

**MOLECULAR PHYLOGENETICS, SYSTEMATICS, REPRODUCTIVE
SAFEGUARDING AND CHROMOSOME EVOLUTION OF THE GENUS
LACHENALIA (ASPARAGACEAE: SCILLOIDEAE)**

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Doctor of Philosophy

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Lachenalia viridiflora in Saldanha Granite Strandveld, St Helena Bay, Western Cape (upper left). *L. nardousbergensis* in Cederberg Sandstone Fynbos, Pakhuis Pass, Western Cape (upper right). *L. aloides* in Swartland Granite Renosterveld, Atlantis, Western Cape (lower right). *L. comptonii* in Ceres Shale Renosterveld, Klein Roggeveld Mountains, Western Cape (lower left). Photographs: Graham Duncan.

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ABSTRACT

The genus *Lachenalia*, comprising 142 species and belonging to the monocotyledonous family Asparagaceae, is morphologically diverse and the most species-rich member of the family in southern Africa. Past attempts to infer a well-resolved and robustly supported phylogenetic tree using Sanger sequencing of candidate loci and/or morphological characters have been largely unsuccessful. A new phylogenetic hypothesis for *Lachenalia* is presented, using multi-locus sequence data sampled from living material of 132 taxa via maximum likelihood and coalescent-based species tree estimation (ASTRAL), as applied to 378 hybrid-enrichment loci. This resolves species-level relationships with good support, thereby providing a sound basis for a new infrageneric classification, revealing several new taxa which are described here, and, most importantly, establishing a solid framework for downstream evolutionary studies. A dated version of the multi-locus phylogenetic tree is employed here to test the hypothesis that pollen limitation, resulting from a low abundance of insect pollinators, promotes the evolution of reproductive safeguarding traits (self-compatibility, autonomous selfing, bird pollination and clonal propagation) in low insect abundance contexts (i.e., infertile fynbos heathland, and species which flower outside of the austral spring season). Ancestral state reconstructions identify an association with non-fynbos vegetation and spring-flowering as ancestral, with the transition to fynbos and non-spring flowering having taken place multiple times. Ordinary and phylogenetic regressions suggest that bird pollination and self-compatibility are selected for in fynbos and in non-spring flowering lineages, with autofertility being positively associated with non-spring flowering. These patterns support interpretation of these traits as reproductive safeguarding adaptations under reduced insect pollinator abundance. The dated multilocus phylogenetic tree is also used to explore the evolution of cytotype and genome size in *Lachenalia*. Novel genome size data for 124 species are reported and reconciled with existing karyotype data to show that genome size variation is largely a product of polyploidization, with polyploidy yielding lineages that are mostly short-lived and recent. By contrast, dysploid change, which is frequent in *Lachenalia* but has little impact on genome size, yields lineages that persist through time and diversify. *Lachenalia* describes a dysploid reduction series with a high ancestral base number.

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CHAPTER 1. INTRODUCTION

1.1. Utility of Next-Generation Sequencing (NGS) data for the resolution of phylogenetic relationships, especially in recently radiating lineages

Since the 1990s, the science of phylogeny inference has relied extensively on DNA sequence data, with comparison of homologous sequence sampled from a set of species proving a powerful tool for the inference of phylogenetic relationships (Doyle and Davis 1998). While the polymerase chain reaction (PCR; Mullis and Faloona 1987) and the Sanger sequencing method (Sanger and Coulson 1975; Sanger et al. 1977) have traditionally been used to sample and sequence candidate loci, this approach, while serving systematics effectively for several decades, is time consuming and may fail to resolve species relationships where lineage divergence has been rapid and/or where there is significant discordance between gene trees (Schuster 2008; Lemmon et al. 2012). Despite being foundational to much of our knowledge of species relationships, Sanger sequencing has in recent years been superseded by the application of high-throughput, next-generation sequencing (NGS) technologies (Kawashima et al. 2005; Lemmon et al. 2012; Behjati and Tarpey 2013; Slatkow et al. 2018; Hu et al. 2021) and these have revolutionized phylogenetics. These technologies enable hundreds of thousands of genetic loci to be simultaneously sequenced at comparatively low cost, thus increasing the amount of information that can be gathered and the number of taxa that can be sampled, and improving the potential for phylogenetic resolution (Soltis et al. 2010; Lemmon et al. 2012; Lemmon and Lemmon 2013). Unsurprisingly, the data generated by NGS have been used successfully to resolve species level relationships in clades in which the application of Sanger sequencing had previously yielded little success (e.g., Fragoso-Martínez et al. 2017; Wanke et al. 2017). Besides massively increasing the amount of character data, these technologies enable the application of species tree inference methods which exploit variance in the trees inferred from independently evolving loci. At the same time, the vast quantities of data they yield often means that sequence assembly and parsing is done bioinformatically, which can compromise the quality of the individual gene trees inferred (e.g., Sayyari et al. 2017).

High-throughput DNA sequencing methodology has rapidly evolved over the past 20 years with the introduction of new methods (Slatkow et al. 2018). For example, it is now possible to sequence a few hundred genes from multiple species within a clade using a novel, hybrid-enrichment, target-capture approach (anchored hybrid enrichment; Lemmon et al. 2012) that

targets highly conserved regions as well as less-conserved flanking sites. Using this method, DNA samples first are converted into sequencing libraries by random shearing of DNA and ligation of sequencing adaptors to the ends of the resulting fragments. The regions of interest are then captured using oligonucleotide probes that are designed to hybridize to specific genomic regions of interest (Lemmon et al. 2012). The use of hybrid-enrichment to target loci for sequencing offers several advantages for the inference of species-level phylogenetic relationships (Lemmon and Lemmon 2013). Beyond being affordable, the datasets generated by this approach offer improved power to resolve species relationships in difficult biological scenarios, this being achieved in three main ways (Lemmon et al. 2012). Firstly, recent studies (e.g., Leaché and Rannala 2011) demonstrate the value of sequencing more loci, with tens or even hundreds of nuclear loci sometimes being required to resolve species relationships in the face of coalescent stochasticity, the latter being especially problematic in recent, rapid radiations and recent divergences (Maddison and Knowles 2006; Edwards et al. 2007; Huang et al. 2010). Secondly, an increase in power and estimation accuracy is also obtained by increasing the number of taxa sampled, this breaking up long branches (Zwickl and Hillis 2002) and helping to resolve gene tree discordance due to coalescent stochasticity (Huang et al. 2010). Thirdly, power and accuracy are also increased by including loci that are informative at appropriate time scales (e.g., Townsend and Leuenberger 2011), this being achieved by selecting specific genes which evolve at rates that maximise the probability of resolving branches at a particular time scale. Target/hybrid sequence capture has been shown to be useful for resolving rapid, recent angiosperm radiations, for example in the tribe Cardueae (Asteraceae; Herrando-Moraira 2018), Neotropical bellflowers (Campanulaceae; Bagley et al. 2020), and at deep phylogenetic levels in the Asterales (Benítez-Villaseñor et al. 2023).

In phylogenetics, reconstructed gene trees frequently disagree with the species tree, with the topologies of individual gene trees also being expected to vary (Pamilo and Nei 1988; Doyle 1992, 1997). In the case of orthologous genes, this has two main causes, the retention of ancestral polymorphisms, resulting in incomplete lineage sorting, and lateral gene transfer, the latter in plants typically being a consequence of hybridization (Doyle 1992, 1997; Baum 2007). Several tests have been used to demonstrate significant gene tree incongruence and it is now widely accepted that gene tree variance must be accommodated when inferring species relationships (e.g., Baum 2007; Edwards 2009; Maddison and Knowles 2006; Knowles 2009). The multispecies coalescent has emerged as a central theoretical framework

for achieving this, being central to most modern methods of species tree inference (e.g., Liu et al. 2009; Heled and Drummond 2010; Mirarab et al. 2014, 2021; Rannala and Yang 2017; Flouri et al. 2018).

Whole genome duplication, or polyploidization, presents potential challenges in genomics and species tree inference, owing to the presence of additional allelic copies at each locus and the independent assortment of alleles at different loci (e.g., Ning et al. 2024). In principle, however, these challenges should be much greater under allopolyploidy than autopolyploidy, owing to the presence of allelic copies from different parent species and the potential for recombination and reticulation (e.g., Oxelman et al. 2017). In the case of autopolyploidy, by contrast, whole genome duplication should merely supply additional copies of the same uniparentally inherited alleles. A comparison of alternative sequencing approaches reveals target-enrichment sequencing to hold the greatest promise for inferring the phylogenetic relationships of polyploid taxa, particularly where these sequences are obtained using fresh leaf material (Ning et al. 2024).

1.2. Utility of highly resolved and well-supported phylogenetic trees for taxonomic classification and for evolutionary research

Up until the late 20th century, angiosperm classification had traditionally been based mainly on morphological similarity (e.g., Cronquist 1981). The development of DNA amplification and sequencing technologies in the 1970s and 1980s, however, transformed angiosperm phylogenetics from the early 1990s (e.g., Chase et al. 1993) onwards, in providing access to thousands of nucleotide characters encoded in both the nuclear and chloroplast genomes. This was followed by a rapid growth in the development of computational methods for analyzing large amounts of sequence data to yield highly resolved and well supported phylogenetic trees. The latter are useful for taxonomic classification because, in revealing groups defined by shared ancestry (i.e., clades), they provide a valuable framework for prediction (Schrire and Lewis 1996; Backlund and Bremer 1998). As such, they can be used both to reveal patterns of trait evolution, to test evolutionary hypotheses, and to develop workable and predictive systems of classification. In particular, they permit the recognition of supraspecific taxa (e.g., genera, sections and subsections), whose monophyly renders them evolutionarily meaningful, and allow the discovery of morphological synapomorphies which enable these taxa to be diagnosed and identified.

1.3. *Lachenalia*, and its historical intractability from a phylogenetic perspective

With 142 species, *Lachenalia* J.Jacq. ex Murray is the most species-rich member of the monocotyledonous family Asparagaceae within the ecologically diverse southern African region (Duncan et al. 2022). Members of the genus are morphologically diverse, showing variation in: plant stature and habit (low-growing and geoflorous up to 400 mm high and more robust); leaf shape (linear to ovate); leaf indumentum (glabrous to stellate-hairy); and leaf number (one to 18). *Lachenalia* also has a diversity of reproductive structures, ranging from condensed to elongate-racemose or spicate, while flowers vary in both perianth colour (e.g., white, yellow, blue, purple, red, or combinations of colours) and form (e.g., widely or narrowly bell-shaped, urn-shaped or tubular), and in the degree of exsertion of the stamens (included to strongly exserted) (Duncan 2012a). An extraordinary level of cytological variation also exists within *Lachenalia*, including differences in basic chromosome number and variable ploidy level (e.g., Crosby 1986; Johnson and Brandham 1997; Kleynhans et al. 2012; Hamatani and Tagashira 2019). Wild hybridization appears to be rare in *Lachenalia*, however, with only one record of a natural hybrid ever being recorded (Duncan 2012a). Several horticulturally outstanding species have been crossbred in cultivation, with cytogenetic and phylogenetic data playing an essential role in guiding crossbreeding strategies (Kleynhans et al. 2009, 2012).

The existence of a practical infrageneric classification of *Lachenalia* has long been needed, on account of the large size of the genus. Building on the foundation laid by Kew botanist J.G. Baker's work, which grouped the species of *Lachenalia* into four (Baker 1871; 29 species) or five (Baker 1897; 45 species) morphologically defined subgenera, recent attempts to establish a phylogenetic system of classification have been hampered by the intractability of species-level phylogenetic relationships within *Lachenalia*. Efforts to infer relationships using morphology (Duncan 2005; Duncan et al. 2005), for example, have yielded unconvincing results owing to a paucity of phylogenetically informative characters and the homoplasious nature of character variation, with successive character weighting (Farris 1969) being needed to achieve resolution. Likewise, low variation in the DNA sequence regions (*trnL-trnF*, ITS) targeted for phylogenetic work using a traditional Sanger sequencing approach has resulted in phylogenetic trees that are poorly resolved and supported (Spies 2004; Hamatani et al. 2008, 2012), even if the clades resolved do show some correspondence to patterns of karyotypic variation.

1.4. Thesis objectives, outline, and structure

This thesis comprises a series of studies aimed at addressing several challenges and questions pertaining to the genus *Lachenalia*, presented in four data chapters (Chapters 2–5). The thesis contains three published papers (Chapters 2–4), and one completed paper that has yet to be submitted for publication (Chapter 5). The primary objectives of the thesis are five-fold. The first (Chapter 2) is to infer a new, well-resolved and robustly supported, multilocus, species-level phylogenetic hypothesis for *Lachenalia* which, beyond confirming the monophyly of the genus, identifies its closest relatives as well as its internal relationships. Based on this hypothesis, a second objective (also Chapter 2) is to develop a new monophyly-based sectional classification, with the identification of morphological synapomorphies which can be used to diagnose the various sections, and to assign all unsampled taxa to sections. A third objective (Chapter 3) is to formally describe and name all taxa identified as new species by the phylogenetic data, and to adjust the taxonomic status of others. Using a dated version of the multilocus phylogeny as a framework for analysis, a fourth objective (Chapter 4) is to explore the evolution of reproductive safeguarding in *Lachenalia*. Specifically, this study aims to test the hypothesis that self-compatibility, autonomous selfing, bird pollination, and clonal propagation represent reproductive safeguarding mechanisms whose evolution has been stimulated by a low abundance of insect pollinators in fynbos heathland vegetation, and in lineages which flower outside the austral spring insect abundance peak. A fifth and final objective (Chapter 5) is to use the phylogeny to explore the evolution of chromosome base number, ploidy, and genome size in *Lachenalia*. Specifically, this study seeks to test the hypotheses: that *Lachenalia* describes a dysploid reduction series; that changes in chromosome base number (dysploidy) have promoted diversification in *Lachenalia*; that genome size variation is largely associated with polyploidizations, the latter yielding lineages that are short-lived and recent, consistent with its interpretation as an evolutionary dead-end; and that low-nutrient environments and/or environments experiencing a short growing season select for small genomes and low ploidy.

CHAPTER 2. MOLECULAR PHYLOGENETICS AND SYSTEMATICS

Published paper: A new sectional classification of *Lachenalia* (Asparagaceae) based on a multilocus DNA phylogeny. *Taxon* 71: 563–586 (2022). <https://doi.org/10.1002/tax.12683>

Abstract

Lachenalia (Asparagaceae; Scilloideae; Hyacintheae) is a large and morphologically diverse genus of more than 140 bulbous species endemic to southern Africa. Previous attempts to infer a well-resolved and robustly supported phylogeny of *Lachenalia* using Sanger sequencing of candidate loci and/or morphological characters have been largely unsuccessful. Consequently, the current infrageneric classification is artificial, and there is a need to explore alternative avenues to produce a phylogenetic classification. In this paper, we present a novel phylogenetic hypothesis for *Lachenalia* inferred using maximum likelihood and coalescent-based species tree estimation (ASTRAL) as applied to 378 hybrid-enrichment loci. Our tree is well resolved and well supported, providing strong support for a monophyletic radiation of the genus in southern Africa and a solid foundation for a revised infrageneric classification. The well-supported placement of *L. isopetala* Jacq. as sister to *Lachenalia* + *Massonia* supports the establishment of a new monotypic genus, *Pseudolachenalia*, to accommodate this species. Conversely, the inclusion of species previously classified as *Polyxena* within the *Lachenalia* clade supports the transfer of these species to *Lachenalia*. Within *Lachenalia*, the delimitation of subgenera and sections is complicated by the highly imbalanced character of the phylogeny and by the high levels of homoplasy shown by most morphological characters traditionally used to delimit species in this group. Nonetheless, we propose an infrageneric taxonomy comprising 10 morphologically distinct, monophyletic sections. The largest of these, *L. sect. Lachenalia*, is further divided into 13 more-or-less diagnosable, monophyletic subsections. Keys to the sections of *Lachenalia*, and to the subsections of *L. sect. Lachenalia*, are provided.

Keywords: high-throughput sequencing; *Lachenalia*; molecular systematics; southern Africa

2.1. Introduction

Lachenalia J.Jacq. ex Murray is a large, southern African genus of bulbous geophytes belonging to the tribe Hyacintheae of the family Asparagaceae, subfamily Scilloideae (APG

III, 2009; Chase et al. 2009; Christenhusz et al. 2017). It is endemic to South Africa and Namibia, where it occurs primarily in the western and southwestern winter-rainfall zones of both countries (Duncan 2012a, b, 2013). In South Africa, however, it also extends inland and eastwards to the summer-rainfall areas of the Northern and Eastern Cape, while in Namibia, it extends to the mountainous summer-rainfall areas of southern Namibia (Duncan 2005, 2012a). With a current total of 136 species (Duncan 2017), *Lachenalia* is the most species-rich member of the family within this ecologically diverse region of southern Africa. The species are deciduous and almost all are winter-growing, with the sole exception of *L. pearsonii* (R.Glover) W.F.Barker, a summer-growing species from the Groot Karasberge of southern Namibia (Duncan and Condry 2013) (Fig. 1A). Although some species are widespread and common, e.g., *L. bulbifera* (Cirillo) Engl. (Fig. 1G), the majority are localized, with several being rare and/or threatened such as the critically endangered *L. convallarioides* Baker (Fig. 1H) (Victor and Dold 2005).



Figure 1. Morphological variation, habitat and pollination in *Lachenalia* and *Pseudolachenalia*. *Lachenalia pearsonii* from southern Namibia, the only summer-growing species (A); the geoflorous *L. pusilla* (B); the urn-shaped flowers of *L. elegans* (C); *L. trichophylla* leaf upper surfaces covered with stellate trichomes (D); the xerophytic

L. zebrina on stony clay flats in Hantam Karoo (E); the pyrophytic *L. sargeantii* after a fire in Overberg Sandstone Fynbos (F); the widespread *L. bulbifera* in Cape Flats Dune Strandveld (G); The critically endangered *L. convallarioides* from the Eastern Cape (H); Cape honeybee *Apis mellifera* subsp. *capensis* visiting a flower of *L. pallida* (I); Southern Double-collared Sunbird visiting a flower of *L. viridiflora* (J); Painted Lady butterfly *Vanessa cardui* visiting a flower of *L. corymbosa* (K); straw-like, fasciculate bulb neck apices of *Pseudolachenalia isopetala* (L); arrow showing unique, papery white bract of *P. isopetala* (M); habitat of *P. isopetala* on arid, stony clay flats in Roggeveld Karoo (N); solitary bee *Amegilla nivea* visiting a flower of *P. isopetala* (O). Photographs: Graham Duncan.

