monophylla* Weim.

Sect. **Dracomontanae** *C. Whitehouse* sect. nov.

Medium erect densely branched shrubs. Young stems with short upwardly adpressed hairs, forming brachyblasts. Sheath glabrous abaxially, markedly hairy adaxially; stipules free, membranous; petiole absent. Leaves trifoliate, glabrous or occasionally with a few hairs. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteole glabrous with entire to ciliate margin, longer than immature receptacle; **sepals 4**, glabrous, female ovate, recurved; **receptacle smooth** or occasionally distinctly ribbed, glabrous; carpels 1; **stigma red to pinkish** or rarely white. Achenes ellipsoid, ribbed, often indistinctly so, brown. **Found from Eastern Cape to Kenya.**

Species: *C. browniana* Burtt Davy, *C. dracomontana* C.M. Whitehouse sp. nov., *C. nitidula* (Engl.) R.E. & T.C.E. Fr., *C. spathulata* Weim.

Sect. **Paucistaminae** *C. Whitehouse* sect. nov.

Low to medium erect densely branched shrubs. Young stems with short upwardly adpressed hairs, forming brachyblasts. Sheath glabrous abaxially; stipules membranous, free, entire or rarely ciliate; petiole absent. Leaves trifoliate, glabrous. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteole with a serrate to shortly ciliate margin, longer than immature receptacle; **sepals 4**, very rarely 3, glabrous; **receptacle distinctly ribbed**, glabrous; carpels 1. Achenes ellipsoid, ribbed, **often pale yellowish brown.**

Species: *C. crassinervis* Weim., *C. paucistaminea* Weim., *C. serpyllifolia* Cham. & Schldl., *C. setifolia* Weim.

Sect. **Tuberculatae** *C. Whitehouse* sect. nov.

Medium erect densely branched shrubs. **Young stems with short upwardly adpressed hairs**, forming brachyblasts. Sheath glabrous abaxially, a few scattered hairs adaxially; stipules membranous, free, entire; petiole absent. Leaves trifoliate, glabrous, rarely with a few scattered hairs beneath, **soft to papery**, usually straight to curved upwards and towards the stem; **leaflets needle-shaped, apex long acuminate, margins minutely serrulate**. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles hairy with a serrate margin, longer than immature receptacle; **sepals 3**, glabrous, female ovate, recurved; receptacle distinctly ribbed, glabrous; carpels 1. Achene ribbed, glabrous, mid to dark brown.

Species: *C. atrata* Weim., *C. tuberculata* (Harv.) Weim.

Sect. **Castanae** *C. Whitehouse* sect. nov.

Medium or sometimes low erect densely branched shrubs. Young stems with short upwardly adpressed hairs, forming brachyblasts. Sheath glabrous abaxially, glabrous or with a few scattered hairs adaxially and a fringe at the apex; stipules free, membranous, entire; petiole absent. Leaves unifoliate or trifoliate, glabrous, **often quite rigid; leaflets needle-shaped, sharply acuminate at the apex**, rarely just shortly acute, **margins usually entire**. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles usually glabrous with entire to serrate margins; sepals 3, female ovate; filaments and anthers usually reddish; bracteoles longer than immature receptacle, receptacle glabrous; carpels 1. Achene ellipsoid, 6–12-ribbed, glabrous, brown to reddish brown.

Species: *C. castanea* Weim., *C. homunculi* C. Whitehouse sp. nov., *C. neglecta* Schltr., *C. robusta* Weim.

Sect. **Filifoliae** *C. Whitehouse* sect. nov.

Low sprawling to tall erect densely branched shrubs. Young stems glabrous or rarely with a few short adpressed hairs, forming brachyblasts. Sheath glabrous adaxially and out and often lacking the fringe of hairs at the apex; stipules membranous, free or rarely joined in front of leaf, entire; petiole absent. Leaves unifoliate or trifoliate, single-nerved to base, glabrous; leaflets needle-shaped, apex acute to long acuminate, margin flat. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles glabrous and

entire or slightly serrate, longer than immature receptacle; sepals 3, glabrous, female usually erect or sometimes becoming recurved; filaments red; receptacle smooth, glabrous; carpels 1. Achenes faintly ribbed, glabrous, brown.

Species: *C. acocksii* Weim., *C. burchellii* Stapf, *C. erectisepala* Weim., *C. filifolia* L.f., *C. repens* Schltr.

Sect. **Cliffortiae**

Syn. *Cliffortia* L. sect. *Inflexae* Weim. in Bot. Not. 1948: 186 (1948) (=*Reflexae* in key & sect. *Alatae*)

Medium erect densely branched shrubs. Young stems with spreading hairs, forming brachyblasts. Sheath hairy abaxially; stipules membranous, free; petiole absent. Leaves trifoliate, **nearly always with hairs beneath, often hairy above**; leaflet elliptic to obovate or needle-shaped, **margins flat**. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles hairy, longer than immature receptacle; sepals 3, hairy on abaxially, female ovate, recurved; Stamens 6–12, filaments red, anthers reddish brown; receptacle distinctly ribbed, usually glabrous; carpels 1; stigma red. Achene broadly ellipsoid, **6-winged or ribbed, if winged then wings incurved**, usually glabrous, often reddish.

Species: *C. cristata* Weim., *C. hexandra* Weim., *C. lanata* Weim., *C. polygonifolia* L., *C. pubescens* C. Whitehouse sp. nov., *C. sericea* Eckl. & Zeyh., *C. subdura* Weim.

Sect. **Multifidae** *C. Whitehouse* sect. nov.

Medium erect densely branched shrubs, usually resprouting after fire. Young stems with short upwardly adpressed hairs, very rarely glabrous, forming brachyblasts. Sheath usually glabrous abaxially and with a few hairs adaxially; stipules membranous, free; petiole absent. Leaves trifoliate or unifoliate or intermediate with deep toothing, curved downwards and away from the stem; leaflet tip acuminate to pungent. Bracteole longer than immature receptacle; sepals 3, female broadly ovate, usually recurved; receptacle glabrous, distinctly ribbed; carpels 1, rarely 2. Achene ribbed, glabrous, brown.

Series *Multiformis*

Young stems hairy. Stipules entire, rarely ciliate. **Leaves usually trifoliate**, only unifoliate and deeply toothed on young growth; **leaflets linear to needle-shaped**, glabrous above, rarely with a few hairs beneath; leaflet margins flat. Flowers solitary, in the axil of ordinary vegetative leaves.

Species: *C. dodecandra* Weim., *C. multiformis* Weim., *C. pterocarpa* (Harv.) Weim.

Series *Ruscifolia*

Leaves usually unifoliate, rarely appearing trifoliate when deeply toothed, multi-nerved to base, usually hairy beneath and glabrous above; leaflets usually lanceolate. Flowers solitary in the axil of ordinary vegetative leaves or conglomerated in to heads with reduced leaves. Bracteole hairy or rarely glabrous; sepals recurved; stigma red or occasionally pinkish.

Species: *C. cymbifolia* Weim., *C. purpurea* (Weim.) C.M. Whitehouse comb. nov., *C. versiformis* C. Whitehouse sp. nov., *C. ruscifolia* L., *C. verrucosa* Weim.

Sect. **Simplices** *Weim.*, Monogr. Gen. Cliffortia: 126 (1934) pro parte

Low to medium, **decumbent to scrambling shrubs**, usually only surviving fire as seed. Sheath glabrous adaxially; stipules membranous, usually ciliate. Leaves unifoliate, single-nerved, curved downwards and away from the stem, usually toothed with teeth recurved. Bracteole hairy, longer than immature receptacle; sepals 3, female sometimes absent or vestigial; receptacle glabrous; carpels 1; stigma red, rarely pinkish. Achene ribbed, glabrous.

Series *Ferruginea*

Young stems forming brachyblasts. Petiole absent. **Leaves oblong-elliptic to obovate or needle-shaped, rigid or leathery**, glabrous or with a few hairs above. Sepals glabrous or rarely hairy, female present; filaments red.

Species: *C. acutifolia* Weim., *C. berberidifolia* Lam., *C. ferruginea* L.f., *C. uncinata* Weim.

Series *Odorata*

Shrubs only surviving fire as seed. Young stems usually with some long hairs. Sheath usually hairy abaxially. **Leaves broadly ovate to subcircular, herbaceous or papery**, markedly toothed. Female sepals erect; anthers usually yellow; receptacle smooth.

Species: *C. hirsuta* Eckl. & Zeyh., *C. odorata* L.f., *C. pilifera* Bolus, *C. reticulata* Eckl. & Zeyh., *C. tricuspidata* Harv., *C. viridis* Weim.

Sect. **Bifoliolae** DC. in Ann. Sc. Nat. 1: 450 (1824); Weim., Monogr. Gen. Cliffortia: 92 (1934)

Low to medium erect shrubs, clonally spreading by roots underground and able to resprout after fire (except for *C. phyllanthoides*). Sheath usually glabrous both abaxially and adaxially except for the fringe of hairs at apex; petiole absent. Leaves **bifoliate** or trifoliate, **often glaucous, glabrous**, curved upwards and towards the stem, midrib not prominent. Flowers solitary, in the axil of ordinary vegetative leaves. Sepals 3, glabrous, female sepals usually erect; filaments and anthers usually reddish; receptacle usually glabrous; carpels 1; stigma red. Achene ribbed or winged, usually glabrous.

Series *Crenata*

Often sparsely branched shrubs. **Brachyblasts usually absent**. Leaves **usually bifoliate**, glaucous, **multi-nerved**; leaflets broadly ovate to subcircular, margins flat. Sometimes filaments greenish white and anthers yellow. Achene ribbed, glabrous, brown.

Species: *C. crenata* L.f., *C. crenulata* Weim., *C. mirabilis* Weim., *C. varians* Weim.

Series *Obcordata*

Leaves trifoliate, **sometimes with middle leaflet highly modified or reduced**. Bracteoles often shorter than immature receptacle; receptacle distinctly ribbed. **Found in coastal regions**.

Species: *C. carinata* Weim., *C. geniculata* Weim., *C. marginata* Eckl. & Zeyh., *C. obcordata* L.f., *C. phyllanthoides* Schltr., *C. tenuis* Weim.

Series *Obovata*

Young stems glabrous, forming brachyblasts. Leaves trifoliate; **leaflets obovate to needle-shaped, margins flat or rounded**. Bracteoles shorter than immature receptacle.

Species: *C. obovata* E. Mey. ex Harv., *C. rigida* Weim., *C. semiteres* Weim., *C. teretifolia* L.f.

Series *Polita*

Young stems forming brachyblasts. Leaves trifoliate; **leaflets needle-shaped to elliptic, margins inrolled beneath**. Bracteole shorter than immature receptacle.

Species: *C. polita* Weim.

Series *Glauca*

Leaves trifoliate or bifoliate; leaflets needle-shaped, elliptic, obovate or subcircular, **apex usually mucronate to acuminate**, margins flat or inrolled beneath.

Species: *C. amplexistipula* Schltr., *C. concinna* Weim., *C. glauca* Weim., *C. longimontana* C. Whitehouse sp. nov., *C. pulchella* L.f.

Section **Multinerviae** *DC.* in Ann. Sc. Nat. 1: 448 (1824); Weim., Monogr. Gen. Cliffortia: 98 (1934) pro parte

Medium to tall scrambling or erect shrubs. Young stems often covered by leaf sheaths and not forming brachyblasts. Sheath glabrous adaxially except sometimes for a fringe at the apex; stipules if present free or rarely joined on reverse side of stem, usually membranous, rarely similar texture to leaf; petiole absent. **Leaves unifoliate, multinerved to base**, glabrous or rarely with scattered hairs, usually markedly toothed, apex sharply acuminate to pungent, usually curved downwards and away from the stem. Flowers solitary in the axil of ordinary vegetative leaves or rarely female flowers in a distinct inflorescence. Bracteoles longer than immature receptacle; sepals 3, glabrous or rarely with a few hairs on the abaxially, female erect; **stamens many to numerous, filaments white and anthers yellow**, only rarely both reddish; carpels 1; **stigma tucked in the axil of the leaves**, usually whitish green, rarely protruding. Achene ellipsoid, ribbed, brown.

Series *Ilicifolia*

Erect shrubs, clonally spreading by roots underground and able to resprout after fire. Young stems glabrous. Sheath glabrous abaxially; stipules present, entire, sometimes joined on reverse side of stem. Leaves green to glaucous, glabrous. Bracteole hairy on keel with a entire margin; sepals glabrous, female broadly ovate to triangular; receptacle glabrous, entire.

Species *C. ilicifolia* L., *C. intermedia* Eckl. & Zeyh., *C. reniformis* (Weim.) C. Whitehouse, *C. schlechteri* (Weim.) C. Whitehouse

Series *Acanthophylla*

Medium erect shrubs. Young stems glabrous, not forming brachyblasts. Sheath glabrous abaxially; stipules present or sometimes absent, entire. Leaves glabrous. Bracteole hairy on keel with a entire margin; sepals glabrous, female broadly ovate to triangular; receptacle glabrous, entire; stigma red or greenish white.

Species: *C. acanthophylla* C. Whitehouse sp. nov., *C. ceresana* C. Whitehouse sp. nov., *C. virgata* Weim.

Series *Phillipsii*

Tall erect shrub with a monopodial growth form. Young stems with short upwardly adpressed hairs or not, **forming brachyblasts**. Sheath hairy abaxially or not; stipules present, entire. Leaves glabrous or with a few hairs along the midrib above or beneath. Bracteoles glabrous; sepals glabrous, female broadly ovate to triangular; receptacle hairy, distinctly ribbed; stigma red. **Achene hairy**.

Species: *C. phillipsii* Weim.

Series *Heterophylla*

Erect shrubs. Young stems glabrous, not forming brachyblasts. Sheath glabrous abaxially; stipules present or sometimes absent, entire. Leaves oblong to lanceolate, glabrous, straight or curved upwards and towards the stem. Female flowers sometimes found in the axil of modified leaves in a distinct inflorescence. Bracteoles hairy or glabrous on the keel with a entire margin; sepals glabrous, female triangular; receptacle smooth; stigma greenish white. Achene glabrous.

Species: *C. heterophylla* Weim., *C. serrata* C. Whitehouse sp. nov.

Series *Grandifolia*

Erect shrubs. Young stems not forming brachyblasts. Stipules present. Leaves oblong to lanceolate, usually glabrous or with a few hairs above. Bracteoles hairy on the keel with a entire margin; sepals glabrous, female triangular; receptacle smooth, hairy towards top; stigma greenish white. Achene glabrous.

Species: *C. grandifolia* Eckl. & Zeyh., *C. lanceolata* Weim.

Series *Denticulata*

Stipules free, entire.

Species: *C. denticulata* (Weim.) C. Whitehouse comb. nov., *C. dregeana* Presl, *C. integerrima* Weim., *C. oligodonta* C. Whitehouse sp. nov., *C. ovalis* Weim., *C. recurvata* (Weim.) C. Whitehouse comb. nov., *C. scandens* C. Whitehouse sp. nov., *C. strigosa* Weim., *C. theodori-friesii* Weim.

Incertae cedis (=sect. **Costatae** *Weim.*, Monogr. Gen. Cliffortia: 34 (1934) pro parte)

Syn. *Cliffortia* L. sect. *Bacciformes* Weim. in Bot. Not. 1948: 184 (1948)

Species: *C. baccans* Harv., *C. bolusii* Diels ex C. Whitehouse sp. nov., *C. cuneata* Aiton, *C. juniperina* L.f., *C. linearifolia* Eckl. & Zeyh., *C. micrantha* Weim., *C. pungens* Presl, *C. stricta* Weim.

This is an unnatural assemblage of species whose placement elsewhere is as yet unconfirmed.

C. baccans Harv.

Low erect densely branched shrub, resprouting after fire. Young stems with short upwardly adpressed hairs, forming brachyblasts. Sheath glabrous both abaxially and adaxially; stipules membranous, free, entire; petiole absent. Leaves trifoliate, glabrous, curved upwards and towards the stem; leaflets needle-shaped, margins entire. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles glabrous with a entire to serrate margin, longer than immature receptacle; sepals 3, glabrous, female ovate, recurved; receptacle smooth, glabrous; carpels 1; stigma red. **Achene subglobose, fleshy, orange**.

tentatively sister to sect. Dracomontanae, and this could possibly be true as it does share some morphological features with those species, in particular *C. spathulata*.

C. pungens Presl

Low to medium erect densely branched shrub, clonally spreading by roots underground and able to resprout after fire. Young stems glabrous, forming brachyblasts. Sheath glabrous abaxially and adaxially; stipules membranous, free, entire; petiole absent. Leaves unifoliate, glabrous, rigid, **curved downwards and away from the stem, thickly needle-shaped**, apex sharply acuminate to pungent, margins curved upwards to form a furrow, entire to minutely serrulate. Flowers solitary, in the axil of ordinary vegetative leaves. Bracteoles glabrous with entire to serrate margins, longer than immature receptacle; sepals 3, glabrous, female broadly ovate to triangular, erect; filaments red, anthers reddish; receptacle smooth, glabrous; carpels 1; stigma red. Achene **narrowly cylindrical and slightly curved**, 6-ribbed, glabrous, brown.