Lachenalia is morphologically diverse, showing variation in: plant stature and habit, which varies from low-growing and geoflorous in *L. pusilla* Jacq. (Fig. 1B) to taller (up to 400 mm high) and more robust in *L. suaveolens* (W.F.Barker) G.D.Duncan; leaf shape, which ranges from linear (e.g., *L. pearsonii*; Fig. 1A) to ovate (e.g., *L. trichophylla* Baker; Fig. 1D); leaf indumentum, which ranges from glabrous (e.g., *L. pusilla*; Fig. 1B) to stellate-hairy (e.g., *L. trichophylla*; Fig. 1D); and leaf number, which varies from 1 to 18. In addition, numerous species are xerophytic (e.g., *L. zebrina* W.F.Barker; Fig. 1E) and three species, including *L. sargeantii* W.F.Barker (Fig. 1F), are pyrophytes (Duncan and Edwards 2007). *Lachenalia* is, however, best appreciated for the diversity of its reproductive structures. Inflorescences range from condensed (e.g., *L. pusilla*; Fig. 1B) to elongate-racemose or spicate (most species; Fig. 1A,C,E–H), while flowers vary in both perianth colour and form, and in the degree of exertion of the stamens. For example, whereas the perianths of *L. elegans* W.F.Barker (Fig. 1C) and *L. bulbifera* (Fig. 1G) are purplish or red and tubular to narrowly urn-shaped, those of *L. pearsonii* (Fig. 1A) and *L. convallarioides* (Fig. 1H) are white and widely or narrowly bell-shaped, respectively. The high level of floral diversity in *Lachenalia* is probably associated with its use of diverse pollinators, with three main pollination syndromes being recognized: mellitophily (Fig. 1I), ornithophily (Fig. 1J) and generalist pollination, involving monkey beetles, blister beetles, hoverflies, march flies, butterflies (Fig. 1K), day-flying moths (Duncan 2005, 2012a) and other insects.

An extraordinary level of cytological variation exists within *Lachenalia*, including differences in basic chromosome number, variable ploidy levels, and the presence of B-chromosomes (supernumerary chromosomes) in some species. Basic chromosome numbers include $x = 5–16, 18, 20–22, 28$ and, while diploidy is the norm, tetra-, hexa-, octo- and higher-order polyploids occur in several species, with B-chromosomes being reported from eight species (e.g., Crosby 1986; Johnson and Brandham 1997; Kleynhans et al. 2012;

Hamatani and Tagashira 2019). Interestingly, wild-hybridization appears to be rare in *Lachenalia*, with only one record of a natural hybrid, a cross between two long-tubed species, *L. callista* G.D.Duncan & T.J.Edwards and *L. vanzyliae* (W.F.Barker) G.D.Duncan, ever being recorded (Duncan 2012a). The extremely low incidence of natural hybrids may reflect the importance of differences in chromosome number, pollinator type, flowering phenology and habitat preference as breeding barriers (Duncan 2005, 2012a). Several horticulturally outstanding species have, however, been crossbred in cultivation, with cytogenetic and phylogenetic data playing an essential role in guiding crossbreeding strategies (Kleynhans et al. 2009, 2012).

The generic and infrageneric classification of *Lachenalia* has traditionally been based on morphology and, up until the late nineteenth century, only two monographs had been produced, both by J.G. Baker at Kew. Prior to Baker's monographs, species currently included in *Lachenalia* had been described under 10 generic names, namely *Chloriza* Salisb., *Coelanthus* Willd. ex J.A.Schultes & J.H.Schultes, *Himas* Salisb., *Lachenalia*, *Monoestes* Salisb., *Orchiops* Salisb., *Periboea* Kunth, *Platyestes* Salisb. and *Polyxena* Kunth. In his first monograph, Baker (1871) placed *Coelanthus* and all of Salisbury's genera in synonymy under *Lachenalia*, reassessing *Chloriza* and *Orchiops* as subgenera. In addition, he described *Brachyscypha* Baker to accommodate the single geoflorous species, *B. undulata* (Thunb.) Baker (= *Lachenalia pusilla*). In his second monograph, Baker (1897) maintained *Chloriza* and *Orchiops* as subgenera, and reassessed *Brachyscypha* as a subgenus. Most subsequent taxonomic work focused on the morphological description of new species and was undertaken mainly by W.F. Barker (e.g., Barker 1978, 1983, 1984, 1987, 1989) and G.D. Duncan (e.g., Duncan 1993, 1996, 1997, 1998, 2012a), with some of the latter's work done in conjunction with T.J. Edwards (e.g., Duncan and Edwards 2002, 2006, 2007). Three new species have been added since (Duncan and Helme 2014; Duncan 2017), bringing the current total to 136.

Phylogenetic studies, making use of plastid sequences and seed morphological characters, have largely confirmed the monophyly of *Lachenalia* as conceptualized by Baker, necessitating only the inclusion of *Periboea* and *Polyxena*, which are embedded within a strongly supported *Lachenalia* clade (Pfosser and Speta 1999; Wetschnig et al. 2002; Pfosser et al. 2003; Manning et al. 2004; Buerki et al. 2012). Plastid sequences also resolve the phylogenetic position of *Lachenalia*, indicating a sister relationship with *Massonia* Thunb. ex Houtt., a genus of 30 species (Manning 2019), within the tribe Massonieae of Hyacinthaceae

(= tribe Hyacintheae of Asparagaceae). Also included in this tribe is *Veltheimia* Gled., a genus of two species, which has been identified as sister to *Lachenalia* (including *Periboea* and *Polyxena*) + *Massonia*. Although *Massonia* has traditionally been distinguished from *Lachenalia* by the basal fusion of its filaments and by its large, leafy basal bracts (Baker 1897), it is more accurately differentiated by its capitate-corymbose or subspicate inflorescences, by its basal bracts typically being at least as long as or longer than the flowers, and by its bell-shaped or tubular flowers with ecaudate tepals (Manning 2019). *Veltheimia*, on the other hand, has traditionally been distinguished from *Lachenalia* by its very short perigone lobes, by its relatively long perigone, by its large, broadly winged capsules, and by its large bulbs (Baker 1897). In addition, *Veltheimia* differs from *Lachenalia* by its perennial fleshy roots, by its possession of bracteoles and almost completely fused perigone lobes, and by its inflated capsules (Duncan and Visagie 2009; Barker et al. 2019).

A workable infrageneric classification of *Lachenalia* has long been desirable on account of the large size of the genus. Building on the foundation laid by Baker's work, which grouped the species of *Lachenalia* into four (Baker 1871; 29 species) or five (Baker 1897; 45 species) morphologically defined subgenera, recent attempts to establish a phylogenetic system of classification have been hampered by the intractability of species-level phylogenetic relationships within *Lachenalia*. Efforts to infer relationships using morphology (Duncan 2005; Duncan et al. 2005), for example, have yielded unconvincing results owing to a paucity of phylogenetically informative characters and the homoplasious nature of character variation, with successive character weighting (Farris 1969) being needed to achieve resolution. Likewise, low variation in the DNA sequence regions (*trnL-trnF*, ITS) targeted for phylogenetic work using a traditional Sanger sequencing approach (Spies 2004; Hamatani et al. 2008, 2012) has resulted in phylogenetic trees that are poorly resolved and supported, even if the clades resolved do show some correspondence to patterns of karyotypic variation. In that context, the most recent infrageneric classification of *Lachenalia* (Duncan 2012a), which addresses 133 species, is of necessity artificial, being aimed primarily at producing workable identification keys. That treatment splits *Lachenalia* into two subgenera based on floral symmetry, with subgenus *Lachenalia* having a zygomorphic perianth and subgenus *Polyxena* (Kunth) G.D.Duncan an actinomorphic perianth. *Lachenalia* subg. *Lachenalia* is further subdivided into five sections on the basis of perianth shape variation, which varies more or less discontinuously between sections, as follows: tubular (section *Lachenalia*), oblong bell-shaped (section *Oblongae* G.D.Duncan), narrowly bell-shaped (section *Angustae*

G.D.Duncan), widely bell-shaped (section *Latae* G.D.Duncan) and urn-shaped (section *Urceolatae* G.D.Duncan).

The central objective of our study is to infer a species-level phylogenetic hypothesis for *Lachenalia*, using multilocus sequence data obtained via a hybrid-enrichment, target-capture approach (anchored hybrid enrichment [AHE]; Lemmon et al. 2012). The use of hybrid-enrichment to target loci for sequencing offers several advantages for the inference of species-level phylogenetic relationships (Lemmon and Lemmon 2013) and has been used successfully to resolve species-level relationships in several other clades where the application of Sanger sequencing has had limited success (Fragoso-Martínez et al. 2017; Wanke et al. 2017). For the purpose of inferring species-level phylogenetic relationships in *Lachenalia*, we employ a combination of species tree summary methods (ASTRAL; Mirarab et al. 2014; Mirarab and Warnow 2015) and concatenation-based maximum likelihood inference (RAxML; Stamatakis 2006). A second objective of the current study is to use the phylogenetic tree presented here as a framework for a revised infrageneric classification of the genus. For this purpose, we use ancestral character state reconstruction of morphological characters to identify putative synapomorphies that characterize the major subclades within *Lachenalia*. Although we adhere to the principle of taxonomic monophyly as a primary criterion in taxon delimitation, we also invoke morphological diagnosability as an auxiliary principle in determining which clades warrant formal recognition.

2.2. Materials and methods

2.2.1. Sampling

Actively growing leaf material of 132 *Lachenalia* accessions, representing 118 species plus a further 5 subspecies, were sampled from the wild-provenance collection of indigenous bulbous taxa in cultivation in the bulb nursery at Kirstenbosch National Botanical Garden (Appendix 1). The balance of the *Lachenalia* samples were replicate collections of selected widespread and/or morphologically variable species. In addition, we sampled, as outgroups, *Massonia bifolia* (Jacq.) J.C.Manning & Goldblatt, *M. depressa* Houtt., *M. pustulata* Jacq. and *Veltheimia capensis* (L.) DC, giving a total of 136 accessions. Voucher specimens of sampled accessions are lodged in the Compton Herbarium (NBG; Appendix 1).

2.2.2. DNA isolation and sequencing

DNA isolation from plant tissue was done according to the protocol of Healey et al. (2014). Two grams of fresh leaf material were frozen and pulverized in liquid nitrogen using a mortar and pestle. As recommended by Healey et al. (2014), all steps were performed in 50 ml Falcon tubes, with the final DNA pellets eluted in 200 µl of Tris-EDTA (TE) buffer. Where pellets did not resuspend fully, an additional amount (typically 100–200 µl) of TE buffer was added until the pellet was fully dissolved. The DNA samples were then purified using the Qiagen Genomic DNEasy Clean and Concentrator Kit (Qiagen N.V., Venlo, Nederland) according to the manufacturer's instructions. Thereafter, the quality and quantity of DNA were assessed, both by running and visualizing samples in a 1% TBE agarose gel, containing ethidium bromide, and using a NanoDrop spectrophotometer.

2.2.3. Sequence data collection

DNA sequences were collected in collaboration with the Center for Anchored Phylogenomics (www.anchoredphylogeny.com). Libraries were prepared following a protocol originally developed by Meyer and Kircher (2010) and later modified by Prum et al. (2015). Extracted DNA was quantified using a Qubit fluorometer (ThermoFisher Scientific, Tallahassee, Florida, U.S.A.), then sonicated to a size distribution of 250–400 bp using a Covaris ultrasonicator. Following end repair, common Illumina adapters were ligated onto the blunt ends, after which sample-specific 8 bp single indexes were introduced using an indexing PCR step. Libraries were pooled in equal concentration in three batches of ca. 16 samples, prior to

enrichment using the AHE Angiosperm kit (v.1) described in Buddenhagen et al. (2016), Mitchell et al. (2017) and L  veill  -Bourret et al. (2018). This kit targets 517 AHE loci. The AHE Angiosperm kit was derived from 25 genomes from species across the Angiosperm clade. The targets for this kit are exons from genes that are low-copy and align well across the angiosperms due to their intermediate levels of variation. These loci were not selected based on function. Although these target exons are somewhat short (each typically <500 bp each), the method also enriches for flanking regions that contain higher levels of sequence variation that is useful for resolving shallow-level divergences. Enriched library pools were then pooled into a single sequencing pool (again, in equal concentration), which was sequenced on a single Illumina HiSeq2500 sequencing lane with a paired-end 150 bp protocol.

2.2.4. Read assembly

Reads were processed following Hamilton et al. (2016). Reads passing the Cassava high-chastity filter were demultiplexed with no mismatches tolerated. Paired reads were then checked for overlap and merged when appropriate, following Rokyta et al. (2012). This process corrects sequencing errors and removes sequencing adapters. Reads were then assembled using the quasi-de novo assembler described in Hamilton et al. (2016), with probe region sequences from *Arabidopsis thaliana* (L.) Heynh., *Billbergia nutans* H.Wendl. ex Regel and *Carex lurida* Wahlenb. serving as divergent references. Following assembly, consensus sequences were produced for assembly clusters containing at least 20 reads. Orthology was assessed for each locus using a neighbor-joining approach that utilized alignment-free distance matrices (see Hamilton et al. 2016 for details). Orthologous sequences were aligned using Mafft v.7.023b (Katoh and Standley 2013), then trimmed and masked following Hamilton et al. (2016). Geneious v.R9 (Kearse et al. 2012) was used to manually inspect the trimmed alignments to check for alignment and trimming errors. The bioinformatics pipeline produced 380 raw alignments, averaging 1337 bp in length (min = 741 bp, max = 4665 bp). After trimming and masking, 378 aligned loci remained, averaging 628 bp in length (min = 98 bp, max = 1994 bp). In total, these alignments contained 231,198 sites (77,330 informative). Only 15% of the characters in these alignments were ambiguous. Raw reads were submitted to the Europe Nucleotide Archive under accession PRJEB48649. All alignments, and Newick tree files can be found at Dryad (<https://doi.org/10.5061/dryad.9kd51c5jf>).

2.2.5. Phylogenetic analysis

A phylogenetic tree was estimated by maximum likelihood, as implemented in RAxML v.2.2.3 (Stamatakis 2006), using default settings. DNA was assumed to follow a general time reversible (GTR) model of evolution, with separate models applied to each locus. The GTR model was preferred over the GTR + Γ model because the latter was over-parameterized for most loci. One hundred bootstrap replicates were performed to assess uncertainty in the phylogenetic estimate. Gene trees, estimated for each locus using the same procedure, were then used to estimate a species tree using ASTRAL (Mirarab and Warnow 2015).

2.2.6. Morphological matrix

All species included in the phylogenetic analysis were scored for 76 characters describing variation in the morphology of bulbs, leaves, seeds, inflorescences and flowers (Appendices 2, 3). For this purpose, we made use of Mesquite v.3.6 (Maddison and Maddison 2018). Forty-eight characters were binary and 28 multistate, with 7 of the latter (i.e., leaf number; leaf orientation; ventral perianth segment extension; seed length; seed width; strophiole length; chalazal collar length) being treated as ordered because they are fundamentally quantitative. Where character states were unknown or inapplicable, they were scored as “?”. Characters were then mapped onto the ASTRAL species tree using parsimony ancestral character reconstruction as implemented in Mesquite. The main purpose of this exercise was to identify clade-specific synapomorphies, for which parsimony mapping is commonly used and was deemed adequate.

2.2.7. Distribution polygons

The distribution polygons were based on field experience (GDD) and on herbarium records. They were configured by connecting the most northerly, southerly, westerly and easterly localities of the species comprising each taxonomic section: where a species is restricted to a single locality, this was indicated by a dot.

2.3. Results

The coalescent species tree and total-evidence maximum likelihood tree are largely identical in terms of both topology and branch support (Figs. 2, 3), with most nodes being strongly supported by both analyses (maximum likelihood bootstrap, $BS \geq 90$; ASTRAL quartet

support, $QS > 75$). While a few nodes are poorly supported by either analysis ($BS < 80$; $QS < 50$), some nodes, particularly within clades J_2 (6 nodes), J_4 (1 node) and J_{11} (20 nodes), enjoy reasonable maximum likelihood bootstrap support ($BS \geq 90$) but are either weakly supported or weakly contradicted by ASTRAL ($QS < 50$). Only in three instances is the total-evidence maximum likelihood topology contradicted by the ASTRAL tree with moderate to strong support: (i) ASTRAL identifies *L. thunbergii* G.D.Duncan & T.J.Edwards as sister to *L. quadricolor* Jacq. + *L. aloides* (L.f.) Engl. with $QS = 96$; (ii) ASTRAL identifies *L. flava* Andrews as sister to *L. thunbergii* + *L. quadricolor* + *L. aloides* with $QS = 85$; (iii) ASTRAL identifies clade J_1 as sister to clade J_2 with $QS = 62$.