Possibly related to sect. Simplices as there are morphological similarities, especially with *C. uncinata*, and phylogenetic analysis of both chloroplast and nuclear data place it close to that group.

C. stricta Weim.

Medium erect densely branched shrub, only surviving fire as seed. Young stems with short to long spreading hairs, forming brachyblasts. Sheath hairy abaxially; stipules free, **brown and scarious**; petiole absent. Leaves trifoliate, hairy above, hairy or not beneath; leaflets needle-shaped, apex acute to sharply acuminate, margins inrolled beneath, entire. Bracteole hairy all over, longer than immature receptacle; sepals 3, hairy on abaxially, female narrowly ovate to linear, erect; filaments white, anthers yellow; receptacle smooth, glabrous; carpels 1; stigma red to pinkish. Achene ellipsoid, 6-ribbed, glabrous, whitish to pale brown.

Morphologically similar to subgenus Eriocephalina, but far removed and in isolated positions in the molecular phylogenetic analyses.

Excluded sections and species:

Sect. *Hermaphroditicae* Weim. in Bot. Not. 1948: 171 (1948)

Species: *C. hermaphroditicae* Weim.

The type of this species has not been found in NBG, where it is supposed to be deposited. The specimen at BOL is of a form of the *C. pterocarpa* complex. This species is probably nothing more than an unusual morph of *C. pterocarpa* with bisexual flowers that occurred once and has since been lost.

Appendix 2. Dichotomous keys to *Cliffortia.*

Key to the sections of *Cliffortia*, or series if the sections are difficult to define morphologically.

1. Female flowers clustered into a serotinous inflorescence ..... subgen. **Arboreae**
   Flowers solitary at the base of ordinary vegetative leaves, or rarely clustered into an inflorescence but then never serotinous 2
2. Achene a berry ..... *C. baccans*
   Achene dry and hard 3
3. Stipules absent, leaves trifoliate, hairy, margins flat ..... *C. bolusii*
   Stipules present or if absent then leaves unifoliate or glabrous or with inrolled margins 4
4. Leaves wedge-shaped with a broad toothed apex ..... *C. cuneata*
   Leaves various but never broadest at the very apex 5
5. Sepals 4 6
   Sepals 3 17
6. Achenes clearly winged, very sparsely to densely hairy, often reddish 7
   Achenes ribbed or smooth, glabrous, brown to whitish 8
7. Achenes small, < 2 mm wide, sparsely hairy ..... ser. **Complanata**
   Achenes large, > 4 mm wide, densely hairy ..... sect. **Alatae**
8. Leaves hairy above and beneath, leaflets broadly ovate, elliptic or obovate, stipules often similar texture to leaf, sepals often fused towards the base ..... sect. **Filicaulae**
   Leaves glabrous or rarely sparsely hairy, stipules membranous, sepals free 9
9. Leaves unifoliate, needle-shaped, margins inrolled 10
   Leaves trifoliate, variously shaped, margins usually flat or rounded 11
10. Stems glabrous, leaves with a single pale stripe beneath ..... ser. **Ericifolia**
    Stems densely hairy, leaves with two pale stripes either side of midrib beneath ..... *C. linearifolia*
11. Leaves needle-shaped, deeply divided and feathery ..... *C. cervicornu*
    Leaves not as above 12
12. Sprawling mat forming semi-herbaceous shrubs ..... ser. **Dentata**
    Erect densely branched shrubs 13
13. Leaves broadly obovate and toothed, carpels often 2 or more, achene swollen ..... *C. micrantha*
    Leaves various, carpels 1, achene ellipsoid 14
14. Leaves curved upwards and inwards, achene thickly 4 or 8 ribbed ..... ser. **Subsetacea**
    Achene smooth or ribbed, but if ribs present then thin and many or indistinct 15
15. Leaves needle-shaped, or sometimes elliptic to obovate, receptacle clearly ribbed 16
    Leaves broadly obovate to narrowly elliptic, sometimes with margins inrolled and then appearing needle-shaped ..... sect. **Dracomontanae**
16. Leaves with flat margins ..... sect. **Paucistaminae**
    Leaves with inrolled margins to raised midrib and with two pale stripes either side ..... *C. linearifolia*
17. Flowers on a short to long peduncle ..... ser. **Pedunculata**
    Flowers sessile at base of leaves 18
18. Leaves petiolate, outer leaflets often sickle-shaped, stems with upwardly adpressed to spreading hairs, achene covered by membranous layer ..... sect. **Petiolatae**
    Leaves without a petiole, or if short petiole present then stems glabrous, outer leaflets straight 19
19. Leaves unifoliate, very long, 2–7 cm, grass-like or needle-shaped, male sepals generally narrowly elliptic ..... 20 (subgen. **Graminea**)
    Leaves much shorter, or if longer then broad and lanceolate, male sepals broadly ovate 21
20. Leaves multi-nerved, toothed, male sepals fused into a hood over the stamens ..... sect. **Graminae**
    Leaves single-nerved, entire, male sepals free ..... sect. **Longifoliae**
21. Leaves bifoliate, leaflets broadly obovate to subcircular ..... sect. **Glaucae**
    Leaves unifoliate or trifoliate 22
22. Leaves unifoliate, although sometimes deeply toothed, and multinerved at the base 23
    At least some leaves trifoliate, or if unifoliate then with a single nerve to the base 24
23. Stems usually hairy, forming brachyblasts, leaves usually hairy, at least below, female sepals recurved ..... ser. **Ruscifolia**

23. Stems usually hairy, forming brachyblasts, leaves usually hairy, at least below, female sepals recurved ..... ser. **Ruscifolia**
Stems and leaves usually glabrous and not forming brachyblasts, female sepals erect ..... sect. **Multinerviae**

24. Stems glabrous or almost so, leaflets glabrous, green, needle-shaped, margins not inrolled, bracteoles glabrous, receptacle smooth 25
Stems hairy, if not hairy then leaves either broader or glaucous or receptacle clearly ribbed to winged 26

25. Leaves bent downwards and out, thick and apex sharply pointed ..... *C. pungens*
Leaves bent upwards and in, often fine ..... sect. **Filifoliae**

26. Decumbent to scrambling shrubs, using surrounding vegetation for support, sheath glabrous inside, leaves unifoliate, bent downwards and out, usually broad and toothed, rarely needle-shaped and then with a hooked tip ..... sect. **Simplices**
Erect densely branched shrubs, leaves trifoliate or occasionally unifoliate, but then greyish hairy with sheath markedly hairy inside or bent upwards and in 27

27. Leaves needle-shaped, curved markedly upwards and in, achene long cylindrical, elongating as it matures ..... ser. **Curvifolia**
Leaves various, achene ellipsoid 28

28. Stems with spreading to curly hairs, sheath hairy on the outside, leaves variously hairy, often dense and greyish 29
Stems glabrous or with short adpressed or rarely spreading hairs, sheath glabrous outside, leaves usually glabrous or with a few scattered hairs 32

29. Leaflet margins flat, achene large, 3–5 mm long, prominently ribbed or winged, wings if present incurved, often reddish ..... sect. **Inflexae**
Leaflet margins usually inrolled beneath, achene smaller, 2–4 mm long, thinly to indistinctly ribbed, pale to mid-brown 30

30. Stipules scarious and brown ..... *C. stricta*
Stipules membranous 31

31. Sheath markedly hairy inside, female sepals usually narrowly linear ..... subgen. **Eriocephalina**
Sheath glabrous or with scattered adpressed hairs inside, female sepals usually ovate ..... ser. **Hirta**

32. Young stems glabrous, smooth ..... sect. **Glaucae**
Young stems hairy or minutely tuberculate 33

33. Leaves oblong to broadly elliptic, sometimes slightly sickle-shaped, filaments red and anthers yellow, achene covered by membranous layer ..... *C. apiculata*
Leaves needle-shaped, or if broader then other characters not as above 34

34. Leaves glabrous, usually glaucous, rarely green, elliptic to obovate or subcircular, if needle-shaped then margins inrolled beneath ..... sect. **Glaucae**
Leaves green, needle-shaped 35

35. Stems minutely tuberculate, underside of leaves minutely tuberculate and margins minutely serrate, stamens many, 20–25, female sepals erect, stigma red ..... *C. juniperina*
Stems smooth, underside of leaves various, stamens 6–18 36

36. Leaves curved downwards and out ..... sect. **Multiformae***
Leaves curved upwards and in 37

37. Leaves very fine, closely overlapping giving the branchlet a feathery appearance, stamens ± 6, filaments white, anthers yellow, stigma greenish white to pinkish, achene not tuberculate ..... *C. exilifolia*
Not as above 38

38. Leaves broad, 0.5–1.5 mm, thick, hard and rigid, margins rounded, bracteole hairy or not on keel ..... sect. **Castanae**
Leaves finer, <1 mm, flexible, margins flat, bracteole hairy on keel ..... sect. **Tuberculatae**

* also keying out here will be *C. homunculi* and certain forms of *C. atrata*.

Key to the species of *Cliffortia*, based on vegetative characters wherever possible, followed by achenes, female flower, male flowers and then growth form and distribution.

1. Leaves all unifoliate, although may be deeply divided 2
   At least some leaves trifoliate or bifoliate 5
2. Leaves wedge-shaped, broadest at the apex and toothed ..... *C. cuneata*
   Leaves various but not broadest at the very apex 3
3. Leaves single-nerved to base ..... Key C
   Leaves multinerved to base 4
4. Leaves forming brachyblasts ..... Key A
   Leaves not forming brachyblasts ..... Key B
5. Leaves bifoliate, or trifoliate and outer leaflets markedly larger than middle leaflet ..... Key D
   Leaves trifoliate, leaflets similar in size or middle larger than outer 6
6. Petiole present ..... Key E
   Petiole absent 7
7. Leaves hairy at least beneath, sometimes glabrescent especially above ..... Key F
   Leaves glabrous above and beneath, even in young leaves 8
8. Middle leaflet markedly toothed or lobed ..... Key G
   Middle leaflet entire 9
9. Young stems glabrous ..... Key H
   Young stems hairy 10
10 Sepals 4, stamens 4–8 ..... Key I
   Sepals 3, stamens 6–25 ..... Key J

Key A

(Leaves unifoliate, multinerved, forming brachyblasts)

1. Leaves long and broad, 10–50 mm × 5–15 mm 2
   Leaves shorter and narrower, 4–15 × 1–5 mm ..... 4 (*C. ruscifolia* complex)
2. Plants very tall, up to 4 m, leaves longer than 25 mm, deeply 2–6 toothed, receptacle and achene hairy ..... *C. phillipsii*
   Plants up to 1.5 m tall, leaves shorter than 30 mm, toothed or entire, receptacle and achene glabrous 3
3. Most leaves more or less entire ..... *C. integerrima*
   At least some leaves clearly toothed ..... *C. intermedia*
4. Leaves entire, flowers clustered in heads with modified reduced leaves ..... *C. ruscifolia*
   Flowers solitary at the base of ordinary vegetative leaves 5
5. Leaves ± glabrous, entire, narrowly lanceolate ..... *C. cymbifolia*
   Leaves hairy at least beneath, often toothed or deeply divided, broader and shorter 6
6. Achenes slightly tuberculate (Swartberg Mts) ..... *C. verrucosa*
   Achenes not tuberculate 7
7. Sheath hairy inside and out (Bokkeveld Escarpment) ..... *C. purpurea*
   Sheath glabrous outside and with only scattered hairs inside (Bot R valley) ..... *C. versiformis*

Key B

(Leaves unifoliate, multinerved, not forming brachyblasts)

1. Tall erect shrubs with very sparse branching, often clustered together at the nodes, leaves large, 50–80 mm long, or if shorter (>30 mm long) then over 25 mm wide, stamens numerous, 50–100 2
   Short to medium shrubs, if tall then leaves smaller, stamens many but usually < 50 5
2. Leaves narrow, 5–10 mm, with very long stipules, >10 mm, except for around the female flowers where they are shorter, broadly ovate, forming an elongated inflorescence and lack stipules ..... *C. heterophylla*
   Leaves broader, 15–40 mm, and not varying on the same plant in the above manner 3
3. Leaves very broadly ovate, 25–40 mm wide ..... *C. denticulata*
   Leaves narrower, 10–20 mm wide 4

4. Leaves very strongly recurved (Kogelberg Mts) ..... *C. recurvata*
Leaves bent downwards and out but not strongly (Langeberg Mts) ..... *C. grandifolia*
5. Tall erect shrub with dense regular horizontally held branches, leaves subcircular held perpendicular to stem, stamens numerous, 50–100 ..... *C. reniformis*
Not as above 6
6. Leaves and stems hairy ..... *C. strigosa*
Leaves and stems glabrous, rarely with a few sparse hairs in the midrib of the leaves above 7
7. Scrambling grass-like shrub, sheath very long, 6–45 mm long, stipules similar texture to leaf ..... *C. graminea*
Medium usually erect shrubs, sheath shorter, <6 mm long, stipules membranous or green 8
8. Leaves broadly ovate, 15–30 mm wide, clasping stem ..... *C. virgata*
Leaves oblong to lanceolate or narrowly triangular, 3-20 mm wide 9
9. Leaves lanceolate (20–35 × 3–7 mm), stipules 6–10 mm long with ciliate margins, anther connectives hairy ..... *C. lanceolata*
Leaves various, stipules shorter, <6 mm long, margins entire, anther connectives without hairs 10
10. Leaf margins entire without teeth 11
Leaves toothed 18
11. Leaves shortly lanceolate, 10–20 × 3–5 mm (Table Mt.) ..... *C. theodori-friesii*
Leaves larger 12
12. Leaves long lanceolate to narrowly oblong, 3–6× longer than wide 13
Leaves broadly ovate to broadly oblong or triangular 15
13. Leaves straight or curved slightly upwards, papery to stiff, 20–50 × 3–10 mm (Kogelberg Mts) ..... *C. serrata*
Leaves curved downwards and out, very stiff and hard (*C. dregeana* complex) 14
14. Plant reseeder; leaves nearly always untoothed (Cederberg & Olifants R Mts) ..... *C. acanthophylla*
Plant reseeder or resprouter; leaves often with teeth (Riviersonderend Mts, Franschhoek to Bain's Kloof Mts & Hex R Mts) ..... *C. dregeana*
15. Stigma usually red, long branches very short and almost forming brachyblasts (Cape Peninsula) 16
Stigma white, long branches normal 17
16. Leaves broadly ovate, never toothed ..... *C. integerrima*
Leaves long oblong, often toothed ..... *C. intermedia*
17. Leaves glaucous, 20–35 × 10–15 mm (Wemmershoek Mts) ..... *C. oligodonta*
Leaves green to glaucous, very variable but if untoothed then usually short and ovate and often clasping stem towards the base (Cape Peninsula or E of Ladismith & Riversdale) ..... *C. ilicifolia*
18. Teeth several to many, 10–50 excluding tip, leaves narrowly lanceolate ..... *C. serrata*
Teeth few, < 10 excluding tip, or if > leaves broadly oblong 19
19. Leaves broadly oblong with a rounded outline, teeth fine and short, < 2 mm long, membranous 20
Leaves narrowly lanceolate or if broad and oblong then teeth broad and incised giving an irregular outline, usually upright erect shrubs 21
20. Teeth 5–14, < 1.5 mm long, lower branches lax, stems scrambling ..... *C. ovalis*
Teeth <5, 1.5–2 mm long, plants erect ..... *C. oligodonta*
21. Leaves short and broad, 9–15 × 5–15 mm, stigma white, often tall shrubs (Cape Peninsula or E of Ladismith & Riversdale) ..... *C. ilicifolia*
Leaves generally longer and narrower, 12–40 × 3–10 mm, low to medium shrubs (Riviersonderend Mts to Cederberg & Olifants R Mts, excluding Cape Peninsula) 22
22. Leaves narrowly lanceolate, 3–5× as long as wide, teeth fine to slightly incised, stigma white (*C. dregeana* complex) 23
Leaves broadly lanceolate, 2–3× as long as wide, teeth broad and deeply incised, stigma red (always?) 24
23. Plant reseeder; leaves nearly always untoothed (Cederberg & Olifants R Mts) ..... *C. acanthophylla*
Plant reseeder or resprouter; leaves often with teeth (Riviersonderend Mts, Franschhoek to Bain's Kloof Mts & Hex R Mts) ..... *C. dregeana*
24. Stipules short, < 3 mm. long, filaments white, anthers yellow (Hex R Mts, Winterhoek Mts & Koue Bokkeveld) ..... *C. ceresana*

Stipules long, 3–7 mm long, filaments red, anthers reddish brown (Riviersonderend Mts) ..... *C. meyeriana*

Key C

(Leaves unifoliate, single nerved)

1. Leaf margins entire to serrulate 2
   Leaf margins markedly toothed 19
2. Stems glabrous 3
   Stems hairy 13
3. Leaf width < 2 mm wide 4
   Leaf width > 2 mm wide 11
4. Leaves < 5 mm long, underside whitish and margins inrolled beneath so that a single white line is visible, sepals 4 5
   Leaves usually > 5 mm long, more or less the same colour above and beneath, sepals 3 6
5. Leaves 1.5-3 mm long, lower branches decumbent but stems ascending ..... *C. brevifolia*
   Leaves 2.5–5 mm long, plants erect ..... *C. ericifolia*
6. Leaves < 11 mm long, stamens ± 6(–9), usually growing in well-drained areas 7
   Leaves 9–50 mm long, stamens 12–25, growing in wet areas 9
7. Plants tall, usually 100–250 cm high, young stems slender, < 0.7 mm wide, female sepals erect ..... *C. erectisepala*
   Plants shorter, usually < 50 cm high, young stems thicker, 0.7–1.1 mm wide, female sepals spreading to recurved 8
8. Leaves hard and rigid, 0.5–1 mm thick, curved downwards and away from the stem (Western Cape) ..... *C. pungens*
   Leaves < 0.5 mm thick, curved upwards and towards the stem (Eastern Cape to Mpumalanga) ..... *C. repens*
9. Leaves < 1 mm thick, often curved downwards at tip to form a small hook, sheath < 2 mm long, stipule margins ciliate (Groot Winterhoek Mts to Cederberg) ..... *C. uncinata*
   Leaves 1–2 mm thick, straight or curved upwards and towards the stem, sheath > 2 mm long, stipule margins entire (Swartberg Mts) 10
10. Leaves more or less terete, stamens 19–24 ..... *C. aculeata*
    Leaves flattened vertically, thicker than wide, stamens 12–18 ..... *C. nivenioides*
11. Leaves hard and rigid, < 30 mm long, curved downwards and away from the stem, plants scrambling, growing in dry areas (Bokkeveld Escarpment) ..... *C. acutifolia*
    Leaves flexible, 25–65 mm long, straight or curved upwards and towards the stem, plants erect, growing in water 12
12. Leaves 30–65 × 4–8 mm, stamens 25–30 ..... *C. longifolia*
    Leaves 25–45 × 1.5–5 mm, stamens 12–18 ..... *C. strobilifera*
13. Leaves glabrous above and beneath, sheath also glabrous on abaxial side 14
    Leaves with hairs above and beneath, sometimes glabrescent but then still hairs on the abaxial side of the sheath 16
14. Leaf margins thickened or inrolled and touching thickened midrib, pale lines present on either side of midrib beneath, curved downwards and away from the stem, sepals 4 ..... *C. linearifolia*
    Leaf margins rounded or flat, leaves uniform in colour, curved upwards and towards the stem, sepals 3 15
15. Leaves hard, usually > 0.5 mm thick, stigma > 3 mm long (Western Cape) ..... *C. neglecta*
    Leaves flexible, < 0.5 mm thick, stigma < 3 mm long (Eastern Cape to Mpumalanga) ..... *C. repens*
16. Leaves 2–4 × 0.5–1 mm, large tree up to 400 cm high, inflorescence a cone-like structure (Bokkeveld Escarpment) ..... *C. dichotoma*
    Leaves 3–15 × 1–5 mm, shrubs under 150 cm high 17
17. Leaves ovate to almost subcircular, margins flat, sepals 4 ..... *C. monophylla*
    Leaves elliptic to oblong or linear, margins inrolled beneath, sepals 3 18
18. Erect shrub, leaves unifoliate to trifoliate, bracteoles 2–5 mm long, stamens ± 6 (SE Cape Mts) ..... *C. dispar*

Sprawling to scrambling shrub, leaves always unifoliate, bracteoles < 2 mm long, stamens ± 12 (Hex R Mts) ..... *C. esterhuyseniae*

19. Leaves 10–55 mm wide, base cordate, often with a short petiole 20
Leaves < 15 mm wide, base tapered to sheath, petiole always absent 24

20. Petiole absent, leaves discolorous, stamens 18–32, filaments 7–15 mm long ..... *C. hirsuta*
Petiole present, leaves usually the same colour above and beneath, but if discolorous then stamens < 18 and filaments shorter than 7 mm 21

21. Young stems thin, usually < 1 mm wide, leaves soft and herbaceous, stamens 20–30, filaments white with yellow anthers ..... *C. pilifera*
Young stems thicker, > 1 mm wide, leaves thicker and more rigid, stamens < 22, filaments usually reddish with yellow anthers 22

22. Leaves elliptic to ovate, glabrous or with only a few hairs on the midrib beneath (Kogelberg Mts) ..... *C. viridis*
Leaves ovate to subcircular, rarely elliptic, hairy especially beneath or sometimes only with some hairs on the midrib 23

23. Leaves 20–65 × 15–55 mm, stipules 6–10 mm long, stamens 12–18 ..... *C. odorata*
Leaves 10–35 × 10–25 mm, stipules < 6 mm long, stamens 18–22 (Groot Winterhoek Mts) ..... *C. reticulata*

24. Leaves and stems distinctly hairy, leaves 3–10 mm long ..... *C. tricuspidata*
Leaves and stems glabrous or with scattered hairs, leaves 7–50 mm long 25

25. Leaves with 10–50 recurved teeth, if fewer then < 20 stamens, anthers yellow 26
Leaves with < 10 straight teeth, stamens 20–40, anthers brownish red 27

26. Plants erect, stamens 17–21 (Brandfontein near Cape Agulhas) ..... *C. berberidifolia*
Plants sprawling or scrambling, stamens 10–16 ..... *C. ferruginea*

27. Leaves 3–6 mm wide, stipules ciliate, < 7 mm long, bracteoles hairy, anther connective with short hairs (Bokkeveld Escarpment) ..... *C. acutifolia*
Leaves, 2–4 mm wide, stipules entire, > 9 mm long, bracteoles glabrous, anther connective glabrous (Riviersonderend Mts) ..... *C. scandens*

Key D

(Leaves bifoliate or outer leaflets markedly larger than middle one)

1. Middle leaflet always present, 3–6 mm long, notched at apex ..... *C. obcordata*
Middle leaflet absent, reduced to a small point or variable across the plant and sometimes fused to one of the outer leaflets 2

2. Stem glabrous, outer leaflets glaucous, middle leaflet reduced to a small point, achenes with 3 wings ..... *C. phyllanthoides*
Stems hairy or glabrous, middle leaflet absent or present but then large and variable, achenes indistinctly ribbed 3

3. Middle leaflet absent, outer leaflets large, 7–20 mm wide, margins often with small teeth, bracteoles glabrous ..... *C. crenata*
Middle leaflet absent or present, outer leaflets < 7 mm wide, margins usually entire or minutely serrulate, bracteoles often hairy 4

4. Stems hairy, middle leaflet always absent 5
Stems glabrous, middle leaflet sometimes present or fused to one of the outer leaflets 6

5. Leaves glaucous, stipules ciliate ..... *C. crenulata*
Leaves green, stipules enitre and glabrous ..... *C. pulchella*

6. Leaves obovate to oblong, apex broadly acute to acuminate, bracteoles glabrous ..... *C. mirabilis*
Leaves broadly obovate, apex rounded to acute, bracteoles sometimes hairy ..... *C. varians*

Key E

(Leaves trifoliate, petiole present)

1. Stems glabrous 2
Stems hairy 5

2. Leaves needle-shaped, flowers sessile 3
Leaves broader, usually distinctly toothed, flowers on a peduncle 4

3. Leaves very short, 3–4 mm long (Paarl to Klapmuts) ..... *C. acocksii*
Leaves longer, 6–17 mm long ..... *C. filifolia*

4. Plants clonally spreading and resprouting after fire, leaves 9–12 × 6–9 mm, distinctly 3-lobed at apex, peduncle short, < 2 mm long ..... *C. triloba*
Plants being killed by fire, leaves < 6 mm wide, or if wider then much longer than 12 mm, toothed or 3-lobed at apex, peduncle longer, 2–20 mm long 5
5. Leaves 14–19 × 1.5–5 mm, toothed or 3-lobed, stamens 15–19 (altitude 1000–2000 m) ..... *C. lepida*
Leaves 10–45 × 3–13 mm, toothed, stamens 20–43 (altitude <1100 m) ..... *C. pedunculata*
6. Leaves hairy, at least beneath, middle leaflet broader and more lobed than outer ones, sepals 4 7
Leaves glabrous all over, all leaflets similar in shape and size but outer ones often sickle-shaped, sepals 3 9
7. All three leaflets lobed, stems tuberculate, flowers not present, stems often minutely tuberculate ..... *C. nitidula subsp. pilosa* (juvenile leaved form)
Middle leaflet lobed but outer leaflets not, flowers can be present, stems hairy but not tuberculate 8
8. Plants semi-erect, branches ascending, < 1 mm long (Western Cape) ..... *C. filicaulis*
Plants decumbent, petiole very short to sessile, petiole 1–2 mm long, persistent (Drakensberg Mts) ..... *C. filicauloides*
9. Leaves broad and oblong, 10–14 × 2.5–4.5 mm ..... *C. drepanoides*
Leaves smaller, 2.5–9 × 0.5–2 mm 10
10. Leaves often 1–2 mm wide, apex rounded to acute, stamens 16–21 (usually coastal, altitude <500 m, often on recent sands or limestone derived soils) ..... *C. falcata*
Leaves usually < 1 mm wide, tips rounded to sharply acuminate, stamens 6–9 (rarely coastal, altitude up to 2400 m, never on recent sands or limestone derived soils) 11
11. Plants sparsely branched, with a long main stem and only a few side-branches (excluding brachyblasts), unable to support itself upright (Agulhas Plain) ..... *C. perpendicula*
Plants densely branched, erect 12
12. Leaves 4–9 mm long, apex sharply acuminate, petiole up to 2 mm long, spreading clonally and resprouting after fire ..... *C. arcuata*
Leaves 2–7 mm long, apex rounded to acute, petiole < 1 mm long, only surviving fire as seed ..... *C. ramosissima*

Key F

(Leaves trifoliate, leaves hairy at least beneath)

1. Margins inrolled beneath 2
Margins flat 14
2. Plants tall, tree-like, leaves 12–20 mm long, inflorescence persistent and woody 3
Plants generally much smaller, leaves 2–20 mm long, not forming an inflorescence 5
3. Leaves markedly toothed, inflorescence a cone on a determinate branch (Anysberg) ..... *C. conifera*
Leaves not toothed, inflorescence (when known) a woody swelling along the main axis of the stem 4
4. Leaves curved downwards and away from the stem (Hantamsberg to Beaufort West) ..... *C. arborea*
Leaves straight to curved upwards and towards the stem (Willowmore Witteberg) ..... *C. sp. cf. arborea*
5. Leaves < 1 mm wide, margins tightly rolled, achenes large, 4–10 × 4–10 mm, reddish, broadly winged 6
Leaves 0.5–5 mm wide, achenes smaller, 1.5–4 × 0.5–2 mm, not winged 7
6. Leaves greyish hairy above, achenes 4–6 mm long (N slopes of Langeberg) ..... *C. alata*
Leaves green and glabrous above, achenes 6–10 mm long (De Hoop Nature Reserve) ..... *C. burgersii*
7. Stems often minutely tuberculate, leaves 3–7 × 0.5–1.5 mm, glabrescent, apex rounded, sheath glabrous abaxially, sepals 4 (Amatola Mts to Mpumalanga) ..... *C. nitidula* subsp. *pilosa*

Stems not tuberculate, leaves various but sheath always with a few hairs, sepals 3 (Cape Floristic Region and Graaf Reinet Mts to Amatola Mts) 8

8. Stipules easily visible, brown and scarious, stamens 6, achene ± 6-ribbed (S coast and mountains, altitude < 1300 m) ..... *C. stricta*

Stipules if visible, membranous to green, stamens 6–30, achene 6–26-ribbed 9

9. Leaves 9–19 × 0.5–1.5 mm (altitude < 350 m) 10

Leaves 2–9 mm long, if longer then 1–5 mm wide (altitude 600–2400 m) 11

10. Leaves hairy above, sheath with a small reddish point at the insertion of the leaflets on the abaxial side, stamens 22–30 (Cape Flats) ..... *C. hirta*

Leaves glabrous above, sheath without small point, stamens 8–10 (De Hoop Nature Reserve) ..... *C. incana*

11. Plants killed by fire, sometimes very tall with a single main stem, up to 2 m, leaves 2–4 × 0.5–1 mm, stamens ± 6 (Swartberg and Graaf Reinet Mts) ..... *C. montana*

Leaves 4–15 mm long, if shorter then plants spreading clonally and resprouting after fire, stamens 6–12 12

12. Plants killed by fire, leaves 3–15 × 1–5 mm, sometimes both unifoliate and trifoliate on same plant, curved downwards and away from stem, stamens ± 6 (SE Cape Mts) ..... *C. dispar*

Plants spreading clonally and resprouting after fire, leaves < 2 mm wide, straight or curved upwards and towards stem, stamens 6–12 13

13. Leaves broadly elliptic to linear, 2–6 mm long, apex obtuse to rounded, stamens 6–12 ..... *C. eriocephalina*

Leaves narrowly linear, 6–9 mm long, stiff, apex acute, stamens 9–12 (Hex R Mts to Groot Winterhoek Mts) ..... *C. weimarckii*

14. Middle leaflet markedly toothed or lobed 15

Leaflet margins entire or minutely serrate 16

15. Plants decumbent with ascending stems, leaves 3–7 mm long, stipule large and leaf-like, 3–6 mm long, sepals 4, achene unribbed ..... *C. filicaulis*

Plants erect, leaves 4–11 mm long, stipules membranous or green, 0.5–3 mm long, sepals 3, achene 3–6-winged, wings recurved ..... *C. polygonifolia* var. *trifoliata*

16. Leaves needle-shaped to linear, 6–16 × 1–1.5 mm, glabrous or almost so above, apex acute to sharply acuminate 17

Leaves smaller, < 9 mm long, < 1 mm wide, apex rounded to acute 19

17. Hairs only found on midrib beneath, achene with rounded ribs (Cape Peninsula) ..... *C. dodecandra*

Hairs if present all over underside of the leaf, achenes shallowly winged or with toothed ribs (SW Cape Mts) 18

18. Leaves evenly hairy beneath, wings shallow and toothed (Hex R Mts to Villiersdorp) ..... *C. cristata*

Leaves almost glabrous, stiff, wings well-developed and recurved (du Toits Kloof) ..... *C. subdura*

19. Brachyblasts closely overlapping, leaves ± glabrous except for a few hairs beneath, bracteoles glabrous (Gansbaai area) ..... *C. anthospermoides*

Leaves hairier, bracteoles hairy 20

20. Leaves oblong to elliptic, 3–7 × 0.5–1 mm, straight or curved downwards and away from the stem, stipules absent, brachyblasts closely overlapping (Graaf Reinet Mts) ..... *C. bolusii*

Leaves usually straight or curved upwards and towards the stem, stipules present 21

21. Leaves 2–4 × 1–2 mm, stipules green and similar texture to leaves, sepals 4, achene unribbed (altitude 1200–1700 m) ..... *C. hantamensis*

Leaves 3–9 × 0.5–4 mm, stipules membranous green, sepals 3, achene sharply ribbed or with recurved wings 22

22. Leaves 6–9 × 0.5–1 mm, green, stamens 10–12 (SW Mts) ..... *C. pubescens*

Leaves 3–7 × 0.5–4 mm, green to silvery hairy, stamens < 10 23

23. Leaves 3–6 × 0.5–1 mm, green, stamens ± 6 (Groot Winterhoek Mts to Gifberg) ..... *C. hexandra*

Leaves 3–7 × 1–4 mm, green to silvery hairy or woolly, stamens 6–9 24

24. Leaves 3–5 × ± 1 mm, densely white woolly (upper Breede R valley) ..... *C. lanata*

Leaves not densely white woolly 25

25. Leaves 3–7 × 1–4 mm, green with coarse hairs ..... *C. polygonifolia*

Leaves 3–7 × 1–2 mm, densely silky hairy ..... *C. sericea*

Key G

(Leaves trifoliate, glabrous, petiole absent, middle leaflet toothed or lobed)