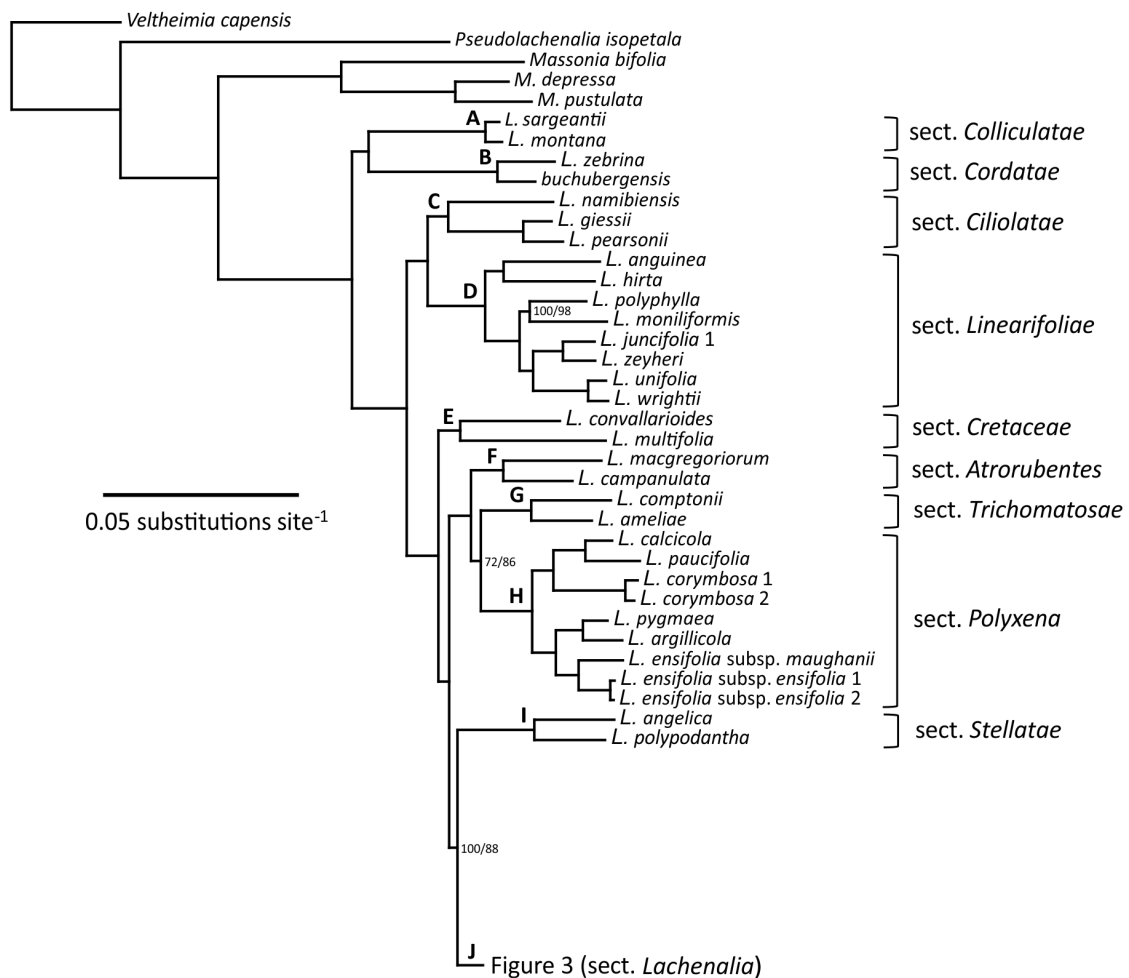


Figure 2. Phylogenetic relationships within genus *Lachenalia* (see Figure 3 for relationships within section *Lachenalia*) inferred using the maximum likelihood criteria as implemented in RAxML. Branch support values (maximum likelihood bootstrap/ASTRAL quartet support) are indicated only on branches for which either support measure was < 100 (i.e., all other nodes have 100/100). Clades corresponding to sections are indicated by letters (A-J) on their subtending nodes and labelled brackets on the right.

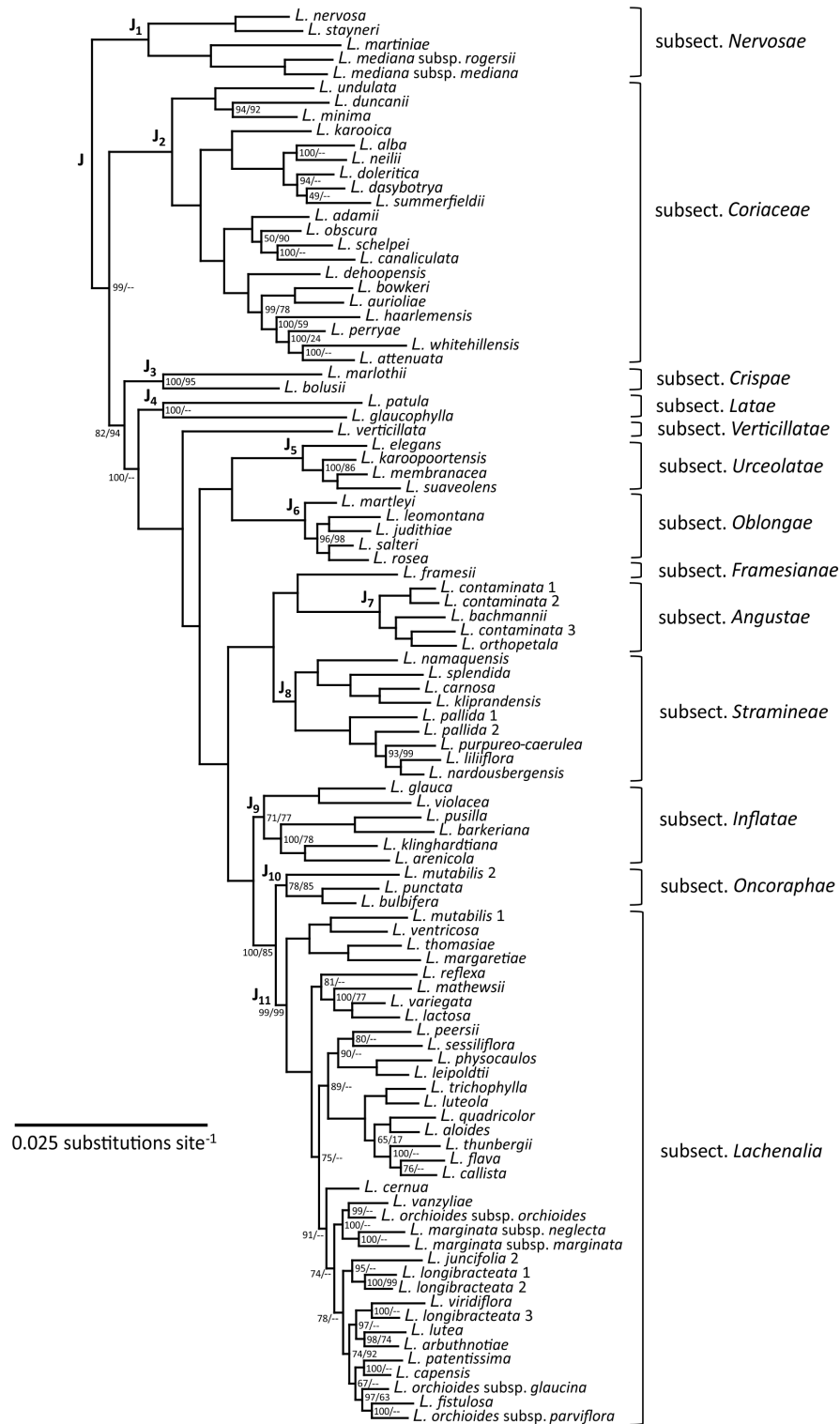


Figure 3. Phylogenetic relationships within section *Lachenalia* inferred using the maximum likelihood criteria as implemented in RAXML. Branch support values (maximum likelihood bootstrap/ASTRAL quartet support) are indicated only on branches for which either support measure was <100 (i.e., all other nodes have 100/100). Clades corresponding to subsections are indicated by letters (J₁ - J₁₁) on their subtending nodes and labelled brackets on the right.

Relative to the four outgroup taxa included (three species of *Massonia* and *Veltheimia capensis*), *Lachenalia* is strongly supported as monophyletic (BS = 100, QS = 100) except for *L. isopetala*, which, dependent on rooting, is resolved either as sister to *Massonia* + *Lachenalia* or as sister to *Veltheimia* with strong support (BS = 100, QS = 100). The monophyly of *Massonia* is also strongly supported (BS = 100, QS = 100). The *Lachenalia* clade (excluding *L. isopetala*) is topologically highly imbalanced, which presents challenges for the delimitation of evenly sized infrageneric taxa, as is perhaps preferable. The 10 sections (nodes A to J), which are defined on the basis of strongly supported monophyly and the possession of diagnostic morphological synapomorphies, are therefore unevenly sized, with nine sections containing between two and eight species and the tenth being much larger (node J: 92 species). Within the latter, we distinguish 13 subsections (nodes J₁ to J₁₁, plus *L. verticillata* W.F.Barker and *L. framesii* W.F.Barker), again based on strongly supported monophyly and the possession of diagnostic morphological synapomorphies. In this case, however, the morphological synapomorphies are slightly more homoplasious and so diagnostically less reliable. The subsections range in size from 1 (*Framesianae*, *Verticillatae*) to 32 species (*Lachenalia*).

Ancestral character state reconstructions revealed most of the morphological characters investigated to be homoplasious. Figures 4 and 5, however, present reconstructions of the least homoplasious characters, which are also used to characterize the sections and subsections delimited in this study.

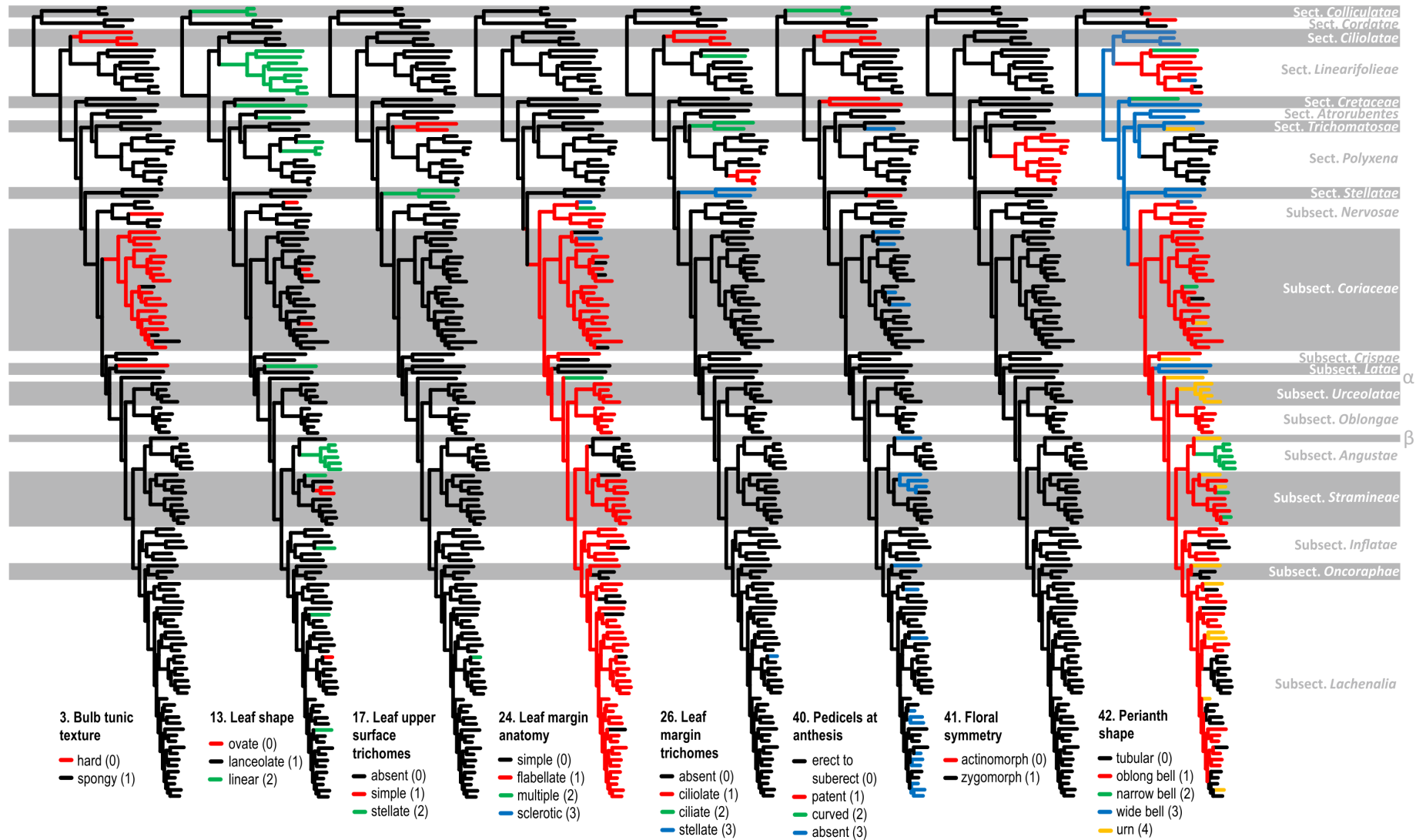


Figure 4. Parsimony (DELTRAN) reconstructions of selected characters (character numbers as in Appendix 2) on the maximum likelihood tree presented in Figures 2 and 3. Sections and subsections (of *Lachenalia* section *Lachenalia*) recognized here are indicated by shaded panels and text on the right, with subsections *Verticillatae* and *Framesianae* respectively represented by “ α ” and “ β ” in the right margin.

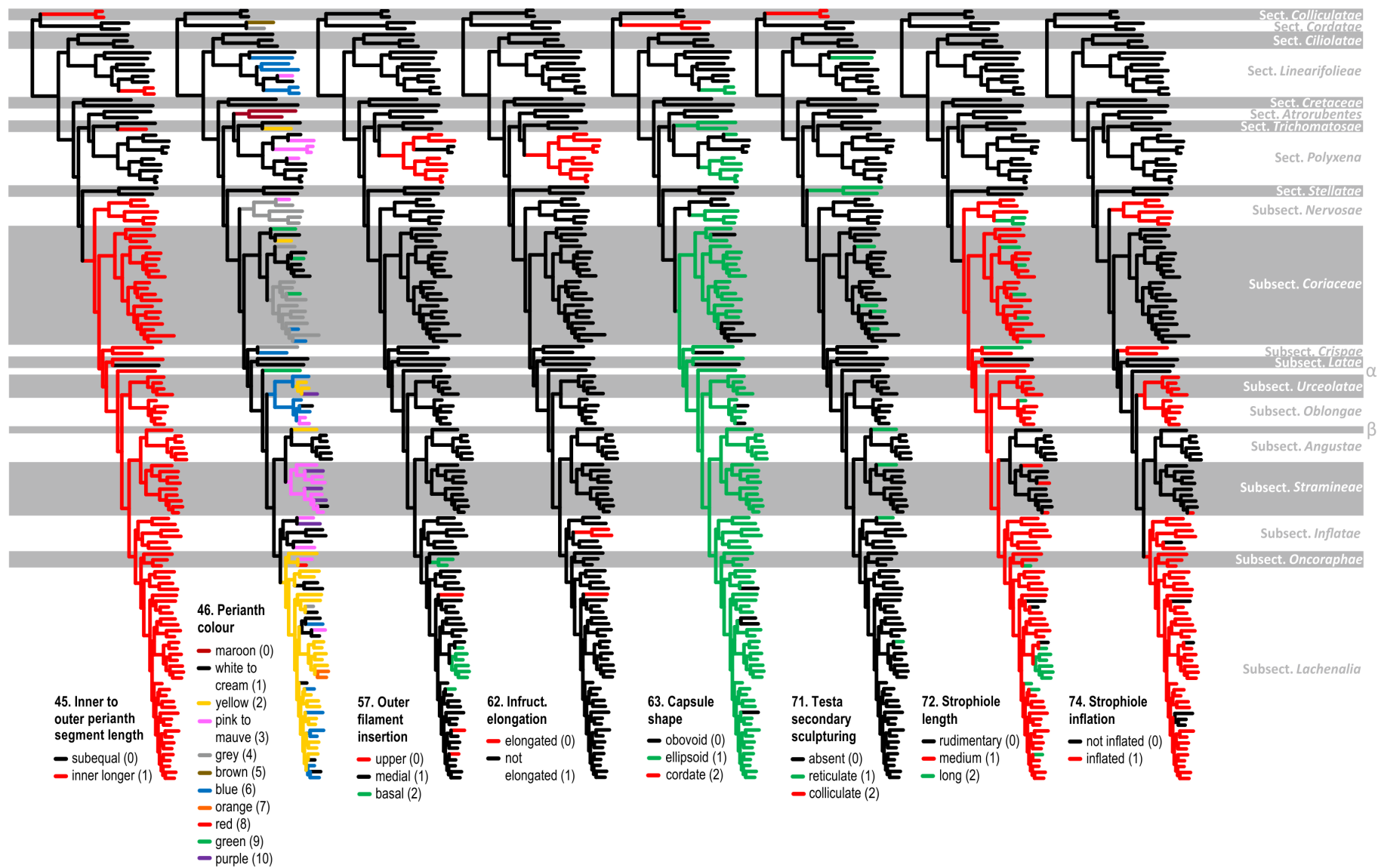


Figure 5. Parsimony (DELTRAN) reconstructions of selected characters (character numbers as in Appendix 2) on the maximum likelihood tree presented in Figures 2 and 3. Sections and subsections (of *Lachenalia* section *Lachenalia*) recognized here are indicated by shaded panels and text on the right, with subsections *Verticillatae* and *Framesianae* respectively represented by “α” and “β” in the right margin.

2.4. Discussion

While our outgroup sampling is limited, strong plastid support for the sister relationship of *Lachenalia* and *Massonia*, and the sister relationship of *Veltheimia* to this pair (Pfosser and Speta 1999; Wetschnig et al. 2002; Pfosser et al. 2003; Manning et al. 2004; Buerki et al. 2012), convincingly roots our phylogenetic analyses either on the branch leading to *Veltheimia*, on the branch leading to *L. isopetala*, or on the branch leading to *Veltheimia* + *L. isopetala*. Under all these arrangements, *L. isopetala* is resolved in an isolated position, distinct from the remainder of *Lachenalia*, which is monophyletic and strongly supported as sister to a monophyletic *Massonia*. Paired with a series of morphological differences that distinguish *L. isopetala* from other *Lachenalia* species, this argues for the segregation of *L. isopetala* as a new monotypic genus. Notwithstanding superficial similarities in floral structure, *L. isopetala* is distinguishable from the rest of *Lachenalia* by its uniquely white, papery bracts, brownish, tubular perianths, larger globose seeds ($1.8\text{--}2.0 \times 1.8\text{--}1.9$ mm), leaf lower surface with protruding, longitudinal veins, and the uniquely straw-like apices of the bulb tunics. Its diploid chromosome number ($2n = 40$) is also very unusual for *Lachenalia*. Based on these differences and its isolated phylogenetic position, we therefore transfer *L. isopetala* to a new genus *Pseudolachenalia*, so named to acknowledge its morphological similarity to *Lachenalia*. This entity occurs on the Bokkeveld and Roggeveld Plateaux, between Nieuwoudtville and Sutherland, and on the Komsberg and Nuweveld Mountains Plateaux in the southern Karoo.

The use of hybrid-enrichment to generate a phylogenetic dataset containing sequence data for hundreds of loci offers unprecedented insights into species-level relationships within *Lachenalia*. Where earlier efforts, based on Sanger sequencing of candidate genes, consistently yielded trees that are poorly resolved and supported (Spies 2004; Hamatani et al. 2008, 2012), the phylogenetic trees presented here are well resolved, with strong total-evidence and coalescent quartet support on most nodes. Almost certainly this is a consequence of the much larger number of variable (111,068) and phylogenetically informative (77,330) sites sampled here. Despite their poor resolution, however, the

phylogenetic trees inferred by both Spies (2004) and Hamatani et al. (2008, 2012) are broadly consistent with the phylogenetic hypothesis presented in this study. Based on her Adams consensus topology, for example, Spies (2004) resolved four principal groupings within *Lachenalia*, two monophyletic (“*L. juncifolia*” and “*Lachenalia* 1” groups) and two paraphyletic/unresolved (“*L. zebrina*” and “*Lachenalia* 2” groups). Where her *L. juncifolia* group corresponds to the *L. namibiensis*–*L. wrightii* clade in our tree (Fig. 2, clades C and D), her *L. zebrina* group corresponds to our *L. sargeantii*–*L. buchbergensis* clade (Fig. 2, clades A and B). Also, where her *Lachenalia* 1 group corresponds to the *L. verticillata*–*L. orchioides* clade in our phylogeny (Fig. 3, *L. verticillata* plus clades J₅–J₁₁), her *Lachenalia* 2 group corresponds to a grade in our tree comprising clades E–I plus clades J₁–J₄ (Figs 2 and 3, *L. convallarioides*–*L. glaucophylla* grade). Within these broad groups there is further correspondence between the subgroups defined by Spies (2004) and the subclades resolved by our analysis. Similarly, there is correspondence between our tree and that of Hamatani et al. (2008, 2012). Clade IV of Hamatani et al. (2008), for example, corresponds to the *L. framesii*–*L. nardousbergensis* clade in our tree (Fig. 3, *L. framesii* plus clades J₇ and J₈), while their “others” clade corresponds to our *L. mutabilis* 2–*L. orchioides* clade (Fig. 3, clades J₁₀ and J₁₁). Finally, clades I and II of Hamatani et al. (2008) match our clades H and D, respectively. Unfortunately, the taxonomic sampling of Hamatani et al. (2008) was very uneven so that many of our clades are not represented in their study at all. Regardless, the strong correspondence of our results to earlier work provides valuable confidence and suggests that our tree provides a robust framework for a revised infrageneric classification of *Lachenalia*, and for downstream evolutionary studies. Although resolution and support in our phylogenetic tree are generally strong, there are one or two areas of uncertainty, particularly the relationships of taxa within the *L. reflexa*–*L. orchioides* clade. Although the cause of this uncertainty remains to be properly assessed, its localized nature ensures that it does not threaten the integrity of the infrageneric classification, which is the focus of this study.

Here, we recognize 10 clades as formal sections on the basis that they are well supported by both the total-evidence (BS = 100) and species tree analysis (QS = 100), and that they have diagnostic morphological synapomorphies. Unfortunately, high levels of homoplasy compromise the diagnostic utility of most morphological characters. Also, the highly imbalanced nature of the *Lachenalia* phylogenetic tree renders the sections highly uneven, with *L. sect. Lachenalia* being much larger than the rest. Briefly, the sections recognized are

as follows (character and character states refer to the numbering presented in Appendix 2) (Figs 2–10):

- Section *Colliculatae*, comprising two pyrophytic species, *L. sargeantii* and *L. montana* Schltr. ex W.F.Barker, from the southern Cape (Fig. 6), is characterized by seed coats with colliculate secondary sculpturing (Fig. 5; character 71: 2). Uniquely, too, the floral pedicels in this clade are characteristically curved at anthesis (Fig. 4; character 40: 2).
- Section *Cordatae*, comprising two xerophytic species, the widespread *L. zebrina* and the southern Namibian- and Richtersveld-endemic *L. buchbergensis* Dinter (Fig. 7) is united by the possession of cordate capsules (Fig. 5; character 63: 2).
- Section *Ciliolatae*, comprising three dwarf species (*L. giessii* W.F.Barker, *L. namibiensis* W.F.Barker, *L. pearsonii*) from southern Namibia and the Richtersveld (Fig. 8), is united by ciliolate leaf margins (Fig. 4; character 26: 1), a feature otherwise found only in *L. ensifolia* (Thunb.) J.C.Manning & Goldblatt (section *Polyxena*). Also, synapomorphic for this clade is the possession of floral pedicels that are patent at anthesis (Fig. 4; character 40: 1), this feature otherwise occurring only in section *Cretaceae* and in *L. polypodantha* Schltr. ex W.F.Barker. Finally, the bulbs of species in this clade are enclosed by hard, leathery tunics (Fig. 4; character 3: 0) that have a fasciculate apex (character 5: 0). This tunic morphology has arisen separately in section *Lachenalia* subsection *Coriaceae*, and in *L. patula* Jacq. and *L. martiniae* W.F.Barker (both section *Lachenalia*).
- Section *Linearifoliae*, comprising eight species mainly from the southwestern and western parts of the Western Cape (Fig. 9), is named for its characteristically linear leaves (Fig. 4; character 13: 2). This feature, however, is homoplasious, having arisen elsewhere in *Lachenalia*. In addition, the lower surfaces of the leaves are banded or covered with papillae. Except for *L. anguinea* Sweet ($2n = 30$) and *L. wrightii* Baker (unknown), species in this clade have a diploid chromosome complement of $2n = 22$.
- Section *Cretaceae*, comprising *L. convallarioides* from the Eastern Cape and *L. multifolia* W.F.Barker from the Tanqua Karoo (Fig. 8), is united by the possession of floral pedicels that are patent at anthesis (Fig. 4; character 40: 1), a feature otherwise found only in section *Ciliolatae* and in *L. polypodantha* (section *Stellatae*).

- Section *Atrorubentes*, comprising *L. macgregoriorum* W.F.Barker from the Bokkeveld Plateau and *L. campanulata* Baker from the Eastern Cape and Karoo (Fig. 6), is characterized by maroon perianth segments (Fig. 5; character 46: 0), a feature unique to this clade.

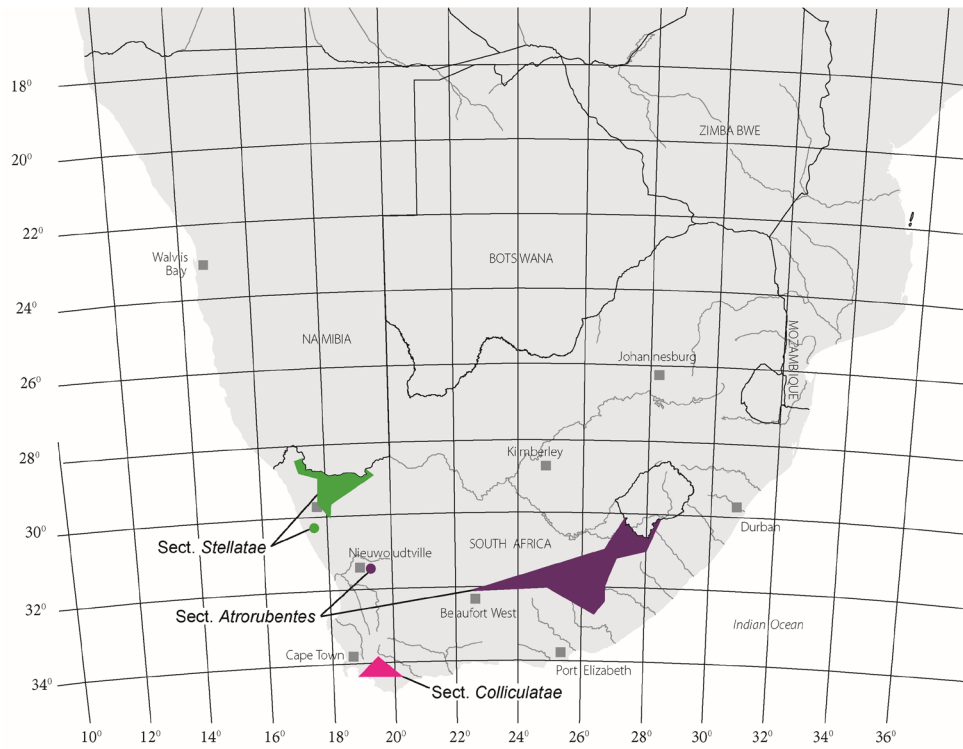


Figure 6. Distribution polygons of *Lachenalia* in South Africa: section *Colliculatae*, section *Atrorubentes* and section *Stellatae*.

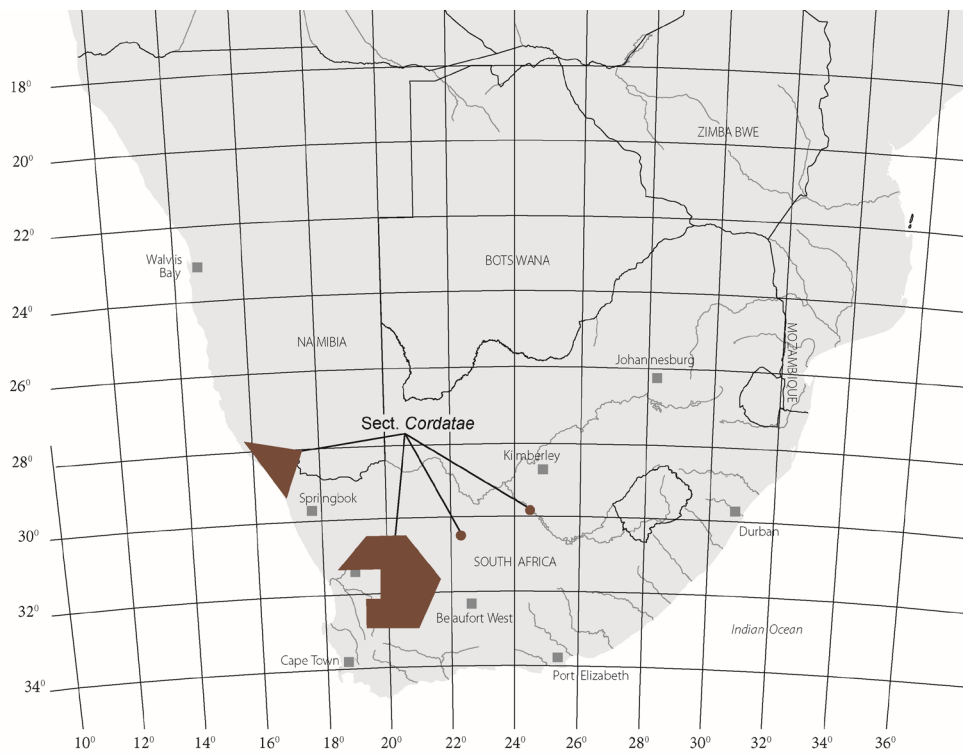


Figure 7. Distribution polygons of *Lachenalia* in South Africa and Namibia: section *Cordatae*.

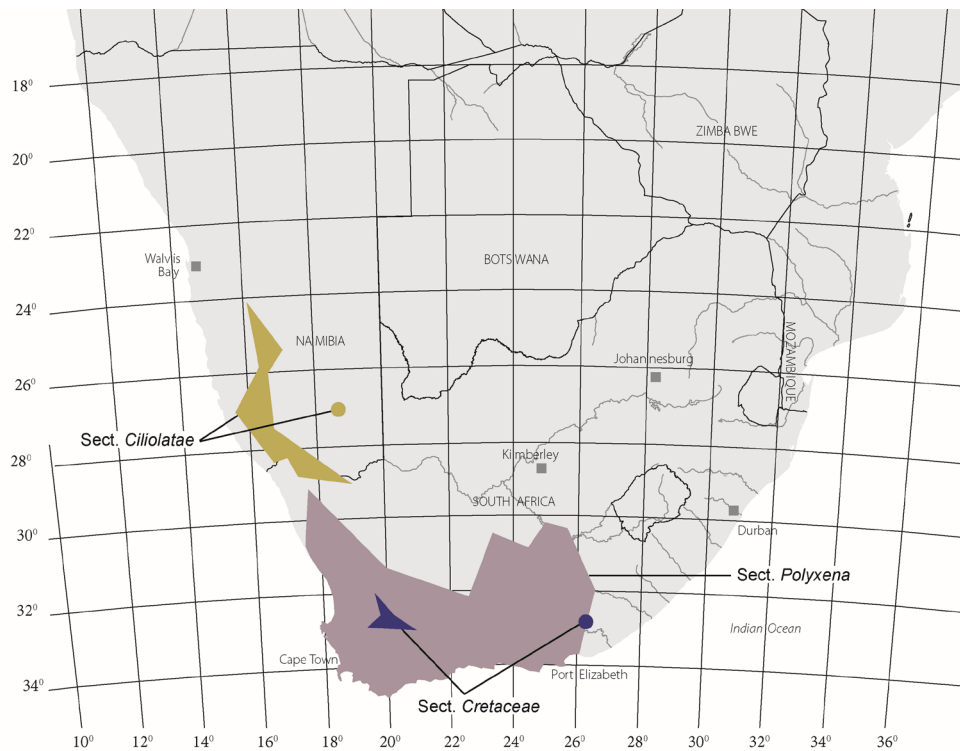


Figure 8. Distribution polygons of *Lachenalia* in South Africa and Namibia: section *Ciliolatae*, section *Cretaceae* and section *Polyxena*.

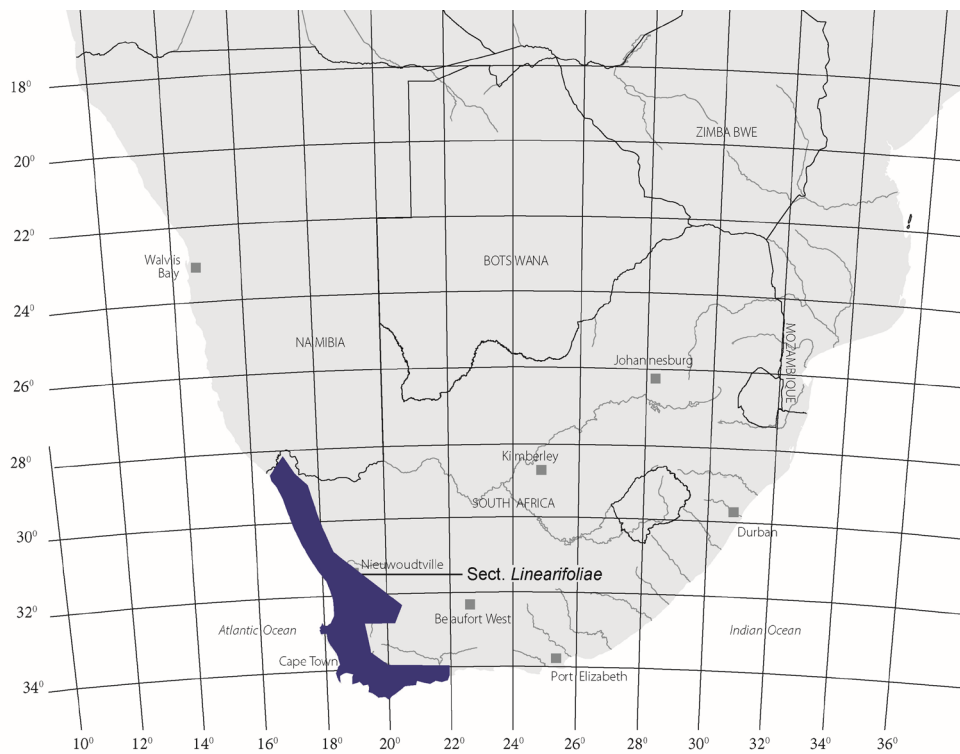


Figure 9. Distribution polygon of *Lachenalia* in South Africa: section *Linearifoliae*.

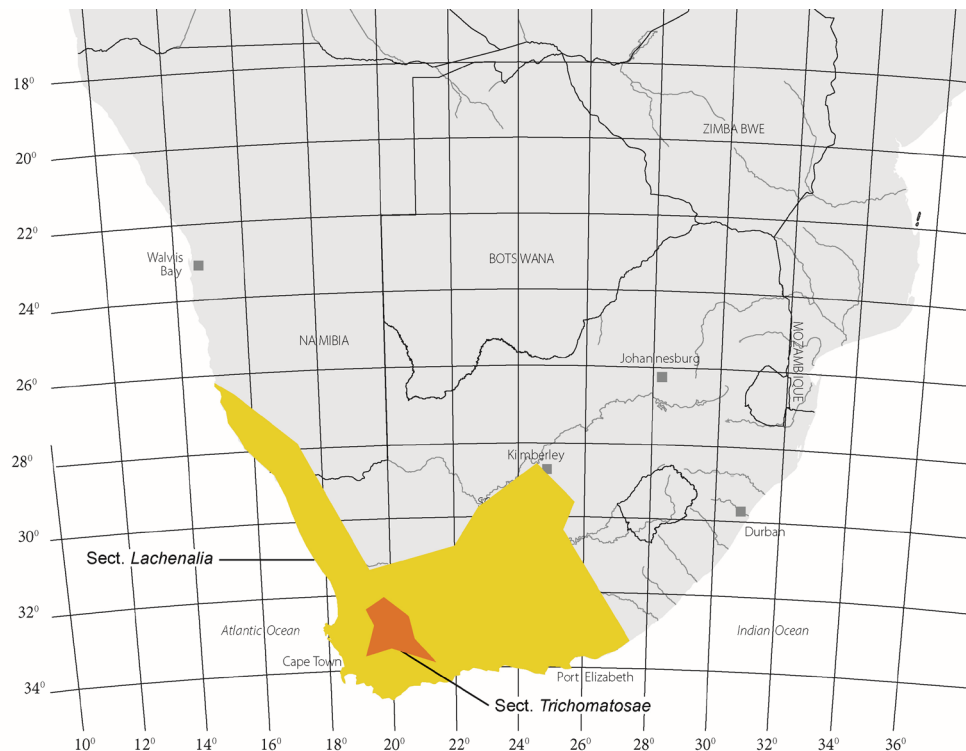


Figure 10. Distribution polygons of *Lachenalia* in South Africa and Namibia: section *Trichomatosae* and section *Lachenalia*.

- Section *Trichomatosae*, comprising the xerophytic *L. comptonii* W.F.Barker and *L. ameliae* W.F.Barker from the Tanqua Karoo (Fig. 10), is united by the possession of simple trichomes on the leaf upper surfaces (Fig. 4; character 17: 1). In addition, the leaf margins in this clade are ciliate (Fig. 4; character 26: 2), a feature otherwise found only in *L. hirta* (Thunb.) Thunb. (section *Linearifoliae*).
- Section *Polyxena*, comprising seven dwarf species previously treated as a separate genus, *Polyxena*, and mostly distributed from the southern Western Cape to the northwestern Northern Cape, but also widespread in the Karoo (Fig. 8), is characterized by actinomorphic flowers (Fig. 4; character 41: 0). In addition, the outer filaments in these species are inserted at the mouth of the perianth tube (Fig. 5; character 57: 0), a feature otherwise found only in *L. reflexa* Thunb., *L. viridiflora* W.F.Barker and *L. patentissima* G.D.Duncan (all section *Lachenalia*). The single exception to this pattern is *L. corymbosa* (L.) J.C.Manning & Goldblatt, whose outer filaments are inserted midway along the perianth tube. Finally, in all members of section *Polyxena* the peduncle elongates, bends at its base and becomes an elongated infructescence appressed to the ground immediately prior to capsule dehiscence

(Fig. 5; character 62: 0). This feature otherwise occurs only in *L. barkeriana* U.Müll.-Doblies & al., *L. pusilla* (both section *Inflatae*) and *L. reflexa* (section *Lachenalia*).