1. Middle leaflet bilobed, emarginate at the apex, glaucous, stems hairy on one side only, sepals 3 ..... *C. obcordata*
   Middle leaflet toothed to 3–5-lobed, stems hairy all round, sepals 4 2
2. Plants trailing, stems decumbent, hairs downwards pointing, leaves thin and membranous, not forming obvious brachyblasts, male sepals with attentuate tips 3
   Plants erect or sprawling, but at least upper branches ascending, stem hairs curled, spreading or upwards pointing, leaves thicker, forming brachyblasts, male sepals acute to acuminate but not long and attenuate .....
3. Leaves 3–6 mm long, 3-lobed (Langeberg and Waboomsberg Mts) ..... *C. gracillima*
   Leaves 4–10 mm long, 3–7-toothed or lobed (SW Mts) .....
4. Leaves 3–7-lobed, middle lobe broader than outer lobes, 1–2 mm wide (Wemmershoek Mts to Helderberg and Cape Peninsula) ..... *C. dentata*
   Leaves 3-lobed, middle lobe narrower than outer lobes, 0.5–1 mm wide (du Toits Kloof Mts) ..... *C. gracilis*
5. Leaves finely divided and feathery, each lobe needle-like and < 0.5 mm wide ..... *C. cervicornu*
   Leaves broader, 1–4 mm wide 6
6. Leaves very small, 1–2.5 × 0.5–1.5 mm, green, bent downwards and away from the stem, stem hairs curled, achene < 2 mm long (Klein Karroo Mts) ..... *C. micrantha*
   Leaves larger, 2.5–6 × 1–4 mm, usually somewhat glaucous, bent upwards and towards the stem, stems adpressed upwards towards the stem, achene 2–4 mm long 7
7. Leaves entire to 3-lobed or toothed, female flowers with bracteoles 0.5–2 mm long and sepals 0.5–1 mm long, carpels usually 2 (Western Cape) ..... *C. propinqua*
   Leaves 3–7-lobed or toothed, female flowers larger with bracteoles 2–3 mm long and sepals 1.5–2.5 mm long, carpels always 1 (Drakensberg Mts) ..... *C. spathulata*

Key H

(Leaves trifoliate, glabrous, petiole absent, stems glabrous)

1. Margins inrolled beneath (E Cape Mts) ..... *C. polita*
   Margins rounded or flat 2
2. Leaves needle-shaped, green, stigma white to red, sometimes hidden by leaves 3
   Leaves flat, ovate to oblong or obovate, usually glaucous, stigma always red and protruding above leaves ..... 10 (*C. glauca* complex)
3. Leaves very long, 12–22 mm, achenes large, 5–7 × 3–4 mm, not winged ..... *C. burchellii*
   Leaves much shorter, < 11 mm long, achenes smaller, < 5 mm long, 3 mm wide 4
4. Leaves green, 4–11 mm long, stamens 9–25, filaments and anthers red or white to yellow, achenes either > 3 mm long or winged and > 2 mm wide 5
   Leaves glaucous, usually < 4 mm long, sometimes up to 7 mm long, stamens 6–12, filaments and anthers reddish brown, achenes < 3 mm long and < 2 mm wide 7
5. Leaves strongly curved upwards and towards the stem, green with faint whitish lines on each margin, stigma white, very short, < 3 mm long, achene narrowly cylindrical, elongating as it matures ..... *C. densa*
   Leaves straight or only slightly curved, green all round, stigma red, 1.5–10 mm long, achene broadly ellipsoid or winged 6
6. Plants low, rarely more than 50 cm high, stem and leaves minutely tuberculate, leaf tips rounded to acute, sepals without a small spine, achene broadly ellipsoid with 6 ribs, often rugose between the ribs ..... *C. juniperina*
   Plants taller, up to 150 cm high, young stems smooth, leaf tips acuminate, sepals with a small spine just beneath apex, achene reddish, variously winged ..... *C. teretifolia*
7. Leaves 4–7 mm long, female sepals 2–3 mm long ..... *C. semiteres*
   Leaves < 4 mm long, female sepals < 2 mm long 8

8. Leaflets ± cylindrical, margins rounded (arid inland mountains, altitude 300–1700 m) ..... *C. amplexistipula*
Leaflets flatter, margins often semi-translucent (coastal plains, altitude < 200 m) 9
9. Bracteoles glabrous, achenes ribbed but not tuberculate, stamens ± 12 (Cape Flats) ..... *C. marginata*
Bracteoles hairy, achenes usually tuberculate, stamens ± 6 (Agulhas plain) ..... *C. tenuis*
10. Leaves 4–10 × 2.5–10 mm, middle leaflet often fused with outer leaflets or one leaflet missing ..... Key D(6)
Leaves < 9 mm long, < 4 mm wide, leaves usually trifoliate throughout the plant 11
11. Leaf tip acute to acuminate or mucronate 12
Leaf tip rounded to acute 15
12. Leaves oblong 13
Leaves ovate to obovate 14
13. Leaves oblong, 1.5–2 mm wide, green, sometimes fused together (Franschhoek valley) ..... *C. rigida*
Leaves narrowly oblong, 1–1.5 mm wide, glaucous (Hex R Valley & Anysberg) ..... *C. semiteres*
14. Leaves small, 3–5 × 1–1.5 (Rooiberg Mt) ..... *C. concinna*
Leaves larger, 6–8 × 3–4 (Langeberg Mts) ..... *C. glauca*
15. Leaves obovate (SW Cape Mts) ..... *C. obovata*
Leaves oblong to ellipsoid 16
16. E Cape Mts, altitude 550–1650 m ..... *C. polita*
Cape Flats to Agulhas Plain, altitude < 200 m 9

Key I

(Leaves trifoliate, glabrous, petiole absent, middle leaflet entire, stems hairy, sepals 4)

1. Leaf margin inrolled beneath 2
Leaf margin flat or rounded 3
2. Midrib thickened so that margins and midrib are touching, always glabrous ..... *C. linearifolia*
Midrib not so thickened, lamina of leaf usually visible beneath, sometimes with hairs ..... *C. nitidula*
3. Leaflets obovate to elliptic, 0.5–4 mm wide 4
Leaflets needle-shaped, < 1 mm wide 8
4. Plants usually small <50 cm high, in shady rock crevices, rarely on open slopes when taller, achenes reddish, hairy, clearly flattened to winged (N Langeberg westwards) 5
Plants usually taller, on open slopes or forest margins, achenes smooth to ribbed, cylindrical and not flattened, glabrous (S Langeberg to Mpumalanga & ?Zimbabwe) 6
5. Leaflets narrowly obovate to elliptic, gradually tapered to base, never toothed, carpels 1 or 2 (Kogelberg to Bain's Kloof) ..... *C. complanata*
Leaflets broadly obovate, abruptly tapered into a pseudopetiole, often toothed, carpels usually 2 (Bain's Kloof to Cederberg and N Langeberg) ..... *C. propinqua*
6. Leaves glaucous, apex rounded, juvenile leaves entire, bracteole margins smooth, 2–2.5 mm long, achene 3–4 mm long (Drakensberg, altitude 2000–3100 m) ..... *C. dracomontana*
Leaves green, juvenile leaves toothed, bracteole margins ciliate, 1–2 mm long, achene 2–3 mm long (Western Cape to Mpumalanga & ?Zimbabwe) 7
7. Leaves elliptic, 2–4 mm long, apex acute, receptacle smooth, achene smooth or faintly ribbed (altitude 1000–2500 m) ..... *C. browniana*
Leaves elliptic to obovate, 3–5 mm long, apex acute to rounded, receptacle and achene clearly ribbed (altitude 0–1500 m) ..... *C. serpyllifolia*
8. Leaves 6–11 mm long, apex acuminate to pungent, 0.5–1 mm long, margins minutely serrulate, stipules 1.5–2.5 mm long 9
Leaves 2–8 mm long, apex rounded to long acuminate, usually < 0.5 mm long, margins ± smooth, stipules < 1.5 mm long 10
9. Leaves curved downwards and away from the stem, 0.5–1 mm wide (Suurberg Mts to Kwazulu-Natal) ..... *C. paucistaminea*

Leaves curved upwards and towards the stem, < 0.5 mm wide (George to Uitenhage) ..... *C. paucistaminea* var. *australis*

10. Plants tall, up to 2 m high, leaves with thickened margins and midrib, varying from curved downwards and away from stem to straight, stipules ciliate (Western Cape to Zimbabwe) ..... *C. linearifolia*
Plants usually less than 50 cm high, leaves without thickened margins, midrib occasionally thickened, always distinctly curved upwards and towards the stem, stipules glabrous (Western Cape) 11
11. Achenes 4 or 8-ribbed (SW Cape) 12
Achenes 9–18-ribbed (Swartberg Mts) 13
12. Leaves 2–4 mm long, brachyblasts not closely overlapping, achene 4-ribbed, cylindrical, ribs evenly spaced, 3.5–5 mm long (Riviersonderend Mts) ..... *C. cruciata*
Leaves 4–8 mm long, brachyblasts closely overlapping giving a feathery appearance to branches, achene 4 or 8-ribbed, slightly compressed with two of the ribs curved laterally, 2.5–3.5 mm long (Cape Peninsula to Cape Agulhas) ..... *C. subsetacea*
13. Leaves 0.5–1 mm wide, with thickened midrib, brachyblasts not closely overlapping, achene 3–4 mm long ..... *C. crassinervis*
Leaves < 0.5 mm wide, without distinct midrib, brachyblasts closely overlapping giving a feathery appearance to branches, achene 2–3 mm long ..... *C. setifolia*

Key J

(Leaves trifoliate, glabrous, petiole absent, middle leaflet entire, stems hairy, sepals 3)

1. Leaf margins curved inwards beneath, thus making an ovate to elliptic leaf appear needle-shaped 2
Leaves either needle-shaped or ovate to obovate or elliptic, margins always flat or rounded 3
2. Bracteole hairy, achene 1.5–3 mm long (Langeberg Mts) ..... *C. longimontana*
Bracteole glabrous, achene 3–4 mm long (mountains from Oudtshoorn to Uitenhage) ..... *C. polita*
3. Leaves ovate to obovate or elliptic, often glaucous in colour 4
Leaves needle-shaped to narrowly lanceolate, green 10
4. Leaves 7–10 × 1–1.5 mm, margins minutely serrulate, bracteoles hairy, 4–5 mm long, achene with incurved wings (du Toits Kloof) ..... *C. subdura*
Leaves various, margins smooth, bracteoles < 3 mm long or if longer then glabrous except sometimes ciliate on the margin, achene shallowly ribbed to smooth 5
5. Leaves oblong to elliptic, 6–12 × 1.5–3 mm, stipules ciliate, bracteoles 5–7 mm long, achene covered with membranous layer ..... *C. apiculata*
Leaves various, stipules usually smooth, bracteoles <3 mm long, achene not covered with membranous layer 6
6. Leaves obovate, apex obtuse to rounded, achene reddish, flattened and winged, hairy, often growing in shady rock crevices ..... *C. complanata*
Leaves various but apex usually acute to apiculate, achene greyish brown, cylindrical shallowly ribbed, glabrous 7
7. Leaves green, rarely glaucous (altitude 400–1700 m) 8
Leaves glaucous (altitude 0–500 m) 9
8. Leaves oblong to elliptic, apex sharply acute (Franschhoek Mts) ..... *C. rigida*
Leaves elliptic to obovate, apex obtuse to acute (Langeberg to Eastern Cape) 2
9. Stamens 12–15 (Cape Peninsula) ..... *C. carinata*
Stamens 6 (Onrus R Mts & Klein R Mts) ..... *C. geniculata*
10. Leaves 2–3 mm long, achene a small orange fleshy berry ..... *C. baccans*
Leaves 3–16 mm long, achene a dry achene 11
11. Leaves strongly curved upwards and towards the stem, 10–16 mm long, with a raised midrib, achene very long and curved, 10–13 mm long (Agulhas Plain) ..... *C. curvifolia*

Leaves not curved strongly upwards, or if so then shorter, < 13 mm long, and midrib not raised or indistinguishable from lamina, achene much shorter, < 5 mm long 12

12. Stems minutely tuberculate, female sepals erect, stigma red, stamens 20–25 13
Stems smooth, female sepals erect to recurved, stigma white to red, stamens 6–18 14

13. Leaves 4–9 mm long, various curved but never strongly so, achenes sometimes rugose between ribs ..... *C. juniperina*
Leaves 8–12 mm long, bent clearly downwards and away from stem, achenes never rugose between ribs ..... *C. pilosula*

14. Leaves 8–15 × 1–4 mm, always bent downwards and away from stem (Cape Peninsula to Elim) 15
Leaves 4–13 × < 1 mm wide, variously curved 17

15. Leaves often fused, needle-shaped to broadly triangular, 8–10 × 1.5–3.5 mm, filaments 8–11 mm long ..... *C. versiformis*
Leaves fused or not, needle-shaped to linear, 8–15 × 1–2 mm, filaments 5–8 mm long 16

16. Leaves always trifoliate, plants usually killed by fire (Cape Peninsula & ?Agulhas coast) ..... *C. dodecandra*
Leaflets variously fused or not, plants clonally spreading and resprouting after fire (Hermanus to Elim) ..... *C. multiformis*

17. Leaves very fine, < 0.5 mm wide, upwardly curved and closely overlapping giving a feathery appearance to the branch, receptacle smooth, stamens 6, filaments and anthers white or cream, achene smooth or very faintly ribbed (altitude 900–1600 m, S facing steep slopes often in shelter of rocks) ..... *C. exilifolia*
Leaves broader, 0.5–1 mm wide, if branches appear feathery then achene clearly ribbed or tuberculate 18

18. Leaves almost cylindrical with smooth margins, upwardly curved towards the stem, receptacle smooth, plant killed by fire, achene narrowly cylindrical, 0.5–1 mm wide, reddish brown in colour, faintly 6-ribbed (altitude 1000–1900 m, shady kloofs in arid mountains from Hex R Mts to Baviaanskloof Mts) ..... *C. castanea*
Leaves variously curved, margins minutely serrulate or angled, achene 1–2.5 mm wide, clearly ribbed or tuberculate 19

19. Plants resprouting from a crown after fire, achenes tuberculate (often growing between rocks) ..... *C. tuberculata*
Plants resprouting or not after a fire, achenes never tuberculate 20

20. Plants killed by fire, achenes broadly ovoid, dark reddish brown, 12–25-ribbed with occasional cross-ribs (altitude 0–1700 m) ..... *C. atrata*
Plants resprouting after fire, achenes narrowly ovoid to cylindrical, pale to dark brown, 6–12-ribbed 21

21. Leaf apex rounded to acute, plants resprouting from a crown, bracteoles glabrous except for margin, stigma red, stamens 11–18, achene ± 6-ribbed (SW Mts) ..... *C. pterocarpa*
Leaf apex long acuminate, plants clonally spreading or not, bracteoles hairy, stigma white to pinkish, stamens 4–6 where known, achenes ± 9-ribbed (George to Drakensberg) 22

22. Achenes pale brown, 2.5–3.5 × 1–1.5 mm, resprouting leaves trifoliate (see Key I, 9) ..... *C. paucistaminea*
Achenes brown, 3–5 × 1.5–2 mm, resprouting leaves unifoliate and toothed (Kammanassie Mts) ..... *C. ×homunculi*

Appendix 3: Character list for DELTA database and morphological matrix

Those marked with an asterix (*) indicate multistate characters used in the Nexus file for phylogenetic analysis of the morphology; all such characters were regarded as unordered for the analysis. Single diagnostic characters for species, which are hence autapomorphic, were excluded from the morphological matrix.

Growth form:

Along with leaf shape and size, the most striking characteristic of the genus *Cliffortia* is the range of growth forms that it encompasses. Ten characters were used in an attempt to try to categorise the growth forms. Only a single character, fire survival strategy, could be described as discrete and even here there are difficulties in classifying it. The others characters are very subjective: the extremes are distinctive but there is little justification in where the boundaries are drawn, as the data cannot easily be quantified. Most of these characters need field studies for verification, and only characters 5–8 can tentatively be determined from dried herbarium material. A further two characters were used to define plant size.

1*. Fire survival strategy of plants

1. plant only surviving fire as seed
2. plant resprouting after fire

A species that does not sprout again after a fire must use a seed bank, whether in the ground or aerial, to survive to the next generation. Directly opposed to this are those species that are not only able to regenerate via seed but are able to send up shoots again from the soil. The boundary is blurred slightly by the facultative resprouters, such as *C. ruscifolia*, where the intensity of the fire affects whether they can resprout or not.

The character can only be reliably determined soon after a fire. Leaves of resprouting shrubs appear soon after the first good rains of winter. Seedlings generally do not appear until the spring and the first true leaves will not be discernible until a while after that. The best evidence that a species does or does not resprout is to find the border of the burn where unburnt plants and old burnt stumps can be seen.

On mature plants an educated guess can be made, but unfortunately branching at the base of the plant is not always indicative of it having resprouted as non-sprouting species are often stunted so that they do not have a clear single stem; nor is a clear single stem always indicative of a non-resprouting species as those that spread clonally often produce just a single stem when they resprout.