- Section *Stellatae*, comprising two xerophytic species, *L. angelica* W.F.Barker from western Namaqualand and *L. polypodantha* from central, northern and eastern Namaqualand (Fig. 6), is united by leaf upper surfaces that are entirely covered with stellate trichomes (Fig. 4; character 17: 2), a feature otherwise occurring only in *L. trichophylla* (section *Lachenalia*). In addition, these species possess prostrate leaves (character 12: 0), a feature otherwise found only in *L. nervosa* Ker Gawl., *L. stayneri* W.F.Barker, *L. kliprandensis* W.F.Barker, *L. nardousbergensis* G.D.Duncan, *L. pusilla* and *L. barkeriana* (all section *Lachenalia*).
- Section *Lachenalia*, comprising 92 species (or 96 taxa), mainly from the southwestern and northwestern parts of the Western and Northern Cape, but also widespread in the Karoo (Fig. 10), is characterized by a predominantly oblong bell-shaped perianth (Fig. 4; character 42: 1) in which the inner perianth segments are distinctly longer than the outer perianth segments (Fig. 5; character 45: 1). In addition, most members of this clade have flabellate, multiple or sclerified leaf margins (Fig. 4; character 24: 1–3), exceptions being subsections *Latae*, *Framesianae* and *Angustae*, and a further 14 species in subsections *Coriaceae*, *Crispae*, *Stramineae*, *Inflatae*, *Oncoraphae* and *Lachenalia*. Finally, except for species in subsections *Framesianae*, *Angustae* and *Stramineae*, almost all species in this clade possess an elongated strophiole (Fig. 5; character 72: 1–2).

Within *Lachenalia* sect. *Lachenalia*, the presence of additional well-supported clades suggests potential for further infrageneric subdivision. Since the morphological synapomorphies of these clades tend to be more homoplasious; however, some of these clades are more difficult to diagnose reliably, especially subsection *Lachenalia* (node J₁₁). For this reason, and because support for some of these clades is moderate or variable between analyses (e.g., nodes J₄, J₉ and J₁₀), we recognize them at the level of subsection. We recognize a total of 13 subsections within section *Lachenalia*, these varying in size from 1 to 32 species. They are as follows:

- Subsection *Nervosae*, comprising five taxa (either four species, one with two subspecies, or five species; see below) from the Western Cape, is united by the possession of seeds with a smooth (character 73: 1), inflated (Fig. 5; character 74: 1) strophiole, this character having arisen three times elsewhere in section *Lachenalia*. In addition, except for *L. nervosa* members of section *Nervosae* are united by having a grey perianth (Fig. 5; character 46: 4), a

feature otherwise found only in *L. buchbergensis* (section *Cordatae*) and in *L. marlothii* W.F.Barker ex G.D.Duncan (subsection *Crispae*), *L. variegata* W.F.Barker (subsection *Lachenalia*), *L. karooica* W.F.Barker ex G.D.Duncan (subsection *Coriaceae*) and the *L. adamii*–*L. attenuata* clade within subsection *Coriaceae*. Finally, the presence of strongly depressed longitudinal grooves on the upper leaf surface (character 16: 1) characterize this clade, but this feature has arisen repeatedly in *Lachenalia*.

- Subsection *Coriaceae*, comprising 20 species mainly from arid or semi-arid habitats of the western Karoo, is united by bulbs that are almost always enclosed by hard, leathery outer tunics (character 3: 0) and whose tunic apices are fasciculate (character 5: 0). This tunic morphology has arisen separately in section *Ciliolatae*, and in *L. patula* (subsection *Latae*) and *L. martiniae* (subsection *Nervosae*). Although a diploid chromosome complement of $2n = 18$ is prevalent in this clade, $2n = 16$ and $2n = 20$ also occur.

- Subsection *Crispae*, comprising the xerophytic *L. marlothii* from the western Karoo and *L. bolusii* W.F.Barker from the Richtersveld and Namaqualand, is united by undulate or crisped leaf margins (character 25: 1), a feature that has, however, originated repeatedly in *Lachenalia*. Also synapomorphic for this group is the possession of seeds with a smooth (character 73: 1), inflated (Fig. 5; character 74: 1) strophiole, this character having arisen three times elsewhere in section *Lachenalia*: in subsection *Nervosae*, on the branch subtending subsections *Urceolatae* and *Oblongae*, and on the branch subtending subsections *Inflatae*, *Oncoraphae* and *Lachenalia*.

- Subsection *Latae*, comprising the xerophytic, dwarf, white-flowered *L. patula* from the Knersvlakte and the cream-flowered *L. glaucophylla* W.F.Barker, mainly from the western Karoo, is united by a widely bell-shaped perianth (Fig. 4; character 42: 3), a feature that is otherwise absent from subsection *Lachenalia*.

- Subsection *Verticillatae* is monotypic, comprising only the xerophytic *L. verticillata* from the Richtersveld. This section is distinctive on account of its trimerous, whorled or verticillate inflorescences (character 39: 1), a feature otherwise found only in *L. obscura* Schltr. ex G.D.Duncan, *L. canaliculata* G.D.Duncan, *L. aurioliae* G.D.Duncan (all subsection *Coriaceae*) and *L. marlothii* (subsection *Crispae*).

- Subsection *Urceolatae*, comprising four species mainly from the western mountain ranges of the Northern and Western Cape, is united by an urn-shaped perianth (Fig. 4; character 42: 4), acute cataphyll apices (character 9: 1) and spicate inflorescences (character 30: 1). All

these features are homoplasious, however, having arisen repeatedly in *Lachenalia*. Members of this subsection have a diploid chromosome complement of $2n = 14$, $2n = 28$ or $2n = 42$.

- Subsection *Oblongae*, comprising five predominantly fynbos-inhabiting species from the southern Western Cape, is named for its oblong bell-shaped perianths, this, however, being synapomorphic for section *Lachenalia* as a whole (Fig. 4; character 42: 1). While the possession of upwardly bending capsules characterizes this clade (character 64: 1), this feature has arisen repeatedly in *Lachenalia*. Also, it is lacking in *L. martleyi* G.D.Duncan.
- Subsection *Framesianae* is monotypic, comprising only the dwarf species *L. framesii* which is endemic to quartz patches of the Knersvlakte.
- Subsection *Angustae*, comprising four white-flowered species from the Western Cape, is united by a narrowly bell-shaped perianth (Fig. 4; character 42: 2), a feature otherwise found only in *L. anguinea* (section *Linearifoliae*), *L. convallarioides* (section *Cretaceae*), *L. adamii* G.D.Duncan (subsection *Coriaceae*) and *L. kliprandensis* and *L. liliiflora* Jacq. (subsection *Stramineae*). An additional feature of subsection *Angustae*, occurring in all species except *L. bachmannii* Baker, which has two linear leaves, is the possession of multiple linear leaves.
- Subsection *Stramineae*, comprising eight species having a combined distribution from northern Namaqualand to the southern Cape, is united by the possession of light to deep yellow bulb tissue (character 2: 1). In addition, a pink to purple perianth (character 46: 3 and 10) is characteristic for this group, though this feature has arisen elsewhere in *Lachenalia*. Except for *L. nardousbergensis*, whose chromosome number is unknown, members of this subsection all have a diploid chromosome complement of $2n = 16$.
- Subsection *Inflatae*, comprising six mainly arid-habitat species and having a combined distribution from western Namibia to the southern Cape, is united by slightly to strongly inflated peduncles (character 32: 1). This feature is otherwise found only in *L. anguinea* (section *Linearifoliae*), in *L. nardousbergensis* (subsection *Stramineae*), in a new Western Karoo species (subsection *Oncoraphae*) and in *L. mutabilis* G.Lodd. ex Schult. & Schult.f., *L. ventricosa* Schltr. ex W.F.Barker, *L. physocaulos* W.F.Barker and *L. leipoldtii* G.D.Duncan (subsection *Lachenalia*). Except for *L. arenicola* G.D.Duncan & Helme and *L. glauca* (W.F.Barker) G.D.Duncan, whose chromosome numbers are unknown, members of subsection *Inflatae* have a diploid chromosome complement of $2n = 14$.

- Subsection *Oncoraphae*, comprising the widespread *L. punctata* Jacq., *L. bulbifera* mainly from coastal parts of the Northern and Western Cape, and a new species from the western Karoo, is united by the possession of seeds with an inflated raphe (character 75: 1). Except for the new species, whose chromosome number is unknown, members of this subsection have a diploid chromosome complement of $2n = 14$ or $2n = 28$, and *L. bulbifera* forms a polyploid complex, with ploidy levels ranging from diploid to octoploid.
- Subsection *Lachenalia*, comprising 35 taxa (32 or 34 species) of which all except *L. mutabilis* and *L. trichophylla* are endemic to the western parts of the Western Cape, is morphologically heterogeneous, accommodating species with a variety of perianth shapes. Although perianth colour is variable, yellow flowers predominate, this feature being a synapomorphy of the clade comprising subsection *Lachenalia* and its sister, subsection *Oncoraphae* (Fig. 5: character 46: 2). Inclusion of the stamens within the perianth tube is also a general feature. Of the 35 taxa included in this subsection, 28 have a diploid chromosome complement of $2n = 14$ or $2n = 28$, that of the remainder being unknown.

In widespread and/or morphologically variable species represented in our analyses by multiple accessions, these accessions were sometimes resolved in phylogenetically isolated positions suggesting that they represent multiple species. The most striking example is *Lachenalia juncifolia* Baker whose two representative accessions are separated across one of the deepest nodes in the *Lachenalia* phylogeny, *L. juncifolia* 1 being resolved within *L. sect. Linearifoliae* and *L. juncifolia* 2 within section *Lachenalia* (Figs. 2, 3). Another is the well-supported segregation of the two *L. mutabilis* accessions between subsections *Oncoraphae* and *Lachenalia* of section *Lachenalia* (Fig. 3), and the fragmentation of both *L. longibracteata* E. Phillips and *L. orchioides* (L.) Ait. within the *L. cernua*–*L. fistulosa* subclade of subsection *Lachenalia* (Fig. 3). Although we do not formally describe any potentially new species in this paper, this being the subject of a partner paper, we note that the distinctness of these entities is in many instances corroborated by morphology. This, therefore, highlights the utility of a well-resolved and well-supported species-level phylogenetic analysis, particularly one that reflects gene tree consilience across multiple loci, in the discovery of new species.

2.5. Conclusion

Our work on *Lachenalia* highlights the utility of next-generation sequence data, representing several hundred loci, for the inference of phylogenetic relationships which were previously intractable using Sanger sequencing of candidate genes with low levels of interspecific sequence divergence. In the case of *Lachenalia* this is likely a consequence of the recentness of its radiation, the genus associating predominantly with arid- and semi-arid renosterveld and succulent karoo environments of Late Miocene–Pliocene age. Our phylogenetic analysis leads to the transfer of *L. isopetala* to a new genus, *Pseudolachenalia*, and, notwithstanding high levels of morphological character convergence, provides the basis for a new, monophyly-based infrageneric taxonomy of *Lachenalia*. Changes at this level include a reduction to synonymy of both previously recognized subgenera and a re-organization of the sections, resulting in an increase from five to 10 sections, and the subdivision of the largest section (section *Lachenalia*) into 13 subsections. In addition, our phylogenetic tree supports the recognition of several new species, some of which currently enjoy recognition at the subspecies level.

2.6. Taxonomic Treatment

2.6.1. New genus

The placement of the unusual *Lachenalia isopetala* as sister to either *Lachenalia* + *Massonia* or *Veltheimia*, with strong support, argues for the transfer of *L. isopetala* to a new monotypic genus. We name this *Pseudolachenalia* in acknowledgement of its morphological similarity to *Lachenalia*.

Pseudolachenalia G.D.Duncan, **gen. nov.** – Type: *Pseudolachenalia isopetala* (Jacq.) G.D.Duncan.

Differs from *Lachenalia* J.Jacq. ex Murray in its unique papery white bracts, brownish tubular perianth, larger globose seed (1.8–2.0 × 1.8–1.9 mm), leaf lower surface with protruding, longitudinal veins and unique straw-like bulb tunic apices.

Pseudolachenalia isopetala (Jacq.) G.D.Duncan, **comb. nov.** $\equiv$ *Lachenalia isopetala* Jacq., *Collectanea* 5: 68–69. 1797 $\equiv$ *Scillopsis isopetala* (Jacq.) Lem. in *Ill. Hort.* 3: 35. 1856 – Holotype: South Africa, Cape, collector and precise locality unknown (†) – Neotype (designated by Duncan, Genus *Lachenalia*: 180. 2012): [illustration] “*Lachenalia isopetala*” in Jacquin, *Icon. Pl. Rar.* 2: t. 401. 1793 or 1794.

Geophyte, winter-growing, 100–300 mm high in flower. *Bulb* globose, 20–30 mm in diameter, solitary; tunic multi-layered, outer layers cartilaginous, thick, light to dark brown; neck distinct, 20–80 mm long, tunics thick, apices fasciculate, straw-like, often protruding above ground; inner cataphyll translucent white, adhering to leaf bases, apex obtuse. *Leaves* 2, lanceolate, 120–200 $\times$ 15–25 mm, spreading to arcuate, intensely glaucous, deeply canaliculate, upper surface plain, lower surface with numerous protruding, longitudinal veins; leaf margins undulate and crisped, cartilaginous; leaf bases 25–40 mm long, lower portion white, shading to light green above; primary seedling leaf terete, erect. *Inflorescence* racemose, many-flowered, dense; sterile apex short; peduncle erect to suberect, 20–50 mm long, glaucous to light brown; rachis light to dark maroonish-brown to greenish-maroon; pedicels suberect, 2–3 mm long, olive green; bracts ovate, 2–6 $\times$ 3–4 mm, papery, bright white. *Perianth* zygomorphic, tubular, spreading to suberect; tube shallowly cup-shaped, 1 mm long, light brownish yellow or dull maroon; outer perianth segments oblong, 12–13 $\times$ 4–5 mm, light brownish yellow or dull maroon, margins creamish-yellow, broad, apical gibbosities dark maroon to brownish-maroon; inner perianth segments linear-oblong, 12–13 $\times$ 4–5 mm, light brownish yellow or dull maroon, apical markings large, dark maroon to brownish maroon. *Stamens* included to shortly exserted, straight; filaments light greenish-white, 9–10 mm long. *Ovary* ellipsoid, 5 $\times$ 2 mm, light green; style included to shortly exserted, straight, 9–10 mm long, light greenish-white. *Capsule* ellipsoid, 10–11 $\times$ 6–7 mm. *Seed* globose, 1.8–2.0 $\times$ 1.8–1.9 mm, glossy, black; strophiole rudimentary, 0.1 mm long, ridged. *Chromosome number* $2n = 30$ (Johnson and Brandham 1997); $2n = 40$ (Spies et al. 2008).

History. – This taxon was first illustrated as *Lachenalia isopetala* in volume 2 of N.J. Jacquin’s *Icones plantarum rariorum* in 1793 or 1794 (year uncertain), from material cultivated at the Imperial Gardens at Schönbrunn, Vienna, Austria, and a formal description by Jacquin followed in volume 5 of *Collectanea* in 1797. No detail exists regarding the collector, and the place of collection was recorded only as “Cape of Good Hope”, however it is likely that material was collected by either Francis Boos or George Scholl, gardeners at

Schönbrunn, while on an expedition to the Cape during the late 1780s. The holotype material was destroyed by fire during World War 2, and the plate in Jacquin's *Icones* was designated as the neotype (Duncan 2012a).

Distinguishing features. – *Pseudolachenalia isopetala* is distinguished from all species of *Lachenalia* primarily by a raceme of unusual yellowish-brown or maroonish-brown, tubular blooms, subtended by unique, prominent white papery bracts (Fig. 1M). It is further distinguished by the combination of features of two arcuate, deeply canaliculate, intensely glaucous leaves with protruding, longitudinal veins along the lower surface, and undulate and crisped, cartilaginous margins; a solitary, globose bulb with the neck covered with straw-like, fasciculate apices that often protrude above ground (Fig. 1L); and a globose, glossy seed that is larger ($1.8\text{--}2.0 \times 1.8\text{--}1.9$ mm) than all other members of the genus *Lachenalia*. The very unusual diploid chromosome number $2n = 40$ was recorded by Spies et al. (2008) for one habitat accession of this species (*Perry 3065* in NBG); however, Johnson and Brandham (1997) recorded $2n = 30$ (also an unusual number) for the same accession (*Perry 3065* in K), and the latter authors also recorded $2n = 30$ for a second habitat accession (*Brandham 3107* in K). *Pseudolachenalia* is distinguished from *Veltheimia* by its much smaller bulb with deciduous roots (versus perennial roots), by always having 2 oppositely arranged leaves (versus a rosette of at least 3 leaves), by its distinctly shorter perianth tubes and longer, brownish or maroon perianth (versus pink or rarely yellow perianth), and by its much smaller, smooth, glossy seeds (versus rough-textured, matt seeds) and non-inflated capsules (versus inflated capsules).

Distribution, habitat, growth cycle and pollination. – *Pseudolachenalia isopetala* is endemic to the Northern Cape, occurring from Nieuwoudtville on the Bokkeveld Plateau in the northwest, to Calvinia and Middelpos in the western Karoo, south-east to the Roggeveld Plateau at Sutherland, and the Komsberg and Nuweveld Mountains Plateaux in the southern Karoo (Duncan 2012a, 2013). Plants occur in the Roggeveld and Hantam Karoo, and in Nieuwoudtville Shale Renosterveld (Mucina and Rutherford 2006). They are winter-growing and proteranthous, flowering in late spring and early summer, after the leaves have started to wither, and the bulbs are dormant in summer. Plants occur as scattered solitary individuals or in small, isolated groups on arid flats in red stony clay (Fig. 1N), and at Sutherland on the Roggeveld Plateau, plants are subject to occasional sub-zero winter temperatures and snowfalls. The solitary bee *Amegilla nivea* (Lepeletier de Saint-Fargeau 1841) has been

observed to pollinate this species near Middelpoort on the Roggeveld Plateau (Duncan 2012a) (Fig. 10).

2.6.2. Infrageneric classification of *Lachenalia*

- Lachenalia*** J.Jacq. ex Murray, Syst. Veg., ed. 14: 314. 1784 – Type: *Lachenalia tricolor* J.Jacq. (= *Lachenalia aloides* (L.f.) Engl.).
- = *Coelanthus* Willd. ex Schult. & Schult.f. in Roemer & Schultes, Syst.Veg.: 46. 1829 – Type: *Coelanthus complicatus* Willd. ex Schult. & Schult.f. (= *Lachenalia reflexa* Thunb.).
- = *Periboea* Kunth, Enum. Pl. 4: 292. 1843 – Type: *Periboea corymbosa* (L.) Kunth (≡ *Lachenalia corymbosa* (L.) J.C.Manning & Goldblatt).
- = *Polyxena* Kunth, Enum. Pl. 4: 294. 1843 – Type: *Polyxena pygmaea* (Jacq.) Kunth (≡ *Lachenalia pygmaea* (Kunth) G.D.Duncan).
- = *Chloriza* Salisb., Gen. Pl.: 21. 1866 – Type: *Lachenalia mediana* Jacq.
- = *Himas* Salisb., Gen. Pl.: 21. 1866 – Type: *Lachenalia angustifolia* Jacq. (= *Lachenalia contaminata* Ait.).
- = *Monoestes* Salisb., Gen. Pl.: 21. 1866 – Type: *Lachenalia unifolia* Jacq.
- = *Orchiops* Salisb., Gen. Pl.: 21. 1866 – Type: *Lachenalia orchiioides* (L.) Ait.
- = *Platyestes* Salisb., Gen. Pl.: 21. 1866 – Type: *Lachenalia purpureo-caerulea* Jacq.
- = *Brachyscypha* Baker in J. Linn. Soc., Bot. 11: 394. 1871 – Type: *Brachyscypha undulata* (Thunb.) Baker (= *Lachenalia pusilla* Jacq.)

2.6.3. Key to the sections of Genus *Lachenalia*

1. Perianth actinomorphic, arising radially from the pedicel sect. ***Polyxena***
1. Perianth zygomorphic, arising obliquely from the pedicel or rachis 2
2. Testa secondary sculpturing colliculate; pedicels curved sect. ***Colliculatae***
2. Testa secondary sculpturing absent or reticulate; pedicels erect/suberect, patent or absent 3
3. Capsules cordate.....sect. ***Cordatae***
3. Capsules ellipsoid or obovoid 4
4. Leaf upper surface and/or margins glabrous 5
4. Leaf upper surface and/or margins trichomatous 8
5. Leaves linear, banded on lower surface or covered with papillae below. sect. ***Linearifoliae***
5. Leaves almost always lanceolate or ovate, rarely banded on lower surface and never with papillae below; rarely linear and unbanded and without papillae below 6
6. Inner perianth segments longer than outer perianth segments; strophiole mostly elongate sect. ***Lachenalia***
6. Inner and outer perianth segments subequal; strophiole rudimentary..... 7
7. Perianth maroon, widely bell-shaped; leaf bases spotted..... sect. ***Atrorubentes***
7. Perianth white, narrowly or widely bell-shaped; leaf bases unmarked..... sect. ***Cretaceae***

- 8. Leaf margins ciliolate; perianth widely bell-shaped; bulb tunics hard sect. *Ciliolatae*
- 8. Leaf margins covered with simple or stellate trichomes; perianth widely bell-shaped or urn-shaped; bulb tunics spongy 9
- 9. Leaf upper surface covered with simple trichomes..... sect. *Trichomatosae*
- 9. Leaf upper surface covered with stellate trichomes sect. *Stellatae*

2.6.4. Sections of *Lachenalia*

The sections (and subsections below) are arranged in order of their phylogenetic sequence (Figs. 2, 3).

* = taxon not included in DNA study; ** = to be described as a new taxon; *** = taxon to be upgraded to species level

***Lachenalia* sect. *Colliculatae* G.D.Duncan, sect. nov.** – Type: *Lachenalia sargeantii* W.F.Barker.

Leaves 2, linear, deeply canaliculate; pedicels curved; perianth zygomorphic, tubular or oblong bell-shaped, white with green markings, arising obliquely from the pedicel; tube cup-shaped, up to 3 mm long; outer perianth segments ovate or oblong; inner perianth segments obovate or oblong-obovate; filaments straight; capsules obovoid; seeds globose, secondary sculpturing colliculate; strophiole rudimentary, ridged.

2 species (*Lachenalia montana* Schltr. ex W.F.Barker, *L. sargeantii* W.F.Barker).

***Lachenalia* sect. *Cordatae* G.D.Duncan, sect. nov.** – Type: *Lachenalia buchbergensis* Dinter.

Leaves 1 or 2, lanceolate, deeply canaliculate or conduplicate; pedicels suberect or curved; perianth zygomorphic, tubular or oblong bell-shaped, brown or grey, arising obliquely from the pedicel; tube cup- or disc-shaped, up to 3 mm long; outer perianth segments oblong or ovate; inner perianth segments oblong or oblong-obovate; filaments straight; capsules obcordate; seeds oblong or ovoid, strophiole non-inflated, rudimentary or medium-length, ridged.

3 species (*Lachenalia buchbergensis* Dinter, *L. nordenstamii* W.F.Barker*, *L. zebrina* W.F.Barker).

***Lachenalia* sect. *Ciliolatae* G.D.Duncan, **sect. nov.** – Type: *Lachenalia namibiensis* W.F.Barker.**

Leaves 2, rarely 1, lanceolate; margins ciliolate; pedicels patent; perianth zygomorphic, widely campanulate, white, arising obliquely from the pedicel; tube cup-shaped, up to 3 mm long; outer perianth segments ovate; inner perianth segments linear; filaments narrowly spreading; capsules obovoid; seeds globose, strophiole non-inflated, rudimentary, ridged.

3 species (*Lachenalia giessii* W.F.Barker, *L. namibiensis* W.F.Barker, *L. pearsonii* (R.Glover)) W.F.Barker.

***Lachenalia* sect. *Linearifoliae* G.D.Duncan, **sect. nov.** – Type: *Lachenalia unifolia* Jacq.**

Leaves solitary, 2 or more, linear, outer surfaces banded, or covered with papillae below; pedicels suberect; perianth zygomorphic, tubular, oblong-, narrowly- or widely bell-shaped, white or cream-coloured, blue or pink, arising obliquely from the pedicel; tube cup-shaped or tubular; outer perianth segments ovate or oblong; inner perianth segments obovate; filaments straight, declinate or recurved; capsules obovoid or ellipsoid; seeds globose, strophiole non-inflated, rudimentary, ridged.

12 species (*Lachenalia anguinea* Sweet, *L. barbarae* G.D.Duncan*, *L. bruynsii* G.D.Duncan*, *L. hirta* (Thunb.) Thunb., *L. juncifolia* Baker (= “*L. juncifolia* 1”), *L. magentea* G.D.Duncan*, *L. moniliformis* W.F.Barker, *L. polyphylla* Baker, *L. schlechteri* Baker*, *L. unifolia* Jacq., *L. wrightii* Baker, *L. zeyheri* Baker).

***Lachenalia* sect. *Cretaceae* G.D.Duncan, **sect. nov.** – Type: *Lachenalia multifolia* W.F.Barker.**

Leaves solitary or numerous, lanceolate or linear; pedicels patent at anthesis; perianth zygomorphic, narrowly- or widely bell-shaped, arising obliquely from the pedicel, chalk-white; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate; filaments straight or narrowly spreading; capsules obovoid; seeds globose, strophiole non-inflated, rudimentary, ridged.

2 species (*Lachenalia convallarioides* Baker, *L. multifolia* W.F.Barker).

Lachenalia* sect. *Atrorubentes G.D.Duncan, **sect. nov.** – Type: *Lachenalia macgregorium* W.F.Barker.

Leaves 2, lanceolate or linear; pedicels suberect; perianth zygomorphic, widely bell-shaped, arising obliquely from the pedicel, light to deep maroon; tube cup-shaped; outer perianth segments ovate, inner perianth segments linear; filaments recurved; capsules obovoid; seeds globose, strophiole non-inflated, rudimentary, ridged.

2 species (*Lachenalia campanulata* Baker, *L. macgregorium* W.F.Barker).

Lachenalia* sect. *Trichomatosae G.D.Duncan, **sect. nov.** – Type: *Lachenalia comptonii* W.F. Barker.

Leaves 1 or 2, lanceolate, upper surfaces and margins covered with simple trichomes; pedicels absent or suberect; perianth zygomorphic, widely bell-shaped or urceolate, yellow or white, arising obliquely from the rachis or pedicel; tube cup-shaped; outer perianth segments ovate, inner perianth segments linear or obovate; filaments recurved or declinate; capsules ellipsoid; seeds ovoid or globose, strophiole non-inflated, rudimentary, ridged.

2 species (*Lachenalia ameliae* W.F.Barker, *L. comptonii* W.F.Barker).

Lachenalia* sect. *Polyxena (Kunth) G.D.Duncan, **stat. nov.** ≡ *Polyxena* Kunth, Enum. Pl. 4: 294. 1843 ≡ *Lachenalia* subg. *Polyxena* (Kunth) G.D.Duncan, Genus *Lachenalia*: 426. 2012 – Type: *Polyxena pygmaea* (Jacq.) Kunth (= *Lachenalia pygmaea* (Jacq.) G.D.Duncan).

Leaves linear, lanceolate or rarely ovate; pedicels erect or suberect; perianth actinomorphic, widely bell-shaped or tubular, arising radially from the pedicel; tube cup-shaped or tubular; outer perianth segments oblong, inner perianth segments linear; filaments straight or recurved; capsules obovoid or ellipsoid; seeds ovoid or globose, strophiole non-inflated, rudimentary, ridged.

7 species/8 taxa (*Lachenalia argillicola* G.D.Duncan, *L. calcicola* (U.Müll.-Doblies & D.Müll.-Doblies) G.D.Duncan, *L. corymbosa* (L.) J.C.Manning & Goldblatt, *L. ensifolia*

(Thunb.) J.C.Manning & Goldblatt subsp. *ensifolia*, *L. ensifolia* (Thunb.) J.C.Manning & Goldblatt subsp. *maughanii* (W.F.Barker) G.D.Duncan, *L. longituba* (A.M.van der Merwe) J.C.Manning & Goldblatt*, *L. paucifolia* (W.F.Barker) J.C.Manning & Goldblatt, *L. pygmaea* (Jacq.) G.D.Duncan).

***Lachenalia* sect. *Stellatae* G.D.Duncan, sect. nov.** – Type: *Lachenalia angelica* W.F.Barker.

Leaf solitary, lanceolate, upper surfaces and margins covered with stellate trichomes; pedicels suberect or patent; perianth zygomorphic, widely bell-shaped, white, or white marked arising obliquely from pedicel; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate; filaments declinate; capsules obovoid; seeds globose, strophiole non-inflated, rudimentary, ridged.

2 species/3 taxa (*Lachenalia angelica* W.F.Barker, *L. polypodantha* Schltr. ex W.F.Barker subsp. *eburnea* G.D.Duncan*, *L. polypodantha* Schltr. ex W.F.Barker subsp. *polypodantha*).

Lachenalia* J.Jacq. ex Murray sect. *Lachenalia – Type: *Lachenalia tricolor* J.Jacq. (= *Lachenalia aloides* (L.f.) Engl.).

Leaves lanceolate or ovate; pedicels absent or suberect; perianth zygomorphic, tubular, oblong bell-shaped, narrowly bell-shaped, widely bell-shaped or urceolate, arising obliquely from the pedicel or rachis; tube cup-shaped or tubular; outer perianth segments oblong or ovate; inner perianth segments oblong, obovate or linear; filaments straight, narrowly spreading, recurved or declinate; capsules obovoid or ellipsoid; seeds globose, ovoid or oblong, strophiole inflated or non-inflated, rudimentary, medium or long, smooth or ridged.

107 species/108 taxa (*Lachenalia adamii* G.D.Duncan, *L. alba* W.F.Barker ex G.D.Duncan, *L. algoensis* Schönland*, *L. aloides* (L.f.) Engl., *L. arbuthnotiae* W.F.Barker, *L. arenicola* G.D.Duncan & Helme, *L. attenuata* W.F.Barker ex G.D.Duncan, *L. aurioliae* G.D.Duncan, *L. bachmannii* Baker, *L. barkeriana* U.Müll.-Doblies & al., *L. bolusii* W.F.Barker, *L. bowkeri* Baker, *L. bulbifera* (Cirillo) Engl., *L. callista* G.D.Duncan & T.J.Edwards, *L. canaliculata* G.D.Duncan, *L. capensis* W.F.Barker, *L. carnosae* Baker, *L. cernua* G.D.Duncan, *L. concordiana* Schltr. ex W.F.Barker*, *L. congesta* W.F.Barker*, “*L. contaminata* 1”** [= *L. sp. nov.*], *L. contaminata* Ait. [= “*L. contaminata* 2 & 3”], *L. dasybotrya* Diels,

L. dehoopensis W.F.Barker, *L. doleritica* G.D.Duncan, *L. duncanii* W.F.Barker, *L. elegans* W.F.Barker, *L. fistulosa* Baker, *L. flava* Andrews, *L. framesii* W.F.Barker, *L. glauca* (W.F.Barker) G.D.Duncan, *L. glaucophylla* W.F.Barker, *L. haarlemensis* Fourc., *L. inconspicua* G.D.Duncan*, *L. judithiae* G.D.Duncan, “*L. juncifolia* 2”** [= *L. sp. nov.*], *L. karooica* W.F.Barker ex G.D.Duncan, *L. karoopoortensis* G.D.Duncan, *L. klinghardtiana* Dinter, *L. kliprandensis* W.F.Barker, *L. krugeri* G.D.Duncan*, *L. lactosa* G.D.Duncan, *L. latimerae* W.F.Barker*, *L. leipoldtii* G.D.Duncan, *L. leomontana* W.F.Barker, *L. liliiflora* Jacq., *L. longibracteata* E.Phillips [= “*L. longibracteata* 1 & 2”], “*L. longibracteata* 3”** [= *L. sp. nov.*], *L. lutea* G.D.Duncan, *L. luteola* Jacq., *L. lutzeyeri* G.D.Duncan*, *L. margaretae*, W.F.Barker, *L. marginata* W.F.Barker subsp. *marginata*, *L. marginata* W.F.Barker subsp. *neglecta* (Schltr.) G.D.Duncan, *L. marlothii* W.F.Barker ex G.D.Duncan, *L. martiniae* W.F.Barker, *L. martleyi* G.D.Duncan, *L. mathewsii* W.F.Barker, *L. maximiliani* Schltr. ex W.F.Barker*, *L. mediana* Jacq. subsp. *mediana*, *L. mediana* Jacq. subsp. *rogersii* (Baker) G.D.Duncan***, *L. membranacea* (W.F.Barker) G.D.Duncan, *L. minima* W.F.Barker, *L. mutabilis* G.Lodd. ex Schult. & Schult.f. [= “*L. mutabilis* 1”], “*L. mutabilis* 2”** [= *L. sp. nov.*], *L. namaquensis* Schltr. ex W.F.Barker, *L. nardousbergensis* G.D.Duncan, *L. neilii* W.F.Barker ex G.D.Duncan, *L. nervosa* Ker Gawl., *L. nutans* G.D.Duncan*, *L. obscura* Schltr. ex G.D.Duncan *L. orchioides* (L.) Ait. subsp. *glaucina* (Jacq.) G.D.Duncan***, *L. orchioides* (L.) Ait. subsp. *orchioides*, *L. orchioides* (L.) Ait. subsp. *parviflora* (W.F.Barker) G.D.Duncan***, *L. orthopetala* Jacq., *L. pallida* Ait. [= “*L. pallida* 1 & 2”], *L. patentissima* G.D.Duncan, *L. patula* Jacq., *L. peersii* Marloth ex W.F.Barker, *L. perryae* G.D.Duncan, *L. physocaulos* W.F.Barker, *L. punctata* Jacq., *L. purpureo-caerulea* Jacq., *L. pusilla* Jacq., *L. quadricolor* Jacq., *L. reflexa* Thunb., *L. rosea* Andrews, *L. salteri* W.F.Barker, *L. schelpei* W.F.Barker, *L. sessiliflora* Andrews, *L. splendida* Diels, *L. stayneri* W.F.Barker, *L. suaveolens* (W.F.Barker) G.D.Duncan, *L. summerfieldii* G.D.Duncan, *L. thomasiae* W.F.Barker ex G.D.Duncan, *L. thunbergii* G.D.Duncan & T.J.Edwards, *L. trichophylla* Baker, *L. undulata* Masson ex Baker, *L. valeriae* G.D.Duncan*, *L. vanzyliae* (W.F.Barker) G.D.Duncan, *L. variegata* W.F.Barker, *L. ventricosa* Schltr. ex W.F.Barker, *L. verticillata* W.F.Barker, *L. violacea* Jacq., *L. viridiflora* W.F.Barker, *L. whitehillensis* W.F.Barker, *L. xerophila* Schltr. ex G.D.Duncan*, *L. youngii* Baker*).

2.6.5. Key to the subsections of *Lachenalia* sect. *Lachenalia*

1. Strophiole ridged, non-inflated, seed almost always ovoid, testa lustre often matt.....2
1. Strophiole smooth, inflated, seed globose, testa lustre almost always glossy 7
2. Bulb tunics almost always hard and tunic apices fasciculate.....subject. *Coriaceae*
2. Bulb tunics spongy, tunic apices non-fasciculate..... 3
3. Bulb tissue light to deep yellow subject. *Stramineae*
3. Bulb tissue white 4
4. Perianth urn-shaped; inner perianth segments magenta-tipped..... 5
4. Perianth widely or narrowly bell-shaped; inner perianth segments white 6
5. Leaves immaculate, green, margins flat, undulate or crisped subject. *Framesianae*
5. Leaves spotted below, glaucous, margins flat, ridged.....subject. *Verticillatae*
6. Perianth widely bell-shaped; filaments recurved or declinate subject. *Latae*
6. Perianth narrowly bell-shaped; filaments straight or spreading.....subject. *Angustae*
7. Raphe non-inflated 8
7. Raphe inflated subject. *Oncoraphae*
8. Perianth often tubular, sometimes oblong bell-shaped, rarely urn-shaped; stamens mostly included within tube; perianth mostly yellow but sometimes orange, multicoloured, white, turquoise or blue.....subject. *Lachenalia*
8. Perianth mostly oblong bell-shaped or urn-shaped, rarely tubular; stamens exserted or included within tube; perianth mauve, pink, blue, purple, grey, yellow or white..... 9
9. Peduncle slightly to strongly inflated..... subject. *Inflatae*
9. Peduncle non-inflated..... 10
10. Leaf upper surface with deep longitudinal grooves subject. *Nervosae*
10. Leaf upper surface without deep longitudinal grooves 11
11. Leaf margins crisped subject. *Crispae*
11. Leaf margins flat 12
12. Perianth urn-shaped; inflorescence spicate subject. *Urceolatae*
12. Perianth oblong bell-shaped; inflorescence racemose subject. *Oblongae*

2.6.6. Subsections of *Lachenalia* sect. *Lachenalia*

Lachenalia subsect. *Nervosae* G.D.Duncan, **subject. nov.** – Type: *Lachenalia nervosa* Ker Gawl.