2*. Rootstock

1. thick caudex or lignotuber
2. clonally spreading via underground horizontal root system

A difficult character to determine correctly but important for a better understanding of the switch between seeding and resprouting life history in *Cliffortia*. More intensive study of the life-cycle of many *Cliffortia* species over time is needed. A discovery of this research has been the predominance of a clonally spreading root system as a major means of vegetative reproduction. Most species that resprout, upon closer examination of their rootstock, have been shown to have this ability. The root system is comprised of a main taproot from which branches come off parallel with the soil surface, some of these roots continue to grow and when they eventually break the soil surface will produce a new plantlet.

Even without digging up the plants, clonality is often very evident. Frequently a species will be common or even dominant in the vegetation for 100 m or so diameter, but be absent from the surrounding vegetation, despite any evidence of habitat change. This is almost invariably due to the spread of a single plant via these underground roots from a presumed point source. However, some species have a tufted appearance, as from a lignotuber, and do not resort to spreading underground. These species tend to grow in habitats protected from fire or with less intense fires. Therefore, these species might be better regarded as facultative resprouters; i.e. reseeders that are able to survive low intensity fires.

3*. Monopodial growth form

1. single main stem with determinate length side branches
2. without a clearly defined main stem

A few of the taller species develop a long main stem and only determinate shorter side branches, these are referred to as monopodial; *C. phillipsii* is the best example of this unusual habit often reaching 4 m tall but rarely more than a metre across.

4*. Lower branches or stem rigidity

1. erect or rigid
2. decumbent to lax

Several species have lower stems that do not support the plant. The lower branches are then decumbent or lie laxly upon the ground, and then the plants can often be prostrate even if the ends of the branches ascend (e.g. *C. filicaulis* and *C. monophylla*). However, most species have erect or rigid stems and the branches are held upright from the base, so that even if they may spread outwards at the bottom they do not lie on the ground in need of support. Older plants which have a single stem can often become prostrate with age but this is due to the weight of the branches they support or the vegetation around them and they should not be regarded as decumbent.

5*. Lower branches rooting

1. sometimes rooting where touching ground
2. not rooting

A character of dubious status as it appears to be closely linked to habitat type and is naturally dependent upon the last character. For a plant to produce adventitious roots it will need constantly moist conditions at the soil surface to encourage their growth. For species that grow in these habitats it is sometimes very difficult to determine whether species have spread horizontally due to underground roots or by rooting above ground where they touch.

6*. Upper branches rigidity

1. erect or ascending
2. scrambling amongst vegetation
3. decumbent

Only a few species can be described as having decumbent upper parts to their branches and this identifies the mat forming or trailing species. Many of the species that have lax lower parts to their stems scramble through vegetation if they have the chance, often aided by their downward pointing leaves and recurved teeth (e.g. *C. ferruginea*). All species that have erect lower branches naturally have erect upper branches too.

7*. Brachyblasts

1. branches forming brachyblasts
2. branches only forming long branches

Growth form is very closely linked to the branching phenology. Much preliminary research was done into this by Fellingham (1999), but the techniques she used required long-term monitoring of the species concerned. This was outside the scope of this project and so branching pattern has been reduced to a simple differentiation between those species that only produce long branches and those that produce short shoots (here after termed brachyblasts following Weimarck). However, the distinction is not always evident and several species produce indistinct brachyblasts. e.g. *C. pedunculata* or *C. dentata*.

8*. Branching density

1. densely divaricately branched
2. sparsely branched

A difficult character to categorise. Certain species have very sparse branching, especially the tall monopodial species such as *C. grandifolia* and *C. heterophylla*. Many species likewise have dense frequent divaricate branching creating those typical ericoid bushes of fynbos. In between are a group of species with infrequent branches but they can often be stunted or entangled and so appear much more densely branched. The presence of brachyblasts, as they are actually short branches, has been taken as being implicit that branching is dense even if the ones that actually go on to form true branches are sparsely distributed.

9*. Branching spread

1. evenly or randomly spread
2. clustered 2–4 together

A few of the species with sparse branching also show clustering of those branches within a few nodes of each other, creating an apparently dichotomous branching pattern. This is related to phenology, the branching phase of growth occurring within a short period of time followed by a long phase of branch extension before the next branching period.

10. Branching angle

1. branches spreading at right angles to main stem
2. branches not as above, usually ascending

A single species, *C. reniformis*, has this characteristic growth form where the branches, as well as the leaves, come out more or less perpendicular from the main stem (it also has a monopodial growth form). *C. schlecteri* and some other species also have a branching pattern that comes close, none are as distinctive as *C. reniformis*.

11. Plant height at maturity

This is a general figure, mainly as a guideline for identification. In cultivation and in exceptional circumstances in the wild where the plants are protected from fire and maybe on richer soils the size can easily exceed these figures, likewise those plants growing in rock crevices or exposed situations can easily become stunted.

12*. Plant height general

1. Short, low or sprawling up to 0.3 m
2. Medium 0.3 to 1.5 m
3. Tall over 1.5 m

This is a multistate character categorising character 10. Although there is a continuous range of sizes, most species follow a basic pattern of short, medium or tall. Short includes all the sprawling, semi-herbaceous, species as well as some of the low shrubby ones that never gain much height. Many species can become dwarfed when exposed but several rarely grow over 30 cm tall unless aided by the vegetation or rocks around them. At the other end of the scale are those species that often consistently reach above head-height. These species are usually found in the wettest mountain fynbos or along streams, although the species in subgenus Arborea contradict this trend found on mountains on the edge of the arid karoo biome. Most species fit into the medium-sized category, which also typifies most shrubs in the fynbos.

Stem characters:

The stems of *Cliffortia* have very few characters and they are highy plastiC. The older stems and branches invariably develop the characteristic flaky bark of all cliffortias and retain few if any useable characters. Therefore, all the characters are based upon the young stems before they start to disintegrate.

13. Young stem diameter 2 cm below apex of stem (mm wide)

Measured at a point where the leaf-sheaths are not present or have been removed. This is a useful character for identification but is too continuous across the range of species to be split up into states for phylogenetic analysis.

14. Young stem colour

1. greenish white
2. reddish tinged

Can vary considerably due to shading or cultivation. Only really useable upon species in their natural environment. Most species still do vary but some species are never reddish tinged and others rarely entirely green. The high plasticity of this character though has meant that it has been excluded from the phylogenetic analysis

15*. Young stems sheathing

1. completely sheathed by leaf-bases
2. visible

In many species that only produce long-shoots, the bases of the leaves can often overlap and the true stem is not visible. Under atypical conditions the stems of these species can often elongate and the true stem becomes visible but these were disregarded. The presence of persistent leaf-sheaths can mask other characters about the stem and sometimes these had to be scored as unknown but often some exposed stem can be detected.

16*. Young stems pubescence

1. more or less glabrous
2. on one side of stem only
3. hairy clearly and evenly

This is generally a very reliable character across a species and sometimes even for a section. Sometimes the pubescence can be lost very quickly and very young shoots need to be checked for the presence of hairs. Very rarely, e.g. *C. obcordata*, only one side of the stem may be pubescent and the side that is pubescent will alternate at each internode.

17*. Stem hairs direction

1. upwardly adpressed
2. downwardly adpressed
3. spreading
4. curled

Along with the last character, this makes for good characters to group related species together. The commonest state is for short straight hairs adpressed upwards along the stem, and only rarely these straight hairs are adpressed in the opposite direction and point downwards. In several species the hairs are only adpressed on the very new growth, which should be disregarded, and soon becoming spreading from the stem or tightly curl up to create a tomentose pubescence.

18. Stem hair length (mm long)

Sometimes very difficult to measure, especially when the hairs are curled. Atypically long hairs are often found at the nodes where the sheath joins the stem and these should not be used.

19*. Stem hairs range of length

1. longest hairs mostly under 1 mm long
2. longest hairs mostly over 1 mm long

A character included for phylogenetic analysis to score those species with exceptionally long stem hairs. Species with long hairs often have intermixed a layer of short hairs too, hence the longest 'typical' hairs should be measured.

20*. Older stem texture

1. minutely tuberculate
2. smooth

Mature stems need to be examined but before they have started to develop their characteristic flaky bark. Tuberculate stems are the result of gland-based hairs that have been lost or never fully developed.

21. Internode length 5th internode from apex of stem (mm long)

A very variable figure because it depends so much on the phenology of the plant at the time of measurement, but it does give some indication of the spacing of the internodes. In species with overlapping leaf-sheaths this character is not determinable. In others the fifth internode is usually easy enough to determine, though the starting point for counting can sometimes appear a bit arbitrary when the shoot is in the process of elongating. This character is also highly dependent upon the degree of exposure or shading the plant is receiving. It is barely worth using as a character for identification.

22*. Distance between brachyblasts

1. brachyblasts closely overlapping even low on branches
2. brachyblasts not or barely overlapping except near tip

An attempt to qualify character 21 by giving some element of scoring to the degree of packing of brachyblasts on the stem. The high variability of the last character meant that analysis to create discrete character states was not viable, so a general opinion was gained from the material at hand. Clearly some species have brachyblasts widely separated from each other, e.g. *C. dentata* or *C. odorata*, while others are so closely packed that the leaves of one overlap the next, usually giving a feathery appearance to the shoot, e.g. *C. paucistaminea* or *C. exilifolia*.

23*. Stem shape

1. stem compressed with leaves flattened against it
2. stem winged
3. stem round, leaves spreading in several directions

All except a few of the more distinctive species have stems that are more or less cylindrical with leaves coming off in all directions. *C. phyllanthoides*, *C. pulchella* and sometimes *C. obcordata*, have their leaves flattened against a slightly compressed stem, while *C. pedunculata* and its relatives have their sheath extending down on to the stem, making it slightly winged.

Leaf morphology:

The leaves of *Cliffortia* easily provide the most useful characters for both diagnosis and predicting relationships. Most sterile specimens of *Cliffortia* can be named with some assurance, only occasionally is further evidence needed in the form of the achene, flowers or life-history to distinguish between two similar species. Anatomical research on the leaves would probably have

added a variety of further characters, but was beyond the scope of this project to assess across the full range of species.

Sometimes, seedling, resprouting or those subtending the flowers (especially in those species with true inflorescences) vary in number, shape and size from mature ones, these therefore should not be used.

The leaf itself can be divided up into its 3 or 4 constituent parts: the sheath, the stipules, petiole and leaflets. The sheaths are invariably 3-veined, and usually clasp the stem in much the same manner. Hence the most useful characters are the degree of hairiness both adaxially and abaxially and the combination of this hairiness is a good character for group diagnoses.

24*. Leaflet number

1. unifoliate
2. bifoliate
3. trifoliate

Dissection of a brachyblast is usually required to confirm whether the needle-leaved species are unifoliate or trifoliate. In a few species the number of leaflets varies, either on the same plant (e.g. *C. multiformis* and *C. varians*), or rarely between populations (e.g. *C. linearifolia*).

The outgroup taxa were all regarded as having trifoliate leaves, as trifoliate is the same as an imparipinnate leaf with only two side leaflets; the other two states being further reduction from a pinnate leaf.

25. Leaflet insertion

1. all three leaflets touching at point of attachment in a triangular arrangement
2. not as above

Species with a petiole have the leaflets meeting in a characteristic arrangement and this has also been observed in some species without a petiole, maybe indicating that it has only recently been lost. Unfortunately this character can only be scored reliably from fresh material, which has not been done consistently across the majority of species and so has had to be omitted from the phylogenetic analysis.

26*. Leaves colour

1. green pale to dark, sometimes red-tinged
2. glaucous often with red tinge to edges

Colour of leaves is difficult to judge, but there is a definite suit of species in *Cliffortia* with grey to glaucous leaves. Species that are greyish because of their dense hairs are regarded as green-leaved because of the colour of the lamina beneath the hairs.

27*. Leaves discolorous

1. ± same colour above and beneath
2. evenly pale whitish beneath usually because of a dense mat of hairs
3. with two pale stripes on either side of midrib

Nearly all leaves are slightly paler beneath, but discolorous implies that the contrast is striking; often this contrast is due to a dense layer of whitish hairs beneath that are not present above. In fresh specimens pale areas or stripes are often evident on either side of the midrib, these can be thin and on the lateral surfaces such as in *C. densa*, or relatively broad and covering most of the under-surface as in *C. falcata* and its allies. This character state is more or less lost in herbarium material and confirming its consistency across the whole range of a species is difficult.

28*. Leaf veining

1. multi-nerved into base
2. single-nerved to base

Weimarck used veining to separate out his two sections of unifoliate species, and this is a useful character, although I have found disagreement with some of his assertions as to number of veins entering the base; e.g. *C. cuneata* and *C. phillipsii* have consistently shown to have more than one vein entering the sheath, whereas one would be hard-pressed to find more than a single vein in *C. aculeata*.

29. Veins into base number

Vein number is best observed by holding a leaf up to the light and all veins that meet at the top of the sheath are counted. This character is clearly related to leaf width.

30. Sheath length (mm long)

Measured from the base of the middle leaflet to the base of the sheath where it joins the stem. If a petiole is present then it is measured from where the stipules leave the petiole to the base of the sheath where it joins the stem. The sheath usually has 3 raised ribs on the reverse that indicate its extent.

31*. Sheath pubescence outside
1. hairy
2. glabrous

Presence or absence of hairs on the reverse of the sheath in the area of the 3 raised ribs. Lamina of stipule is not counted although this can sometimes have hairs when the rest of the sheath reverse is glabrous (e.g. *C. drepanoides*). Hairiness of the sheath on the reverse is linked to stem hairiness, a species with glabrous stems will never have hairy sheaths, but also a few hairs from the stem that happen to creep up on to the bottom of the sheaths should not be counted.

32*. Sheath pubescence inside excluding fringe at apex
1. markedly hairy
2. scattered adpressed hairs
3. glabrous

Apparently independent of the pubescence of the sheath outside, the hairiness of the adaxial surface of the sheath also varies. Presence or absence of hairs inside the sheath is scored, but excluding the apex which is nearly always has at least a short fringe of hairs (see next character). Hairs if present can either be long and straight or curled, but this character has not been consistently scored and appears dependent upon the age of the leaf examined, so has been omitted for the time being. Often the sheath inside is not entirely glabrous but shows some evidence of a few straight hairs, hence an intermediate state was included.

33*. Sheath apex hairs
1. with fringe of hairs
2. without fringe of hairs

Really just an extension of the glabrous state of the last character. A fringe of hairs is nearly always present (and counted as such if densely hairy inside) but a few species seem to have a complete absence of any hairs inside the sheath. *C. heterophylla* is unique in having a fringe of hairs on the outside of the sheath at the insertion point of the leaf. As this is not useful phylogenetically and is a very minor character for such a distinctive species it has not been scored as different.

34. Sheath production
1. produced at insertion of leaves
2. not produced

Character diagnostic to *C. hirta*, which appears to have a small, often reddish, glandular extension of the sheath on the reverse just beneath the insertion of the leaflets. As it is autapomorphic it has been excluded from the phylogenetic analysis.

35*. Stipule presence
1. present
2. absent

Some evidence of a stipule is usually present although sometimes the more modified leaves subtending flowers or in the middle of a brachyblast can appear to have lost them. Only if they are never evident should it be regarded as absent.

36*. Stipule texture
1. membranous to clear or with only green veins
2. green and texture similar to lamina
3. brown and scarious

The stipules are usually membranous with only the veins showing much colour, but can sometimes be completely green (or greyish hairy) and textured in a similar manner to the leaf itself; in a single species, *C. stricta*, they are diagnostically brown and scarious.

38. Stipule length (mm long)

Measured from the apex of the stipule, excluding any hairs, to the point of the stipule where the sheath and leaflets meet.

37*. Stipule range of length

1. less than 3 mm long
2. 3 to 6 mm long
3. more than 6 mm long

A multistate character to account for the variation shown in the previous character. Justification for the separation of the states is tentative but there is some evidence of discontinuities.

39*. Stipule margin

1. ciliate
2. entire to serrulate

A consistent character but clearly related to sheath hairiness. The margin of the stipule is often consistently ciliate or not for a species but sometimes individual stipules can be hairless so a general consensus for the plant is needed. It is only scored as polymorphic if some plants never have cilia while others have some.

40*. Stipules joining

1. touching or joined at top of sheath in front of leaf insertion
2. joined on reverse side of stem
3. free

Usually the stipules sit on either side of the leaf and the sheath is split on the reverse side of the stem to a greater or lesser degree. On rare occasions the leaves so encircle the stem that the stipules actually coalesce with one another on the opposite side to the leaf, and can indeed often look then like a small leaflet, e.g. *C. crenata* and *C. reniformis*. In a few unifoliate species, e.g. *C. ferruginea* and *C. odorata*, the stipules do the reverse and tend to touch each other or even join in front of the point of insertion of the leaflet.