Leaves lanceolate or ovate, upper surfaces with strongly depressed longitudinal grooves; perianth oblong bell-shaped or widely bell-shaped, mostly grey-blue, rarely pink; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate; filaments straight, declinate or recurved; capsules ellipsoid or obovoid; seeds globose, strophiole inflated, smooth, medium or long.

5 species (*Lachenalia martiniae* W.F.Barker, *L. mediana* Jacq. subsp. *mediana*, *L. mediana* Jacq. subsp. *rogersii* (Baker) G.D.Duncan***, *L. nervosa* Ker Gawl., *L. stayneri* W.F.Barker).

Lachenalia subsect. ***Coriaceae*** G.D.Duncan, **subsect. nov.** – Type: *Lachenalia undulata* Masson ex Baker.

Bulbs almost always with hard outer tunics and fasciculate tunic apices; leaves lanceolate or ovate; pedicels absent or suberect; perianth oblong bell-shaped, rarely tubular or urn-shaped, mostly greenish, grey, light blue or cream-coloured, rarely yellow; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate; filaments straight, declinate or recurved; capsules ellipsoid or obovoid; seeds ovoid or globose, strophiole non-inflated, medium or long, ridged.

22 species (*Lachenalia adamii* G.D.Duncan, *L. alba* W.F.Barker ex G.D.Duncan, *L. attenuata* W.F.Barker ex G.D.Duncan, *L. aurioliae* G.D.Duncan, *L. bowkeri* Baker, *L. canaliculata* G.D.Duncan, *L. concordiana* Schltr. ex W.F.Barker*, *L. dasybotrya* Diels, *L. dehoopensis* W.F.Barker, *L. doleritica* G.D.Duncan, *L. duncanii* W.F.Barker, *L. haarlemensis* Fourc., *L. karooica* W.F.Barker ex G.D.Duncan, *L. maximiliani* Schltr. ex W.F.Barker*, *L. minima* W.F.Barker, *L. neilii* W.F.Barker ex G.D.Duncan, *L. obscura* Schltr. ex G.D.Duncan, *L. perryae* G.D.Duncan, *L. schelpei* W.F.Barker, *L. summerfieldii* G.D.Duncan, *L. undulata* Masson ex Baker, *L. whitehillensis* W.F.Barker).

Lachenalia subsect. ***Crispae*** G.D.Duncan, **subsect. nov.** – Type: *Lachenalia bolusii* W.F.Barker.

Leaves lanceolate, margins crisped; pedicels suberect; perianth oblong bell-shaped or urn-shaped, light blue; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate; filaments straight; capsules ellipsoid or obovoid; seeds ovoid or globose, strophiole inflated, medium or long, smooth.

2 species (*Lachenalia bolusii* W.F.Barker, *L. marlothii* W.F.Barker ex G.D.Duncan).

Lachenalia subsect. ***Latae*** G.D.Duncan, **stat. nov.** ≡ *Lachenalia* sect. *Latae* G.D.Duncan, Genus *Lachenalia*: 346. 2012 – Type: *Lachenalia patula* Jacq.

Leaves linear or lanceolate; pedicels suberect; perianth widely bell-shaped, white or cream-coloured; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate or linear; filaments declinate or recurved; capsules ellipsoid or obovoid; seeds globose or ovoid,

strophiole non-inflated, rudimentary or medium, ridged.

2 species (*Lachenalia glaucophylla* W.F.Barker, *L. patula* Jacq.).

Lachenalia subsect. ***Verticillatae*** G.D.Duncan, **subsect. nov.** – Type: *Lachenalia verticillata* W.F.Barker.

Leaf solitary, lanceolate, glaucous, lower surface spotted, blotched or plain; margins ridged, flat or undulate and crisped; pedicels absent; perianth urn-shaped, arranged in distinct 3-flowered verticils, greenish with magenta tips; outer perianth segments ovate; inner perianth segments obovate and purple-tipped, lower inner perianth segment protruding; filaments declinate, included; capsules ellipsoid; seeds globose or ovoid; strophiole non-inflated, medium, ridged or smooth.

2 species (*Lachenalia krugeri* G.D.Duncan*, *L. verticillata* W.F.Barker).

Lachenalia subsect. ***Urceolatae*** G.D.Duncan, **stat. nov.** ≡ *Lachenalia* sect. *Urceolatae* G.D.Duncan, Genus *Lachenalia*: 378. 2012 – Type: *Lachenalia elegans* W.F.Barker.

Leaves 1 or 2, lanceolate or ovate; pedicels absent, but sometimes present in inflorescence sterile apex; perianth urn-shaped, light to deep yellow, blue or purple; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate, margins often undulate; filaments straight; capsules ellipsoid or obovoid; seeds globose, strophiole mostly inflated, medium and smooth, rarely non-inflated, rudimentary and ridged.

5 species (*Lachenalia congesta* W.F.Barker*, *L. elegans* W.F.Barker, *L. karoopoortensis* G.D.Duncan, *L. membranacea* (W.F.Barker) G.D.Duncan, *L. suaveolens* (W.F.Barker) G.D.Duncan).

Lachenalia subsect. ***Oblongae*** G.D.Duncan, **stat. nov.** ≡ *Lachenalia* sect. *Oblongae* G.D.Duncan, Genus *Lachenalia*: 191. 2012 – Type: *Lachenalia salteri* W.F.Barker.

Leaves 1 or 2, mostly lanceolate, rarely linear; pedicels suberect; perianth oblong bell-shaped, white, cream-coloured, pink or blue; tube cup-shaped; outer perianth segments ovate, inner perianth segments obovate; filaments straight or declinate; capsules ellipsoid or obovoid; seeds globose; strophiole inflated, mostly medium, rarely long, smooth.

7 species (*Lachenalia judithiae* G.D.Duncan, *L. leomontana* W.F.Barker, *L. lutzeyeri* G.D.Duncan*, *L. martleyi* G.D.Duncan, *L. rosea* Andrews, *L. salteri* W.F.Barker, *L. youngii* Baker*).

Lachenalia subsect. ***Framesianae*** G.D.Duncan, **subject. nov.** – Type: *Lachenalia framesii* W.F.Barker.

Leaves 1 or 2, lanceolate, canaliculate, margins undulate; pedicels absent; perianth urn-shaped, greenish yellow, magenta-tipped; tube cup-shaped, outer perianth segments ovate, inner perianth segments obovate, magenta-tipped, and lower inner perianth segment longer and protruding; filaments declinate; capsules ellipsoid; seeds ovoid; strophiole non-inflated, medium, ridged.

1 species (*Lachenalia framesii* W.F.Barker).

Lachenalia subsect. ***Angustae*** G.D.Duncan, **stat. nov.** ≡ *Lachenalia* sect. *Angustae* G.D.Duncan, Genus *Lachenalia*: 326. 2012 – Type: *Lachenalia orthopetala* Jacq.

Leaves mostly 3 or more, rarely 2, linear; pedicels suberect; perianth narrowly bell-shaped, white or cream-coloured; tube cup-shaped, outer perianth segments ovate, inner perianth segments obovate or linear; filaments straight or narrowly spreading; capsules ellipsoid; seeds mostly globose, rarely ovoid; strophiole non-inflated, rudimentary, ridged.

4 species (*Lachenalia bachmannii* BakLer, *L. contaminata* Ait. [= “*L. contaminata* 2 & 3”], “*L. contaminata* 1”** [= *L. sp. nov.*], *L. orthopetala* Jacq.).

Lachenalia subsect. ***Stramineae*** G.D.Duncan, **subject. nov.** – Type: *Lachenalia nardousbergensis* G.D.Duncan.

Bulb tissue light to deep yellow; leaves 1 or 2, lanceolate or ovate, rarely linear; pedicels

absent or suberect; perianth urn-shaped or oblong bell-shaped, rarely narrowly bell-shaped, mostly pink, mauve, blue or purple, rarely white, yellow or greenish with magenta tips; outer perianth segments ovate, inner perianth segments obovate; filaments mostly declinate, rarely narrowly spreading; capsules ellipsoid; seeds globose or ovoid; strophiole mostly non-inflated, rudimentary and ridged, rarely inflated, medium length and smooth.

9 species (*Lachenalia carnosa* Baker, *L. kliprandensis* W.F.Barker, *L. liliiflora* Jacq., *L. namaquensis* Schltr. ex W.F.Barker, *L. nardousbergensis* G.D.Duncan, *L. pallida* Ait. [= "*L. pallida* 1 & 2"], *L. purpureo-caerulea* Jacq., *L. splendida* Diels, *L. valeriae* G.D.Duncan*).

***Lachenalia* subsect. *Inflatae* G.D.Duncan, **subject. nov.** – Type: *Lachenalia klinghardtiana* Dinter.**

Leaves 1, 2 or numerous, mostly lanceolate, rarely linear; peduncle slightly to strongly inflated; pedicels suberect, rarely erect; perianth oblong bell-shaped or tubular, white, cream-coloured, mauve or purple; tube mostly cup-shaped, rarely disc-shaped; outer perianth segments ovate or oblong; inner perianth segments obovate or linear; filaments straight or declinate, exserted; capsules ellipsoid; seeds mostly globose, rarely ovoid; strophiole mostly inflated, rarely non-inflated, medium length, smooth, rarely ridged.

8 species (*Lachenalia arenicola* G.D.Duncan & Helme, *L. barkeriana* U.Müll.-Doblies & al., *L. glauca* (W.F.Barker) G.D.Duncan, *L. klinghardtiana* Dinter, *L. nutans* G.D.Duncan*, *L. pusilla* Jacq., *L. violacea* Jacq., *L. xerophila* Schltr. ex G.D.Duncan*).

***Lachenalia* subsect. *Oncoraphae* G.D.Duncan, **subject. nov.** – Type: *Lachenalia punctata* Jacq.**

Leaves 1 or 2, lanceolate; pedicels suberect or absent; perianth tubular or urn-shaped, mostly pink or red, rarely yellow; tube cup-shaped or tubular; outer perianth segments ovate or oblong; inner perianth segments obovate or oblong; filaments straight or declinate; capsules ellipsoid; seeds globose or oblong; strophiole inflated, medium or long, smooth; raphe inflated.

3 species (*Lachenalia bulbifera* (Cirillo) Engl., “*L. mutabilis* 2”** [= *L. sp. nov.*], *L. punctata* Jacq.).

Lachenalia J.Jacq. ex Murray subsect. ***Lachenalia*** – Type: *Lachenalia tricolor* J.Jacq. (= *Lachenalia aloides* (L.f.) Engl.).

Leaves 1 or 2, mostly lanceolate, rarely ovate or linear; pedicels absent or suberect; perianth mostly tubular or oblong-campanulate, rarely urn-shaped, mostly yellow but sometimes orange, multicoloured, white, turquoise or blue; tube cup-shaped or tubular; outer perianth segments ovate or oblong; inner perianth segments obovate or oblong, rarely linear; filaments straight or declinate, rarely narrowly spreading; capsules ellipsoid, rarely obovoid; seeds mostly globose, rarely ovoid; strophiole mostly inflated, rarely non-inflated, mostly smooth and medium or long, rarely rudimentary and ridged.

37 species/38 taxa (*Lachenalia algoensis* Schönland*, *L. aloides* (L.f.) Engl., *L. arbutnotiae* W.F.Barker, *L. callista* G.D.Duncan & T.J.Edwards, *L. capensis* W.F.Barker, *L. cernua* G.D.Duncan, *L. fistulosa* Baker, *L. flava* Andrews, *L. inconspicua* G.D.Duncan*, “*L. juncifolia* 2”** [= *L. sp. nov.*], *L. lactosa* G.D.Duncan, *L. latimerae* W.F.Barker*, *L. leipoldtii* G.D.Duncan, *L. longibracteata* E.Phillips [= *L. longibracteata* 1 & 2’], “*L. longibracteata* 3”** [= *L. sp. nov.*], *L. lutea* G.D.Duncan, *L. luteola* Jacq., *L. margaretae* W.F.Barker, *L. marginata* W.F.Barker subsp. *marginata*, *L. marginata* W.F.Barker subsp. *neglecta* Schltr. ex G.D.Duncan, *L. marlothii* W.F.Barker ex G.D.Duncan, *L. mathewsii* W.F.Barker, *L. mutabilis* G.Lodd. ex Schult. & Schult.f. [= “*L. mutabilis* 1”], *L. orchioides* (L.) Ait. subsp. *glaucina* (Jacq.) G.D.Duncan***, *L. orchioides* (L.) Ait. subsp. *orchioides*, *L. orchioides* (L.) Ait. subsp. *parviflora* (W.F.Barker) G.D.Duncan***, *L. patentissima* G.D.Duncan, *L. peersii* Marloth ex W.F.Barker, *L. physocaulos* W.F.Barker, *L. quadricolor* Jacq., *L. reflexa* Thunb., *L. sessiliflora* Andrews, *L. thomasiae* W.F.Barker ex G.D.Duncan, *L. thunbergii* G.D.Duncan & T.J.Edwards, *L. trichophylla* Baker, *L. vanzyliae* (W.F.Barker) G.D.Duncan, *L. variegata* W.F.Barker, *L. ventricosa* Schltr. ex W.F.Barker, *L. viridiflora* W.F.Barker).

2.6.7. New species and status

The four new taxa, “*Lachenalia mutabilis* 2” (Duncan 666), “*L. juncifolia* 2” (Duncan 459), “*L. longibracteata* 3” (Duncan 119) and “*L. contaminata* 1” (Malan 117), will be published

elsewhere, in conjunction with the elevation of three subspecies to species level (*L. mediana* Jacq. subsp. *rogersii* (Baker) G.D.Duncan, *L. orchioides* (L.) Ait. subsp. *glaucina* (Jacq.) G.D.Duncan, *L. orchioides* (L.) Ait. subsp. *parviflora* (W.F.Barker) G.D.Duncan). With the transfer of *L. isopetala* to *Pseudolachenalia*, this will bring the total number of species in *Lachenalia* to 142.

2.7. Author Contributions

Design of the research: GAV. Performance of the research: GDD. Data collection: GDD, ARL, EML. Data analysis: GAV, ARL, EML, GDD. Data interpretation: GAV, CDS, FF, AGE, GDD. Writing: GDD, GAV, ARL.

2.8. Acknowledgements

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2.9. Addendum

Following the publication of the above paper (<https://doi.org/10.1002/tax.12683>) it became evident that errors in nomenclature, typification, and taxonomic status had occurred, however, correcting these errors in the thesis before doing so in the published literature risks generating taxonomic confusion. I therefore undertake to publish a paper, as soon as possible, in which these errors will be formally addressed in a taxonomic journal. The errors are listed as follows:

(a) Addressing the nomenclature and typification of the genus *Pseudolachenalia*, the type species of which, *P. isopetala*, already has a synonym in the genus *Scillopsis*, of which the latter has yet to have a designated type and be listed as a synonym of *Lachenalia*.

(b) Addressing changes in the taxonomic status of certain sections of the genus *Lachenalia* (i.e., sect. *Angustae*, sect. *Latae*, sect. *Oblongae* and sect. *Urceolatae*), which are now recognised as subsections, but which also represent new combinations.

(c) Addressing the genus *Monoestes* which has the same type as the already published *Lachenalia* sect. *Linearifoliae*, and *Monoestes* may therefore be used at sectional rank.

CHAPTER 3. SYSTEMATICS: NEW SPECIES AND TAXONOMIC ADJUSTMENTS

Published paper: Four new species and three taxonomic adjustments in *Lachenalia* (Asparagaceae: Scilloideae) from southern and western South Africa. *Phytotaxa* 585: 261–273 (2023). <https://doi.org/10.11646/phytotaxa.585.4.2>

Abstract

A novel phylogenetic hypothesis for *Lachenalia* (Asparagaceae; Scilloideae; Hyacintheae) based on multilocus DNA sequence data has revealed the existence of four morphologically, ecologically and/or geographically distinct species, and supports the elevation of three existing subspecies to the species level. The new species and combinations are presented here. The new species are *Lachenalia komsbergensis* from the Komsberg in the southern Great Karoo (Northern Cape), *Lachenalia inflata* from the Hantam-Roggeveld escarpment in the western Great Karoo (Northern Cape), *Lachenalia granitica* from the Cape West Coast (Western Cape) and *Lachenalia filamentosa* from the Cape West Coast, Breede River Valley and Overberg (Western Cape). The taxa elevated from subspecies to species level are *Lachenalia rogersii* (previously *L. mediana* subsp. *rogersii*) from the Breede River Valley (Western Cape), *Lachenalia glaucina* (previously *L. orchioides* subsp. *glaucina*) from the southern Cape Peninsula (Western Cape) and *Lachenalia parviflora* (previously *L. orchioides* subsp. *parviflora*) from the northeastern Cape Peninsula (Western Cape).