41*. Petiole presence

1. present
2. absent

The petiole is generally just present or absent, occasionally it is reduced to a short stump, but then it is still regarded as present, e.g. *C. ramosissima*. Often the seedling leaves, and sometimes the resprouting ones too, of a species with show evidence of a petiole that is lacking on the adult leaves, e.g. this feature has lead to resprouting and juvenile plants of *C. nitidula* subsp. *pilosa* being frequently misidentified as *C. filicauloides*, but these are disregarded here.

42. Petiole length (mm long)

The petiole is measured from the base of the leaflets to where the stipules join the top of the sheath.

43. Leaf texture

1. soft and membranous
2. flexible and chartaceous
3. hard and rigid

A few species have a very thin herbaceous nature to the leaves, e.g. *C. dentata*, while many others are clearly much more rigid, especially the stiffly spiny species such as *C. dregeana* and *C. ruscifolia*. Measurements were made of the thickness of the leaves but the variation was more or less continuous and the states described above are hard to define. This character was therefore omitted from the phylogenetic analysis.

44. Leaflet shape

1. subcircular
2. elliptic
3. oblong
4. linear to needle-shaped
5. narrowly triangular to lanceolate
6. ovate
7. obovate

Leaflet shape, although relatively consistent within species, is hard to separate into discrete states. While useful for identification, it could not be used in the phylogenetic analysis.

The character defines the general outline of leaflets ignoring any lobes or teeth. Elliptic, ovate and obovate differ in where the widest point is (middle, lower half, upper half respectively). subcircular is a very broad form of elliptic that is more or less as wide as it is long and often wider; oblong implies that the leaflets have more or less parallel sides, linear to needle-shaped is a much

narrower version of oblong (at least 3× as long as wide); lanceolate to narrowly triangular implies that the leaf has its broadest point more or less near the base and then tapers gradually to its apex, it is not dissimilar to narrowly ovate. Usually more than one state is chosen to describe the variation found within the leaves adequately.

45*. Comparison of middle and outer leaflet shapes
1. middle and outer leaflets more or less similar in shape
2. middle leaflet significantly broader than outer leaflets
3. middle leaflet smaller than outer leaflets or reduced to a small point

Although the inner and outer leaflets generally vary slightly, in some species the inner one can be distinctly broader than the two outer ones, e.g. *C. filicaulis*. Only in two species is the inner one smaller than the two outer ones: *C. obcordata*, where the inner leaflet is folded in two and notched at the apex, and *C. phyllanthoides*, where it is reduced to just a small point.

46*. Leaflet base
1. leaflet base not contracted significantly but may be tapered evenly
2. leaflet contracted abruptly at base to form a pseudopetiolule
3. leaflet base cordate or clasping stem
4. leaflet base noticeably swollen and bulbous

Most leaf-bases join the top of the sheath and taper out gradually to the widest point. In a few species the lamina of the leaflet narrowly extends for a short distant before expanding so that it looks like a short petiole joining the leaflet to the sheath and is here termed a 'pseudopetiole'; it is generally associated with inrolling of the margins. In other cases the leaves extend back beyond the point of attachment to the sheath or petiole and so the base is described as 'cordate'. Rarely the base of the leaflet on narrow-leaved species can be swollen slightly.

47*. Leaf pubescence above
1. hairy
2. a few hairs only along the midrib
3. glabrous

Several species have hairs that are lost early on, so young leaves need to be examined. Generally, there are longer hairs along the midrib and sometimes only a few of these persist towards the base of the leaf, e.g. *C. dregeana*.

48. Leaf hair length above (mm long)
Excluding any abnormally long hairs that are often found along the midrib.

49*. Leaf pubescence beneath
1. hairy all over
2. hairy only on midrib or margins
3. glabrous

The leaves are usually more hairy beneath than above. Sometimes the leaf can be quite densely hairy beneath with short curled hairs which give a white tomentose appearance. Even then there are often some long hairs on the midribs and veins, so that they stand out in contrast against the rest of the lamina. Only a few species have sparse long hairs present only on the midribs and these can often be easily lost.

50. Leaf hair length beneath (mm long)

Very difficult to measure in some species where the margins inroll over the lamina beneath or the hairs are very crisped. The long straight hairs along the midrib are also excluded.

51*. Leaf tubercles
1. leaf underside with minute raised tubercles
2. leaf underside smooth

An unsatisfactory character, which is hard to delimit and almost impossible to determine accurately from herbarium material. True tubercles are only found on a few needle-leaved species, but others have minute dots which are not raised, although probably have the same origin.

52*. Leaf margin rolling
1. inrolled beneath
2. clearly canaliculate with margins turned upwards along their entire length
3. flat or rounded

The overall shape of the leaflets is greatly influenced by the behaviour of the margins. Most species have edges of the leaf that lie flat or are needle-leaved with rounded margins. Several species have their margins distinctly curled downwards and underneath. Less easy to define is those species where the general bend of the leaf sides is upwards creating a central channel.

53*. Leaf margin toothing
1. entire
2. minutely serrulate to scabrid
3. markedly toothed

The margins are often toothed, either coarsely, which include 'lobes' (see character 54), as short spines or as a fine serration. The latter is very hard sometimes to determine and separate from the 'entire' state, especially in herbarium material.

54*. Middle leaflet lobing
1. lobed
2. unlobed

When the toothing is deep, sparse and at the apex the leaf can appear lobed, e.g. *C. dentata* and *C. filicaulis*. The lobes are regarded as toothing for all other purposes as the distinction is too vague otherwise.

55. Teeth number, excluding tip

Not counted unless the toothing is clear (i.e. scabrid to serrulate leaflets are scored as missing).

56*. Leaflet teeth, range of number
1. 0–9
2. 10–100

A delimitation of the above character into states for phylogenetic analysis.

57. Teeth length (mm long)

Measured from the apex of the tooth to the edge of the leaf of the side of the tooth closest to the midrib.

58*. Teeth curving
1. straight
2. recurved

It is usually quite clear whether the teeth are straight or recurved under the leaf. The former are generally used for protection, while the latter for helping the plant scramble over its neighbouring vegetation.

59*. Leaf-tip shape
1. sharply long acuminate to pungent
2. acute to broadly acuminate or mucronate
3. obtuse to rounded

Pungent includes all the spiny tipped species, but also included in this category are those species which have a long tapering point to their apex but are only weakly spiny or even soft. Obtuse to rounded includes all those species that have no evidence of a point and acute to mucronate covers the rest of the species, but the boundaries are not very clear.

60. Leaf-tip length (mm long)

Measured from the tip to the point where the leaf becomes green.

61*. Leaf-tip, range of length
1. under 0.9 mm long
2. usually longer than 0.9 mm

Clearly related to character 59 and base upon the last character but used to separate out those species that are more clearly spiny.

62*. Leaflet curvature
1. outer leaflets sickle-shaped
2. leaflets ± straight

Leaflets are only described as sickle-shaped when they have a planar surface and are curved along the horizontal axis, e.g. *C. falcata*. Needle-shaped leaves are not regarded as sickle-shaped

when curved as it is difficult to tell the plane that they are curved in and they are probably curved along the vertical axis (see next character).

63*. Leaf direction

1. bent upwards and in
2. ± straight
3. bent downwards and out

One of the hardest characters to score with any certainty, but certainly appears to be useful in separating some closely related species. Many species leaves curve up and towards the stem, while others definitely curve down and away from the stem, which can give a star shaped appearance to the brachyblast. But reliably determining this character from herbarium sheets is tricky and field observations are not always consistent for a given species as it can depend upon the age of leaves on the shoot, e.g. young leaves are nearly always bent inwards to protect the new buds.

64. Middle leaflet length (mm long)

Measured from the base of the leaflet where it joins the sheath or petiole to the tip, including the spine if present.

65. Middle leaflet width (mm wide)

Measured across the widest point of the leaflet, but excluding the spine of any teeth (but including the green laminar part of the teeth).

66. Middle leaflet lobes number

Including the middle lobe, hence all leaflets can be regarded as having a 'single lobe' and the default is '1'. Lobing is only taken in to account on a few species: leaves where clear fusion between leaflets has occurred do not count as being 'lobed'; species that are 'lobed' usually still have separate outer leaflets (which sometimes also can be lobed). This character and the next two are only included to help determine *C. dentata* and its relatives.

67. Middle leaflet lobe length (mm long)

Measured from the tip, including a spine if present, to the deepest incision of the lobe.

68. Middle leaflet lobe width (mm wide)

Measured across the broadest part of the lobe.

69. Outer leaflet length (mm long)

This character and the next are rarely needed for identification except in the bifoliate species, where middle leaflet size cannot be scored as it is absent. Measured from the base of the leaflet where it joins the sheath or petiole to the tip, including the spine if present.

70. Outer leaflet width (mm wide)

Measured across the widest point of the leaflet.

71*. Midrib prominence

1. midrib beneath prominent against lamina all the way to apex
2. midrib barely prominent only towards the base or not differentiated from lamina

This should only be scored from fresh material as the midrib often becomes more prominent upon drying.

The midrib can be barely differentiated from the rest of the lamina but is often raised and prominent, even to the extent in *C. linearifolia* of meeting up with the inrolled or thickened margins so that the intervening lamina is barely visible. Weimarck regarded most of the needle-leaved species as originating from "an intense reduction in the width of the lamina, often to such an extent that the midrib takes up almost the whole of the underside". They are therefore scored as ambiguous for this character unless a clear separation between lamina and midrib can be made.

72. Leaf thickness across midrib (mm thick)

Leaf thickness is hard to measure accurately without a high-powered microscope. Leaf thickness is quite dependent upon conditions that the plant is growing in and the age of the leaf chosen. See character 43 for a easier, although less reliable, character to score.

73. Leaf thickness across lamina when different from midrib (mm thick)

Only scored for those species where the midrib is prominent above the lamina.

Juvenile leaves:

A very useful suite of characters, which unfortunately at present is far from complete and so has been omitted from the phylogenetic analysis. On the few species that have been observed growing from seedlings it is clear that the juvenile leaves can vary considerably from the adult ones and can give an interesting insight into their origins. Several of the species have been seen to revert from trifoliate to unifoliate or vice versa between the seedling and adult forms of the leaves, as well as varying their degrees of toothing. A petiole can also be present in the juvenile leaves, but become lost in the mature ones. Others have leaves identical to, though smaller than, the mature plants.

74. Seedling and resprouting leaf variation
    1. similar to mature foliage
    2. markedly different

75. Seedling and resprouting petiole presence
    1. present
    2. absent

76. Seedling and resprouting leaflet number
    1. trifoliate
    2. unifoliate

77. Seedling and resprouting leaf toothing
    1. toothed or lobed
    2. entire

Inflorescence structure:

Fellingham (1999) tried to describe *Cliffortia* inflorescence structure, but only examined 8 species and did not go further at attempting to categorise the other species into different groups. Only a very few species could be regarded as having an 'inflorescence', most species have scattered flowers situated at the base of normal vegetative leaves. Fellingham discovered that all species produce their flowers on short shoots, even if those short shoots are so reduced that they appear to be absent. For her work, she used the terminology of 'bract' to describe any leaf that actually subtended a flower (as opposed to those that subtend the short shoot upon which the flower grows). This terminology gets complicated though as often the subtending leaf is no different from the normal vegetative ones and therefore to distinguish the two seems arbitrary upon whether the plant is flowering at the time or not! In this account therefore, the leaves of both fertile and vegetative shoots as regarded as equal unless they are morphologically different.

78*. Inflorescence structure
    1. flowers apparently solitary at the base of ordinary vegetative leaves
    2. flowers clustered together, male and female in similar clusters
    3. at least the female flowers gathered in a distinct inflorescence structure, males also present or scattered over rest of plant

    Fellingham described several different inflorescence types, but species are split up into just three here. The commonest inflorescence type is those that lack any structure and flowers are scattered apparently uniformly over the plant. The congested inflorescence type covers those species where both male and female flowers can be found conglomerated into a small area, such as in *C. ruscifolia* or *C. odorata*. In *C. ruscifolia* the leaves subtending the flowers are markedly reduced; in *C. odorata* the flowers look as though they are subtended by an ordinary leaf, but in fact the subtending leaves have actually been lost. The third type is the female structured inflorescence, this is found in sect. *arborea* and in *C. heterophylla*. In this type the female flowers are collected together in a distinct structure, with the subtending leaves of the flowers markedly different from the normal foliage, while the male flowers are scattered across the rest of the plant. (The outgroup taxa are also included in this last category as having distinct inflorescences.)

79*. Flowers perfect or not
    1. separate male and female flowers, although sometimes with rudimentary parts of the other remaining, and not found in same inflorescence
    2. male and female parts always found in same flower
    3. male and female flowers found on same inflorescence

    A character purely included to separate out the outgroup from Cliffortia. All Cliffortia species have separate male and female flowers. Weimarck described a separate section called

Hermaphroditicae, based upon a single species, *C. hermaphroditica*, which itself was based upon a single specimen that had perfect flowers, but this species has been abandoned here.

80*. Peduncle presence
1. absent
2. present holding a solitary flower
3. present holding a compound flowerhead

The term 'peduncle' used here is quite likely not homologous. The 'peduncle' in *C. pedunculata*, *C. lepida*, and *C. triloba*, which gets successively shorter in each, only ever carries a single flower and so terming it a peduncle is rather misleading. It is thus defined to distinguish the elongation of the flower bearing stalk on one side of the bracteoles to the 'pedicel' on the other. The other state could be referred to as a 'true' peduncle and is found in the outgroup taxa as well as the cone bearing stalks of *C. conifera*.

Flowers:

Both the male and female flowers have a similar basic structure, consisting of bracteoles, a receptacle, which is more or less vestigial in the male, and sepals. A few characters are applicable to both the male and female flowers, while others are usually exclusive to one sex or the other, although they are sometimes discernible in a vestigial form in the other.

81*. Bracteole pubescence, excluding margin
1. hairy
2. glabrous

The hairiness of the bracteoles is a good character that is usually consistent within a species and often across a section. The bracteoles, independent of the margins, can be hairy or glabrous although if there are hairs present they are often confined to a small area towards the lower half of the keel.

82*. Bracteole margin
1. clearly ciliate
2. serrate or very shortly ciliate
3. entire

The margin of the bracteole can have varying degrees of hairiness, apparently independent of whether the rest of the surface is hairy or not. The margin is often distinctly ciliate but sometimes the ciliae are more like short round projections along the margin and classified as serrate; others are clearly entire or slightly wavy. The three categories are usually consistent and distinguishable enough to define.

83*. Sepals number
1. 3
2. 4

Although several species have been described as having 3 or 4 sepals the species are in fact almost always consistently either three or four in number within a species. Only *C. paucistaminea* has shown signs of variation in sepal number between populations, although other species may have the odd flower here and there lacking or gaining a sepal. The number is consistent between male and female flowers too and it is a very good and reliable character at the sectional level.

84*. Sepals pubescence
1. hairy on outside
2. glabrous except for small patch near apex inside

Often the sepals will only have a few, but long, hairs towards the tip of the sepals (on the outside, the tuft of short hairs inside the apex that hold the sepals together in bud is not taken in to account as it is present in all species). But the presence of even a couple is a good enough indicator of hairiness; species that have glabrous sepals are always glabrous. A good diagnostic character but a hand lens is needed to see the presence or absence of hairs properly and they are usually clearer on the male than the female flowers (and if present on one sex they are presumed present on the other).

Male flowers:

The male flowers are all rather uniform in their structure and there are few discrete characters to be scored as they all have a similar design for the basic purpose of dangling their stamens out to allow the

wind to take the pollen away.

85. Male peduncle length (mm long)

Measured from the base of the subtending leaf to the base of the bracteoles.

86. Male bracteole number

1. 1, but often nested with other flowers
2. 2
3. 3+

The number of bracteoles is nearly always two, but a few species have been observed to have either an extra one, or lost one (the other then being greatly enlarged and enclosing the whole flower, and sometimes more than one flower). This character has not been consistently scored and so at present is unreliable and omitted from any analysis.

87. Male bracteole length (mm long)

Best measured removed from the pedicel. Measure from base of bracteole to tip, excluding any hairs.

88. Male pedicel length (mm long)

This includes the vestigial receptacle of the male flowers, on top of which the sepals sit, as well as any true pedicel. To separate the two would have been rather tricky as a means of identification, although the latter can be distinguished by the presence of an articulation (see next character). Bracteoles need to be removed to measure this character accurately and it is measured from above where the bracteoles join to the base of the sepals.

89. Pedicel articulation

1. pedicel short (i.e. the vestigial receptacle)
2. pedicel long and articulated (i.e. a true pedicel is present)

Mostly what is termed a pedicel is actually the vestigial receptacle of the male flowers, but a true pedicel is evident in a few species by the presence of an articulation. This articulation needs to be present because a few species, e.g. *C. ruscifolia*, have an elongated vestigial receptacle and so the flower appears truly pedicellate. This and the next character have not been scored consistently across the range of species and so are omitted for the time being from any analysis.

90. Pedicel hairiness

1. glabrous
2. hairy

This character if it was to be used in phylogenetic analysis would need more clearly defining. As the pedicel can either be a true pedicel or the vestigial receptacle, thus hairiness is not necessarily homologous. Usually if the receptacle is hairy in the female flower then so is the pedicel of the male, but also in certain species which have a true pedicel, that part can be hairy while the vestigial receptacle is glabrous. At present this character is only scored for identification purposes.

91. Male sepal length (mm long)

Measured from the top of the pedicel to the apex of the sepals.

92. Male sepal width (mm wide)

Measured across the widest point of the sepals.

93*. Male sepal shape

1. broadly ovate (less than 4x as long as wide)
2. narrowly elliptic (more than 4x as long as wide)

Most species have relatively broad male sepals, but a few species in subgenus *Graminea* have distinctive narrowly elliptic sepals. It is important not to use old sepals that may have shrunk and wrinkled when assessing this character.

94*. Male sepal tips

1. acute to acuminate
2. small spine just beneath apex
3. long acuminate becoming filiform

Most species have acute to acuminate sepal tips. In *C. teretifolia*, the tip of the sepals protrudes just beneath the true apex where they join in bud, a diagnostic character for that species;

this is also true of the female sepals but is most clearly noticeable on the male. In a few species the tips of the male sepals have become attenuated, filiform and slightly curled, which is especially noticeable in bud, e.g. *C. dentata*.

95*. Male sepals splitting and fusion
1. completely free at anthesis
2. separating on one side only at anthesis forming a hood over the stamens
3. not completely separating at tip at anthesis
4. joined into a short tube for lower third to half

In *C. graminea*, the sepals of the male only split on one side and form a hood over the stamens, a diagnostic character (as if this species needs any more!). In a few species the sepals do not split completely at anthesis but the tips stay fused together by the small patch of hairs at their apex inside. This may not be consistent, in some cases the sepals may separate and recurve as most species do, and this state needs more field observations as is hard to determine correctly from herbarium material. Finally, a few species have a short area of fusion towards the base of the sepals so that a short tube is formed. Again it only appears to occur in a few species, e.g. *C. monophylla*, but may not be consistent within them.

97. Stamen number

Usually best to count in freshly opened flowers or mature buds as stamens can easily be lost in older flowers.

96*. Stamens, range of number
1. few, 4–8
2. intermediate, 9–18
3. many, 19–50
4. numerous, 50+

A delimitation of the above character for phylogenetic analysis, as well as being useful for quick identification. It is a relatively consistent character within a species as can be seen by the generally small ranges. The smallest state is justified on the basis of being one or two whorls of stamens, thus a species with 8–9 stamens but three sepals would be better placed in state 2, while a species with the same number of stamens and four sepals would be more appropriate in state 1. As it happens, species with four sepals always have 4–8 stamens.

98. Filament length (mm long)

Measured from the base of the flower to the attachment point on the anther.

99*. Filaments colour
1. red
2. white to greenish cream

Stamen colour is also a useful character, and although several species are polymorphic, there are certain suite of species for which filament and anther colours are constant and quite diagnostic.

100*. Anthers colour
1. brownish red
2. cream to yellow

Occasionally sterile anthers will be 'colourless' when the rest are tinged reddish brown; the overall trend is usually obvious.

101*. Anther connective hairiness
1. glabrous
2. hairy

The presence of hairs on the anther connective was only recorded by Weimarck for *C. lanceolata* but this study discovered it consistently on collections of the apparently unrelated *C. acutifolia*.

Female flowers:

The female flowers appear to follow two basic patterns for capturing the male pollen from the air. One is that typical of most wind-pollinated plants, and the flower structure is designed to hang out the stigmatic surface into the wind streams, the other is more unusual in that the flowers are hidden within the leaves or even tucked down right at the base. Clearly in the latter pattern the leaves have developed to create the right eddies in the wind to allow the pollen to drop out of the air and on to the stigma.

102. Female peduncle length (mm long)

Measured from the base of the subtending leaf to the base of the bracteoles.

103. Female bracteole number

1. 1, often nested with other flowers
2. 2
3. 3+

The number of bracteoles is nearly always two, but a few species have been observed to have either an extra one, or lost one (the other then being greatly enlarged and enclosing the whole flower, and sometimes more than one flower). This character has not been consistently scored and so at present is unreliable and omitted from any analysis.

104. Female bracteole length (mm long)

Best measured removed from the pedicel. Measure from base of bracteole to tip, excluding any hairs.

105*. Female bracteole range of length

1. short, less than 2 mm long
2. medium, between 2 and 5.5 mm long
3. long, over 5.5 mm long

A multistate character to account for the variation shown in the previous character.

106*. Female bracteole to receptacle ratio

1. shorter than immature receptacle
2. as long or longer than immature receptacle

Clearly closely linked with the last character and far from independent, but included as it defines whether the bracteoles are there as a protective measure for the developing ovary or not. One needs to ensure that the receptacle is immature, which is usually best discerned by the presence of an unwithered stigma, as the receptacle soon grows beyond the size of the bracteoles as it matures in many species.

107*. Female sepals presence

1. present
2. absent or vestigial

Present in most species although they might fall early on in the development of the fruit, but one or two species, e.g. *C. odorata*, have only vestigial sepals or they are lost while the stigma is still developing.

108*. Female sepals joining

1. all fused at base
2. free or occasionally one or two joined but not all at same point

The sepals are usually free, though two may become fused the third or fourth are free; only in a few species are the sepals truly fused all the way around, e.g. *C. monophylla* and relatives. Clearly linked to state 4 of character 95, but due to the difference in clarity of observing this feature in the male and female flowers both have been included. As a result the consistency of this character in both sexes has been questioned and more observations are needed on fresh specimens to determine whether the variation is uniform within and across species.

109*. Female sepal shape

1. narrowly to broadly ovate to obovate
2. ± triangular broadest point at base or just above base
3. oblong to linear ± parallel sided for a greater part of their length

Sepals are all generally ovate, but inrolling of the margins can make the shape more linear to oblong or the lower part of the sepal is greatly shortened so that the widest part is near the base and the shape looks more triangular. Intermediates are found between all these states and the distinctions are not clear.

110. Female sepal length (mm long)

Measured from the top of the receptacle to the apex of the sepals.

111. Female sepal width (mm wide)

Measured across the widest point of the sepals when flattened out.

112*. Female sepals stature

1. erect
2. spreading but not recurved
3. recurved

Erect sepals stand ± perpendicular to the top of the receptacle. Spreading sepals diverge at more than 30° from the top of the receptacle, but the sepals are still straight. Recurved sepals are usually reflexed at 180° and bent back on oneself. Most species fall neatly into the first and third states, but a few appear to fit into the second, especially sect. Petiolatae, with possible overlap with one of the other two. Mature flowers need to be used, even ones where the fruits have started to develop, as all sepals are erect and held together in bud and the bracteoles can also prevent the sepals from spreading and recurving.

*113**. Carpel number

1. 1
2. 2+

Weimarck subdivided the genus into two subgenera dependent upon the number of carpels, this character is often easiest to see by examining the number of stigmas in the female flowers, which match the number of carpels. However, stigmas can often be lost easily in dried material and sectioning a few mature achenes also helps to determine the carpel number.

114. Stigma length (mm long)

One needs to ensure that one has a fully mature, but not withering, stigma as they elongate during development. Measure from point of attachment to the receptacle to top of the highest feathery extension. In species where the short stigma appears bent, measure to point furthest from the base if it cannot be straightened out.

115*. Stigma colour

1. red
2. pinkish white
3. white to greenish

The stigma usually changes between bright red or a greenish white, but occasionally there is a more intermediate colour of white with a reddish tinge in certain bits of the stigma giving a pinkish hue. In some species the stigma colour is variable and probably dependent upon habitat, but often the colour is consistent within a species and between closely related species.

116*. Stigma branching

1. highly branched and feathery
2. band-like or thinly and sparsely short-branched

The stigma branching is variable amongst the species but generally very hard to quantify. In the few species related to *C. arborea* it is reduced to a thin strap-like band with little or no branching.

117*. Stigma exsertion

1. stigma prominent above leaves and sepals
2. stigma hidden by leaves

Although clearly linked to character 114, this is not a straight classification of that character into discrete states because stigma exertion is dependent upon leaf size and curvature as well as stigma length. This character is a bit arbitrary in where the separation between the states are drawn but was included because of its clear relationship to the means of pollen capture employed by each species.

118. Stigma twisting

1. stigma held erect
2. stigma twisted to one side of the leaves

Linked to the last character and is possibly confined only to *C. graminea*, which manages to exert its stigma from the foliage by twisting it to one side. It has therefore been excluded from the phylogenetic analysis until field observations have been made.

119. Receptacle length (mm long)

Measured from point of attachment to bracteoles to the base of the sepals. Can vary quite widely depending upon how mature the flower is but do not choose flowers where the stigma has started to wither.

120. Receptacle width (mm wide)

Measured across the widest axis of the receptacle. Can vary quite widely depending upon how mature the flower is but do not choose flowers where the stigma has started to wither.

121*. Immature receptacle hairiness

1. glabrous
2. hairy

The receptacles show characters similar to the mature achenes, but some species have hairs on the receptacles that are lost as they mature, on the other hand, if there are hairs on the achenes they are always present on the receptacles too. Sometimes the hairs are only near the apex, and often they are very short so that a hand-lens is needed to see them properly.

122*. Immature receptacle ribbing

1. smooth
2. clearly ribbed

Ribbing should only be regarded in terms of clearly raised portions, not just differentiation in banding colour of the receptacle. Those species that have smooth receptacle but where coloured bands are present will eventually develop ribs on their achenes, though not as distinct as those that had ribbed receptacles originally. This character is useful to help classify the type of ribbing on the achene that is present in the species. If a species has smooth achenes it will always have smooth receptacles.

Achenes:

The achenes of a *Cliffortia* species are very often diagnostic, and when both leaves and achenes are present most species can be named with some certainty. But turning the distinctive overall look into discrete character is hard and often direct comparison with a picture or herbarium material is much better.

123*. Achene pedicel

1. on a short recurved pedicel so that achene hangs downwards
2. held erect

Only the two species, *C. alata* and *C. burgersii*, allow their achenes to hang on a recurved pedicel, all other species, barring those that bore the flowers on long peduncles anyway, have them tucked away at the base of their leaves.

124*. Achene shape

1. straight
2. curved
3. irregular

Irregular achenes are generally oblong in outline and are not cylindrical to ellipsoid in general outline (i.e. excluding ribs and wings); curved achenes are slightly banana-shaped.

*125. Achene length (mm long)

Measured free from their bracteoles; from the top to bottom including the often shrunken elaiosome at the base.

126. Achene width (mm wide)

Measure achenes free from their bracteoles and across the widest axis including any ribs or wings.

127. Achene colour

1. medium to dark brown
2. greyish brown
3. whitish to yellowish or pale brown
4. orange
5. red to reddish brown

Achene colour is also distinctive but difficult to classify. Several species have reddish achenes, but this will often dry to a dull brown so recording the correct colour is very dependent upon obtaining fresh material. The fleshy fruits of *C. baccans* are a bright orange, but most species

vary between a greyish to light or dark blackish brown. Because of the difficulty of needing to use fresh achenes across all the species, this character was omitted from any phylogenetic analysis.

128. Achene texture
1. fleshy
2. dry

A diagnostic character for *C. baccans*. *C. micrantha* was once regarded as having fleshy achenes but in fact the achene looks swollen due to the number of carpels it contains and is not fleshy.

129*. Achene pubescence
1. hairy
2. glabrous

Hairs if present usually require a hand-lens to observe as they can often be very short.

130. Achene hair length (mm long)

A variable and difficult to score character with not much usefulness in identification as the presence of hairs alone will have been very diagnostic.

131*. Achene ornamentation
1. ribbed or winged
2. unribbed

Ribs if present are generally obvious, even if often low or irregular. Occasionally they can be obscured by an inflated waxy transparent layer (see character 140) that needs to be removed before the ribs are visible.

132. Achene ribs number

Difficult to count generally, especially when numerous, but often best counted by looking down on the top of the achene, whereby a starting and ending point can more easily be established. Wide variation can be found in the counting of ribs due to the frequent presence of lesser ribs or indistinct wrinkling of the surface rather than clear ribs.

133*. Rib number
1. 2–4
2. 6–12
3. 12+

An attempt to delimit the above character but as that character was hard to score consistently it was more of a general circumscription. Most species fall in the 6 to 12 ribs range, even with indistinct or the lesser ribs present. Those species with fewer than 6 ribs usually have very distinct ribs, and when there are more than 12 it is usually considerably more and the achenes are relatively large.

134. Achene rib width (mm wide)

Measured across the widest point on a typical rib or the thickness of a wing.

135*. Achene rib form
1. ribs rounded to sharply acute
2. clearly winged

Wings are here regarded as narrow ribs with acute edges that have extended. The boundary of the character is rarely unclear.

136*. Achene wings
1. incurved
2. straight

Close examination of some ribbing is required to determine whether they are not true ribs but wings that are curved inwards upon themselves. This character is confined to *C. polygonifolia* and its relatives.

137*. Achene tubercles
1. present
2. absent

Tubercles on mature achenes are usually clearly evident with the naked eye and easily with a hand-lens.

138. Achene symmetry
1. ribs curved transversely
2. ± equal

A diagnostic character for *C. subsetacea*. The two largest ribs are curved transversely across the achene, so that the one of the ribs of the achene appears to be bulging out between the others forcing them apart.

139*. Achene inter-rib ornamentation
1. transversely rugose between ribs
2. not as above

Transverse ribs are sometimes present between the main ribs giving the achene surface a rugose texture.

140*. Achene covering
1. achene covered by membranous layer
2. achene not covered

Several species do have a semi-transparent membranous layer, which often comes away from the achene in older fruits. The layer is often inflated as the fruit matures and so it can appear smooth and unribbed. This feature was noted by Weimarck for *C. arcuata*, but has become apparent, although not as marked, in several other closely related and some apparently unrelated species.

141. Altitude (m)

Maximum and minimum altitude ranges for the species across its entire distribution.

142. Flowering time peak

Description of the months in which most of the plants are in flower.

143. Male flowering time
1. January
2. February
3. March
4. April
5. May
6. June
7. July
8. August
9. September
10. October
11. November
12. December

Any month in which a male flower has been recorded.

#144. Female flowering time
1. January
2. February
3. March
4. April
5. May
6. June
7. July
8. August
9. September
10. October
11. November
12. December

Any month in which a female flower has been recorded.

Classification and taxonomy:

145. Subgenus

1. Arborea
2. Eriocephalina
3. Graminea
4. Cliffortia

146. Section
1. Gramineae
2. Longifoliae
3. Heteromorphae
4. Tuberculatae
5. Complanatae
6. Petiolatae
7. Costatae
8. Castanae
9. Filicaulae
10. Dracomontanae
11. Paucistaminae
12. Cliffortiae
13. Filifoliae
14. Multifidae
15. Alatae
16. Simplices
17. Bifoliolae
18. Multinerviae

147. Subgenus Weimarck 1934–1954
1. Digraphidium Weim.
2. Monographidium (Presl) Weim.

148. Section Weimarck 1934–1954
1. Complanatae Weim.
2. Hermaphroditicae Weim.
3. Petiolatae Weim.
4. Costatae Weim.
5. Bacciformes Weim.
6. Alatae Weim.
7. Inflexae Weim. - also called 'Reflexae' in key
8. Arboreae Weim.
9. Bifoliolae DC.
10. Multinerviae DC.
11. Simplices Weim.

Those species described subsequent to Weimarck's classification were placed into the correct section as best as could be determined from his key.

149. Synonyms, taxa included in the current description

Only taxa accepted by Weimarck in his monograph were included.

150. Taxonomic notes

General notes upon how best to distinguish the species, especially in the field, from similar looking ones.

151. Distribution notes

Outline of the boundaries of the geographic range for the species.

152. Habitat

A generalized habitat description.

153. Conservation status category
1. Ex
2. CR
3. EN
4. VU
5. LR(cd)

6. LR(nt)
7. LR(lc)
8. DD
9. NE

IUCN Red data list categories for conservation

154. Conservation status criteria and notes

IUCN Red data list criteria and justification for the placement within the particular category.

155. Etymology

Derivation of the Latin specific epithet.

156. Common names

List of any common names that could be found for those few species that have them. New common names were not made up.

157. Illustrations

Bibliography of where illustrations of particular species may be found.

Appendix 4: Voucher specimens for DNA extractions

Taxon	Collection	Date	Locality
Acaena cylindristachya	ex Hibbs		
Acaena latebrosa	Whitehouse 122	31-May-00	Sutherland District, escarpment at top of Komsberg Pass
acanthophylla	Whitehouse 248	16-Aug-01	Clanwilliam District, Olifants R Mts, Die Berg road, S of Bloubos
aculeata	Whitehouse 160	20-Oct-00	Oudtshoorn District, Swartberg Mts, peak between Waboomsberg and Swartberg Pass, gully on NE side
acutifolia	Whitehouse 254	19-Aug-01	Calvinia District, ± 5 km W of Nieuwoudtville along Vanrhynsdorp road
alata	Whitehouse 94	11-Mar-00	Riversdale District, Garcia's Pass, just S of Muiskraal
amplexistipula1	Whitehouse 62	13-Oct-99	Ceres District, top of pass on to Zuurvlakte, road between Ceres-Clanwilliam road and Katbakkies
amplexistipula2	Whitehouse 210	22-Feb-01	Laingsburg District, Anysberg Nature Reserve, S slopes above kloof leading from Goedehoop, near top of kloof
anthospermoides	Whitehouse 219	17-May-01	Caledon District, Grootbos Nature Reserve, just N of public road, ± opposite Bobbejaansfontein farm
apiculata	Whitehouse 165	29-Nov-00	Caledon Division, Arieskraal, S facing slopes on Bokkeveld shale above Arieskraal Dam
arborea	Whitehouse 121	31-May-00	Sutherland District, escarpment at top of Komsberg Pass
arcuata	Whitehouse 24	20-Mar-99	Oudtshoorn District, Great Swartberg, path from Die Hoek to Gouekrans Hut
atrata	Whitehouse 7	20-Feb-99	Paarl District, Wemmershoek Mts, Agter Tafelberg (Elandsvlakte), S of Mountain Club hut, valley bottom
baccans	Whitehouse 60	13-Oct-99	Ceres District, Swartruggen Mts, Groenfontein Farm, near campsite just N of Stompiesfontein
berberidifolia	Whitehouse 41	4-Jul-99	Bredasdorp District, Rhenosterkop
brevifolia1	Whitehouse 120	10-May-00	Bredasdorp District, road to Die Dam from Wolvengat, N of Ratelrivier farm
brevifolia2	Whitehouse 260	3-Sep-01	Bredasdorp District, road to Brandfontein, just before Springfield turning, W edge of Soutpan
browniana	Whitehouse 296	28-Nov-01	Underberg District, Sani Pass, just W of South African border post
burchellii	Whitehouse 91	10-Mar-00	George District, Outeniqua Pass, by side of road at summit
burgersii	Whitehouse 226	20-May-01	Bredasdorp District, De Hoop Nature Reserve, 1.3 km N along main road from turn-off to campsite, next to old track
carinata	Whitehouse 240	4-Aug-01	Cape Peninsula, Slangkop Mt, above Kommetjie, along track towards ruins of coastguard house
castanea1	Whitehouse 124	1-Jun-00	Laingsburg District, Witteberg, S slopes, ravine above Nooitgedacht
castanea2	Whitehouse 90	10-Mar-00	Willowmore District, Baviaanskloof, Willowmore end, Adamskraal, on edge of plateau above Nuwekloof
ceresana	Whitehouse 269	22-Sep-01	Tulbagh District, Groot Winterhoek Wilderness Reserve, N facing S slopes of Sneeugat
cervicornu	Whitehouse 163	22-Oct-00	Prince Albert District, Swartberg Pass, slopes above road, SW of Malvadraai
complanata	Whitehouse 15	21-Feb-99	Paarl District, Wemmershoek Mts, top end of Elandsvlakte, along stream behind Wemmershoek Tafelberg, W of Olifants Peak
conifera	Whitehouse 208	22-Feb-01	Laingsburg District, Anysberg Nature Reserve, E end above Prinspoort
crassinervis	Whitehouse 105	8-Apr-00	Laingsburg District, Besemfontein, Klipkraal Trail, jeep track to radio mast, saddle
crenata	Whitehouse 108	8-Apr-00	Laingsburg District, Besemfontein, Klipkraal Trail, ridge down to Verlorenhoek Kloof
cristata	Whitehouse 213	10-Mar-01	Worcester District, Stettynsberg, Stettyn farm, track between Heuningneskloof & Elandskloof
cruciata	Whitehouse 137	15-Sep-00	Worcester District, Jonaskop, Wildepaardeberg, just below sandy plateau
cuneata	Whitehouse 21	6-Mar-99	Stellenbosch District, Jonkershoek, car park at start of Swartboskloof Trail
cuneata var. microcarpa	Whitehouse 166	29-Nov-00	Caledon Division, Arieskraal, S facing slopes on Bokkeveld shale above Arieskraal Dam
curvifolia	Whitehouse 221	18-May-01	Caledon District, Waterford Farm, on ridge of hill to W of dam at bottom of Waterkloof
cymbifolia	Whitehouse 81	15-Feb-00	Caledon District, Pringle Bay, by road to Missile Test Site
densa	Whitehouse 97	11-Mar-00	Riversdale District, Garcia's Pass, footpath to Sleeping Beauty, above waterfall
dentata	Whitehouse 14	20-Feb-99	Paarl District, Wemmershoek Mts, on S slope of Winterberg above saddle between Winterberg & Tafelberg
denticulata	Whitehouse 19	6-Mar-99	Stellenbosch District, in small upland valley on E side of Somerset West Triplets, on Swartboskloof Trail
dichotoma	Whitehouse 252	18-Aug-01	Calvinia District, Papkuilsfontein Farm, Oorlogskloof escarpment, just N of waterfall where De Hoop river flows in to Oorlogskloof
dispar	Whitehouse 156	19-Oct-00	Uniondale District, Prince Alfred's Pass, N side just below summit
dodecandra1	Whitehouse 69	22-Dec-99	Cape Peninsula, Silvermine N.R., above Kalk Bay, valley between Ou Kraal and Kalk Bay Mt., edge of Bona Spei forest

dodecandra2	Whitehouse 241	4-Aug-01	Cape Peninsula, Slangkop Mt, above Kommetjie, along track towards ruins of coastguard house
dracomontana	Whitehouse 286	21-Nov-01	Underberg District, Royal Natal National Park, summit of Ampitheatre near Tugela Falls
dregeana	Whitehouse 8	20-Feb-99	Paarl District, Wemmershoek Mts, lower E slopes of Winterberg
drepanoides	Whitehouse 83	23-Feb-00	Uitenhage District, Groendal Wilderness Area, jeep track to Spitskop, above path to Blindekloof
erectisepala1	Trinder-Smith		Gifberg
erectisepala2	Whitehouse 57	10-Oct-99	Port Elizabeth District, Suurberg Mts, Addo Elephant National Park 4 hour trail, by track to W of Brandrug
ericifolia1	Whitehouse s.n.		Cape Flats, Rondevlei Nature Reserve
ericifolia2	Whitehouse 261	3-Sep-01	Bredasdorp District, road to Brandfontein, just before Springfield turning, W edge of Soutpan
eriocephalina1	Whitehouse 25	20-Mar-99	Oudtshoorn District, Great Swartberg, path from Die Hoek to Gouekrans Hut
eriocephalina2	Whitehouse 177	10-Jan-01	Graaff Reinet District, Nardouwsberg, S slopes of saddle between two summits
esterhuyseniae	Versfeld in Whitehouse 284	10-Nov-01	Worcester District, shale band beneath Milner Peak
exilifolia	Linder in Whitehouse 67	21-Nov-99	Caledon District, Riviersonderend Mts, Pilaarkop, S slopes
falcata	Whitehouse 43	4-Jul-99	Bredasdorp District, Brandfontein
ferruginea	Whitehouse 38	3-Jul-99	Bredasdorp District, Hermanus to Elim, S base of Koue Berg Pass
filicaulis	Whitehouse 23	12-Mar-99	Cape Peninsula, path between University & Rhodes Memorial
filicauloides	Whitehouse 293	25-Nov-01	Estcourt District, Monk's Cowl Reserve, valley to W of Cathkin Peak, ± 2 km N of Keith's Bush Camp
filifolia	Whitehouse 54	3-Oct-99	Paarl District, Perdeberg, Lemoenkloof Farm, plateau between Lemoenkloof and Skelmklip Peak
geniculata	Whitehouse 49	4-Sep-99	Caledon District, Fernkloof Nature Reserve, rise above car park at end of Rotary Way
glauca	Whitehouse 93	11-Mar-00	Riversdale District, Garcia's Pass, just S of Muiskraal
gracillima	Whitehouse 98	11-Mar-00	Riversdale District, Garcia's Pass, near car park at start of footpath to Kristalkloof
graminea	Whitehouse 18	2-Mar-99	Cape Peninsula, Silvermine N.R., at far end of dam
grandifolia	Whitehouse 102	12-Mar-00	Swellendam District, Marloth Nature Reserve, path to Twelve o'clock Peak, just above Die Plaat path
hantamensis	Whitehouse 125	1-Jun-00	Laingsburg District, Witteberg, S slopes, ravine above Nooitgedacht
heterophylla	Whitehouse 45	21-Jul-99	Caledon District, hills behind Kleinmond from Fairy Glen, gully beneathTweelingpieke
hexandra	Whitehouse 131	24-Jun-00	Clanwilliam District, Cederberg Mts, path from Sanddrif to Wolfberg Cracks
hirsuta	Whitehouse 64	23-Oct-99	Caledon District, Riviersonderend Mts, between Greyton and Macgregor, Boesmanskloof Trail in valley bottom between Oakes Falls and Die Galg
hirta	Whitehouse 76	7-Feb-00	Cape Peninsula, Rondebosch Common, SW corner
×homunculi	Whitehouse 199	18-Feb-01	Uniondale District, Kammanassie Nature Reserve, plateau to N of Buffelsberg
ilicifolia	Whitehouse 195	17-Feb-01	Uniondale District, Kammanassie Nature Reserve, lower S slopes of Mannetjiesberg
incana	Whitehouse 227	20-May-01	Bredasdorp District, De Hoop Nature Reserve, Potberg Section, 2 km W of Cupidoskraal along road
integerrima	Whitehouse 47	5-Aug-99	Cape Peninsula, NE slopes of Devil's Peak, just beneath King's Blockhouse
intermedia	Whitehouse 191	13-Dec-00	Cape Peninsula, Kirstenbosch, Silver Tree Hill, above dam
juniperina	Whitehouse 22	6-Mar-99	Stellenbosch District, Jonkershoek, car park at start of Swartboskloof Trail
lanata	Meyer 741	4-Oct-94	Tulbagh District, Wolseley, Kluitjieskraal Forest Station, Watervalsberg
lanceolata	Whitehouse 101	12-Mar-00	Swellendam District, Marloth Nature Reserve, path to Twelve o'clock Peak, just above Die Plaat path
lepida Weim.	Whitehouse 232	24-Jul-01	Blokkop, ridge to summit N of valley above Villiersdorp Wild Flower Garden, damps seeps beneath cliffs at summit
linearifolia1	Whitehouse 66	5-Dec-99	Victoria East Division, Amatola Mts, Hogsback, circular walk on W side of Heads
linearifolia2	Linder 3976	14-Nov-86	Zimbabwe, Nyanga, Inyangani, NW of summit
longifolia	Whitehouse 228	20-May-01	Bredasdorp District, De Hoop Nature Reserve, Potberg Section, in stream beside Hamerkop
longimontana1	Whitehouse 128	10-Jun-00	Montagu District, Bloupunt Hiking Trail, W facing slopes beneath Bloupunt
longimontana2	Whitehouse 301	9-Jan-02	Robertson District, Langeberg Mts above Robertson, ridge just W of Arangieskop
marginata	Parker 3475	26-Mar-40	Stellenbosch District, Somerset West
meyeriana	Whitehouse 140	15-Sep-00	Worcester District, Jonaskop, Wildepaardeberg, slopes above plateau
micrantha	Whitehouse 109	9-Apr-00	Laingsburg District, Besemfontein, Verlorenhoek Trail, gorge slopes above Verlorenhoek Pool

scandens	Linder in Whitehouse 68	21-Nov-99	Caledon District, Riviersonderend Mts, Pilaarkop, S slopes
schlechteri	Whitehouse 225	18-May-01	Caledon District, Waterford Farm, by dam at bottom of Waterkloof
semiteres	Whitehouse 231	24-Jul-01	lower slopes of Blokkop, N slope of valley above Villiersdorp Wild Flower Garden
sericea	Whitehouse 44	21-Jul-99	Caledon District, Sir Lowry's Pass, above Steenbras Siding, by old road
serpyllifolia1	Whitehouse 58	11-Oct-99	Barrydale District, Tradouw Pass, S of summit, by parking area next to road
serpyllifolia2	Balkwill et al. 1243	8-Feb-84	Umzinto District, Vernon Crookes Nature Reserve
serrata	Whitehouse 46	21-Jul-99	Caledon District, hills behind Kleinmond from Fairy Glen, gully beneathTweelingpieke
setifolia	Whitehouse 205	20-Feb-01	Prince Albert District, Swartberg Nature Reserve, Blesberg summit, just W of radio tower
sp. cf. arborea	Viviers & Vlok 211	4-Jul-87	Witteberg, peak N of Worlds View Farm, steep S facing slope
sp. cf. eriocephalina	Whitehouse 188	27-Jan-01	Worcester District, Matroosberg, flats between summits
sp. cf. glauca1	Whitehouse 78	15-Feb-00	Caledon District, Sir Lowry's Pass, by old road to Gantouw Pass
sp. cf. glauca2	Whitehouse 214	11-Mar-01	Worcester District, Stettynsberg, Stettyn farm, mid E facing slopes of 4x4 track above farm
sp. cf. juniperina	Whitehouse 61	14-Oct-99	Ceres District, Swartruggen Mts, Groenfontein Farm, rocky flats above canyon
sp. cf. pterocarpa	Whitehouse 126	10-Jun-00	Montagu District, Bloupunt Hiking Trail, just N of saddle at top of Rietkloof
sp. cf. verrucosa	Whitehouse 89	10-Mar-00	Willowmore District, Baviaanskloof, Willowmore end, Adamskraal, Nuwekloof
sp. cf. virgata	Whitehouse 264	16-Sep-01	Cape Peninsula, Silvermine Nature Reserve, nek between Constantiaberg and Noordhoek Peak, above Blackburn Kloof,
spathulata	Whitehouse 287	21-Nov-01	Qwa-Qwa District, Witsieshoek, N slopes of Sentinel peak, zig-zags on Sentinel hiking trail
stricta	Whitehouse 36	3-Jul-99	Bredasdorp District, Hermanus to Elim, Koue Berg Pass
strigosa	Whitehouse 85	2-Mar-00	Paarl District, Bain's Kloof, S face of Limietkop, above stream
strobilifera	Whitehouse 30	22-Mar-99	Oudtshoorn District, De Hoek campsite, Hoeksrivier, where path to Gouekrans Hut crosses
subsetacea	Whitehouse 17	28-Feb-99	Caledon District, Perdeberg Trail from Highlands State Forest, in valley just before ascent to Perdeberg
tenuis	Whitehouse 40	3-Jul-99	Bredasdorp District, along road to Rietfontein, between Vlooi Kraal & Rietfontein
teretifolia1	Whitehouse 135	14-Sep-00	Paarl District, lower slopes of Perdeberg, just above Lemoenkloof Farm
teretifolia2	Whitehouse 50	24-Sep-99	Porterville District, Grootfontein Farm, near Beaverlac campsite, track to Olifants R along Ratel R
theodori-friesii	Whitehouse 88	7-Mar-00	Cape Peninsula, Table Mt., Valley of Isolation, N slopes
tricuspidata	Whitehouse 16	21-Feb-99	Paarl District, Wemmershoek Mts, top end of Elandsvlakte, along stream behind Wemmershoek Tafelberg, W of Olifants Peak
triloba	Whitehouse 251	18-Aug-01	Clanwilliam District, Cederberg Mts, N of Algeria, Nieuwoudt Pass, opposite forester's village
tuberculata	Whitehouse 48	9-Aug-99	Paarl District, Franschhoek Pass, car park at summit of pass at start of path to Du Toit's Kop
uncinata	Whitehouse 249	17-Aug-01	Clanwilliam District, Cederberg Mts, S of Algeria, just S of summit of Uitkyk Pass
varians	Whitehouse 281	18-Oct-01	Caledon District, Riviersonderend Mts, Die Galg, at end of old pass as trail descend down in to Boesmanskloof
verrucosa	Whitehouse 162	21-Oct-00	Prince Albert District, Swartberg Mts, above waterfall below Gamkaskloof road, at top end of Grootkloof
versiformis	Whitehouse 145	27-Sep-00	Caledon District, Kogelberg, S slopes of Mt. Horeb, Kleinmond-Highlands road
virgata	Whitehouse 267	22-Sep-01	Tulbagh District, Bergplaas Farm, path up to Sneeugat, around Nooiensverdriet
viridis	Whitehouse 273	10-Oct-01	Caledon District, Kogelberg Nature Reserve, Kogelberg Trail, W facing gulley at NW end of Platberg
weimarckii	Whitehouse 187	27-Jan-01	Worcester District, Matroosberg, Spekrivierskloof, stony E slopes above Ski Hut