Keywords: Hyacintheae; narrow endemics; southern African flora

3.1. Introduction

Lachenalia J.Jacq. ex Murray (1784) is a large, southern African genus of bulbous geophytes which is included either in the family Asparagaceae, subfamily Scilloideae, tribe Hyacintheae (APG III 2009; APG IV 2016; Chase et al. 2009; Christenhusz et al. 2017) or formerly in the family Hyacinthaceae, subfamily Hyacinthoideae (APG II 2003). The genus is endemic to South Africa and Namibia where it occurs primarily in the western and southwestern winter rainfall zones of both countries, though it is also represented in the interior, summer-rainfall areas of South Africa (Northern and Eastern Cape provinces) and in mountainous, summer-rainfall areas of southern Namibia (Duncan 2005, 2012a, 2017; Duncan et al. 2005). With a current total of 142 species, *Lachenalia* is the most species-rich member of the Asparagaceae

within this ecologically diverse region of southern Africa (Duncan et al. 2022). All species are deciduous and almost all are winter-growing, with the single exception of *L. pearsonii* (R.Glover) W.F.Barker, a summer-growing plant from the Groot Karasberge of southern Namibia (Glover 1915; Barker 1969; Duncan and Condry 2013). A previous, largely artificial classification of *Lachenalia*, based entirely on morphological traits, recognised two subgenera based on floral symmetry: subgen. *Lachenalia* (Duncan 2012a) with zygomorphic perianths and subgen. *Polyxena* (Kunth) G.D.Duncan (2012a) with actinomorphic perianths. Guided by a new phylogenetic hypothesis, based on 378 randomly sampled DNA loci, however, Duncan et al. (2022) dispensed with the pre-existing subgenera and proposed a new infrageneric taxonomy comprising 10 morphologically distinctive sections (*Colliculatae*, *Cordatae*, *Ciliolatae*, *Linearifoliae*, *Cretaceae*, *Atrorubentes*, *Trichomatosae*, *Polyxena*, *Stellatae* and *Lachenalia*) (listed here according to their phylogenetic sequence). The largest of these, sect. *Lachenalia*, was further subdivided by Duncan et al. (2022) into 13 subsections (*Nervosae*, *Coriaceae*, *Crispae*, *Latae*, *Verticillatae*, *Urceolatae*, *Oblongae*, *Framesianae*, *Angustae*, *Stramineae*, *Inflatae*, *Oncoraphae* and *Lachenalia*) (listed here according to their phylogenetic sequence). The analyses for this new phylogenetic hypothesis included multiple accessions of certain widespread and/or morphologically variable species, of which some were resolved in phylogenetically isolated positions, suggesting that they represent multiple species, and the distinctness of these entities was corroborated by morphology. All four new species described in the current paper, as well as three subspecies elevated to species level, fall within sect. *Lachenalia* (Duncan et al. 2022).

3.2. Materials & Methods

Morphological studies were made from wild and cultivated plants of all four new species proposed here, as well as from recent collections of all three subspecies recommended for elevation to species level. For the four new species, eight specimens each of *L. komsbergensis* and *L. inflata* were collected, and seven specimens of *L. granitica* and nine of *L. filamentosa*. For the three taxonomic adjustments, seven specimens of *L. rogersii*, eight of *L. glaucina* and seven of *L. parviflora* were collected. Material was cultivated in pots in the bulb nursery at Kirstenbosch National Botanical Garden. Pressed material in the herbaria BOL, K, NBG and SAM was examined (Thiers 2019). I include the grid reference system and orthography of geographical names in accordance with Leistner and Morris (1976). Morphological measurements were made from wild and cultivated plants, using a digital

calliper; these included the height of the plants in flower, bulb diameter, leaf length and width, flower head and peduncle length, bract and pedicel length, perianth tube length, tepal length and width, filament and style length, and ovary and capsule length and width. Six living plants each per new species and taxa investigated for taxonomic adjustment were sampled, using mean values. Mean values of seed length and width, and strophiole length, were obtained using a light microscope.

3.3. Taxonomy: new species

3.3.1. *Lachenalia komsbergensis*

Lachenalia komsbergensis G.D.Duncan, *Phytotaxa* 585(4): 262 (2023). (Figs. 11, 12)

Type:—SOUTH AFRICA. Northern Cape: Komsberg Pass, 2.1 km north of entrance to farm De Plaat on R354 from Matjiesfontein to Sutherland (3220DC), solitary plants and scattered clumps on flats among low scrub, in deep, beige alluvium above dry riverbed, in full sun, elev. 1144 m, 8 October 2001, *Duncan 459* (holotype NBG).

This new species differs from *L. juncifolia* Baker (Baker 1871) in its leaves that are deeply canaliculate for their entire length, its spreading, white perianth with narrowly spreading stamens, its slightly larger globose seeds ($1.2\text{--}1.3 \times 1.3\text{--}1.4$ mm) with a matt black testa and rugose primary sculpturing, a longer strophiole 0.5–0.6 mm long, and in its taller stature up to 300 mm high in full flower.

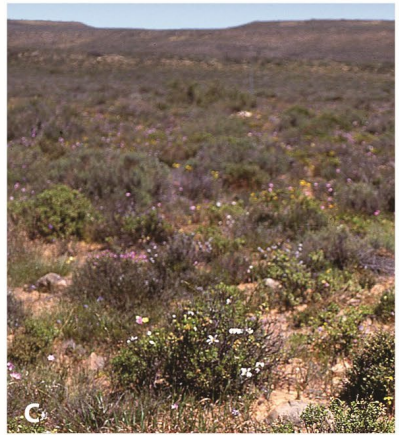


Figure 11. Four new species of *Lachenalia* from southern and western South Africa.

Lachenalia komsbergensis: detail of three flowering racemes in cultivation (A); single plant flowering in beige alluvium, Komsberg Pass (B); general view of Central Mountain Shale Renosterveld habitat, Komsberg Pass (C). *Lachenalia inflata*: single flowering plant in red sand north of Middelpoort (A); single flowering plant in red clay west of Calvinia (B); general view of Roggeveld Karoo habitat north of Middelpoort (F). *Lachenalia granitica*: detail of flowering spike in cultivation, with arrow showing a very long bract (G); flowering plant from Vredenburg in cultivation (H); spike of flowering and fruiting plant in Saldanha Granite Strandveld habitat near Vredenburg (I). *Lachenalia filamentosa*: flowering plants from Riviersonderend in cultivation (J); single flowering plant from De Hoop Nature Reserve in cultivation (K); two plants in early fruiting stage in De Hoop Limestone Fynbos habitat (L). Photographs: A-D, F-K (Graham Duncan); E (Adam Harrower); L (David Gwynne-Evans).

Deciduous, winter-green geophyte 150–300 mm high. Bulb subglobose, 15–20 mm in diam., offset-forming (1 or 2 offsets formed per bulb, but not necessarily every year); tunic multi-layered, outer tunics spongy, dark brown, inner tunics membranous, light brown; cataphyll translucent white, adhering to leaf bases, apex obtuse. Leaves 2, linear, 200–260 × 1–15 mm, suberect or recurved, deeply canaliculate, upper surface light green, lower surface light green in upper two thirds, heavily magenta- or purple-barred in lower third; apex acute; primary seedling leaf terete, erect. Inflorescence racemose, 10–48-flowered, flower head 40–120 mm long; peduncle erect to suberect, 100–155 mm long, rigid, light green, heavily speckled with dull purple; rachis light green and heavily purple-speckled in lower two thirds, white in upper third; lower bracts ovate, 1–4 × 2–3 mm, upper bracts lanceolate, 1–2 × 1 mm, white; pedicels suberect, 5–10 mm long, white. Perianth zygomorphic, oblong-campanulate, suberect; tube cup-shaped, 3 mm long, white; outer perianth segments ovate, 4–5 × 3–4 mm, apices flat, apical gibbosity deep pink; inner perianth segments obovate, 5–7 × 3–5 mm, translucent white, median keel light green in upper half, apices recurved. Stamens well exserted, narrowly spreading; filaments 8–10 mm long, white; anthers 0.8–1.0 mm long, pollen yellow. Ovary obovoid, 3 × 2 mm, light green; style well exserted, straight, 9–10 mm long, white; stigma minutely capitate. Capsule obovoid, 6–7 × 4–5 mm. Seed globose, 1.2–1.3 × 1.3–1.4 mm, matt, black; primary sculpturing rugose; strophiole 0.5–0.6 mm long, ridged. Flowering time: September to October.

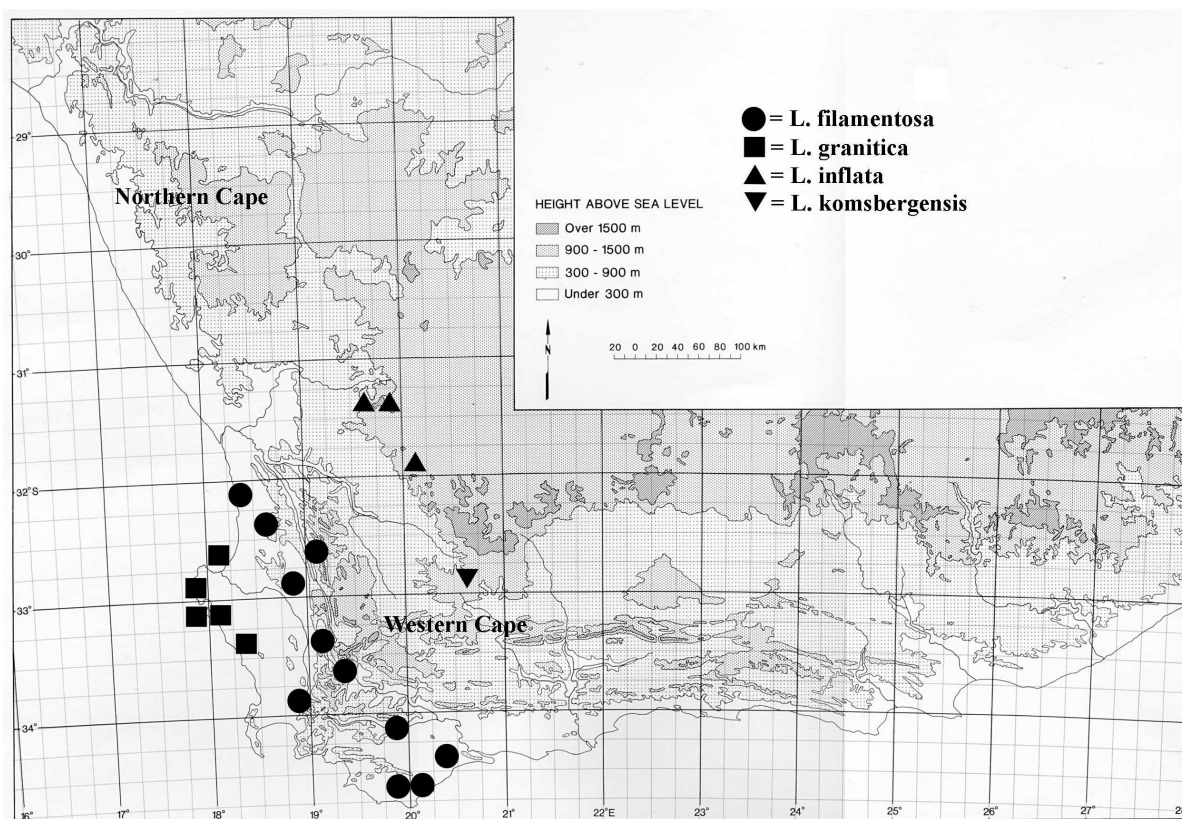


Figure 12. Distribution map of four new species of *Lachenalia* from southern and western South Africa: *L. komsbergensis* in the southern Great Karoo of the Northern Cape (inverted black triangle); *L. inflata* in the western Great Karoo, Northern Cape (black triangles); *L. granitica* along the Cape West Coast, Western Cape (black squares); *L. filamentosa* along the Cape West Coast, Breede River Valley and Overberg in the Western Cape (black dots).

Etymology:—The specific epithet *komsbergensis* commemorates the Komsberg in the southern Great Karoo, to which this species is endemic.

Other material examined:—SOUTH AFRICA. Northern Cape: Komsberg Pass, 2.1 km north of entrance to farm De Plaat on R354 from Matjiesfontein to Sutherland (3220DC), solitary plants and scattered clumps on flats among low scrub, in deep, beige alluvium above riverbed, in full sun, elev. 1144 m, 1 September 2000, *Summerfield s.n.* (NBG); Komsberg Pass, farm Damslaagte (3220DC), at river crossing, 19 October 2008, *Saunders & Manning 3196* (NBG).

Notes:—The first collection of flowering specimens was made at the type location by G.C. Summerfield in September 2000. A subsequent record of flowering plants was made at the same location by G.D. Duncan on 8 October 2001, when the type specimens were collected and habitat photographs were taken. Material from this collection has since flowered every

year in cultivation in the bulb nursery at Kirstenbosch. A third collection at late flowering stage was made close to the type location by R. Saunders and J. Manning on 19 October 2008.

Diagnostic features and affinities:—*Lachenalia komsbergensis* is recognised in flower by an erect or suberect, rigid, light green peduncle that is heavily speckled with dull purple, bearing a raceme of small oblong-campanulate, spreading, white and pink flowers carried on white, suberect pedicels (Fig. 11A). It has shallowly cup-shaped perianth tubes and narrowly spreading perianth segments. The outer perianth segments have a prominent deep pink median keel and apical gibbosity, and the strongly protruding inner perianth segments have a dull pink median keel. The flowers have bracts that are ovate at the base of the inflorescence and lanceolate above, and well exserted, narrowly spreading, white stamens. The inflorescence emerges towards the end of the growing season, as the leaves start to wither. The species is further recognised by two linear, suberect or recurved, deeply canaliculate, conduplicate leaves, with acute apices. The upper and lower surfaces are light green, and the lower surfaces are heavily magenta- or purple-barred in the lower third, and green-barred in the upper two thirds. The primary seedling leaf is terete, with erect orientation. The fruit is an obovoid capsule containing globose, matt black seeds with rugose primary sculpturing, and ridged strophioles.

Lachenalia komsbergensis is included within subsect. *Lachenalia* of sect. *Lachenalia* (Duncan et al. 2022). It was initially considered to be a tall-growing form of *L. juncifolia*, but in phylogenetic analysis it was retrieved as sister to two accessions of *L. longibracteata* Phillips (1931), in a phylogenetically strongly isolated position from *L. juncifolia*, the latter falling within sect. *Linearifoliae* (Duncan et al. 2022). *L. komsbergensis* differs from *L. juncifolia* in having leaves that are deeply canaliculate for their entire length (versus slightly canaliculate in the lower two thirds and terete in the upper third), a spreading, mostly white perianth (versus cernuous and cream-coloured, pink, magenta or purple), narrowly spreading stamens (versus straight), a taller stature up to 300 mm high (versus up to 200 mm high), slightly larger seeds ($1.2\text{--}1.3 \times 1.3\text{--}1.4$ mm) with a matt black testa and rugose primary sculpturing (versus $1.1\text{--}1.2 \times 1.3$ mm, with a glossy black testa and smooth primary sculpturing), and a substantially longer strophiole 0.5–0.6 mm long (versus 0.2 mm long). Furthermore, *L. juncifolia* has a much longer flowering period (August to December) and a much wider distribution from Calvinia in the western Great Karoo to Herbertsdale in the southern Cape (Duncan 2012a).

Distribution and habitat:—*Lachenalia komsbergensis* occurs in the Succulent Karoo biome in the southern part of the Northern Cape, and is endemic to the Komsberg, a component of the Hantam-Roggeveld Centre of Plant Endemism (Van Wyk and Smith 2001; Mucina and Rutherford 2006; Clark et al. 2011). It is highly localised to open flats adjacent to a seasonal river in the southern Komsberg, at an elevation of 1144 m (Fig. 12). The area receives most of its rainfall in winter but is transitional between winter and summer rainfall regimes (Clark et al. 2011). The plants occur as scattered solitary individuals or in small groups of up to four plants on flats in deep, beige alluvium. The bulbs are shallowly seated and occur among low, sparse, scrubby vegetation including perennials of the family Asteraceae, in Central Mountain Shale Renosterveld, a vegetation type which occupies a borderline position straddling the Fynbos, Succulent Karoo and marginally the Nama Karoo biomes (Mucina and Rutherford 2006) (Fig. 11B,C). The species flowers late in the spring season (late September to October), at a time when the leaves are already beginning to desiccate.

3.3.2. *Lachenalia inflata*

Lachenalia inflata G.D.Duncan, *Phytotaxa* 585(4): 265 (2023). (Figs. 11, 12)

Type:—SOUTH AFRICA. Northern Cape: Akkerendam Nature Reserve, Calvinia (3119BD), stony clay flats and lower slopes of Hantamsberg Mountains, among low scrub, elev. 1139 m, 22 July 1961, *Barker 9319* (holotype NBG!).

This new species differs from *L. mutabilis* Loddiges ex Schultes & Schultes (Linnaeus (1830)) in having a bright yellow perianth with the upper two inner perianth segments equal in length to the lower inner perianth segment, and in its shorter filaments and oblong, narrower seeds with an inflated raphe.

Deciduous, winter-green geophyte 80–220 mm high. Bulb subglobose, 15–22 mm in diam., solitary; tunic multilayered, outer tunics spongy, dark brown, inner tunics membranous, translucent light brown; cataphyll translucent white, apex obtuse. Leaf 1, rarely 2, lanceolate, 100–150 × 10–20 mm, suberect, conduplicate, leathery, upper and lower surfaces green or glaucous, sometimes heavily flushed with brownish purple, or heavily purple- or green-blotched along median; margins thickened/cartilaginous, slightly to strongly undulate, sometimes crisped; leaf bases clasping, 10–20 mm long, light green, sporadically or densely marked with purplish or green blotches on outer surface; primary seedling leaf prostrate, flat.

Inflorescence spicate, 10–35-flowered; peduncle erect or suberect, moderately or strongly inflated above, maroon, or dull mauve-flushed in upper half, light green and mauve-spotted in lower half; rachis inflated and mauve-flushed in lower half, shading to brownish green in upper half; sterile apex 10–20 mm long, light green or light mauve; lower bracts cup-shaped, 1 × 2 mm, translucent white, upper bracts lanceolate, 2–5 × 1 mm, translucent white. Perianth zygomorphic, urceolate, slightly cernuous; perianth tube cup-shaped, 2–3 mm long, light greenish yellow or bright yellow; outer perianth segments ovate, 6–7 × 4 mm, bright yellow or greenish yellow, apices straight, apical gibbosity dark yellow, brown or brownish green; inner perianth segments obovate, 7–9 × 3–4 mm, bright yellow or greenish yellow, apices slightly recurved, median keel dull brown or brownish green. Stamens declinate, included; filaments 5–6 mm long, white. Ovary ellipsoid, 4 × 2 mm, light green; style declinate, included, 5 mm long, white, stigma minutely capitate. Capsule ellipsoid, 8–9 × 4–5 mm. Seed oblong, 1.2 × 0.9 mm, glossy, smooth, black; strophiole 0.7 mm long, glossy, inflated, smooth; raphe inflated. Flowering time: July to September.

Etymology:—The specific epithet *inflata* is descriptive of the inflated upper part of the peduncle.

Other material examined:—SOUTH AFRICA. Northern Cape: 12 km west of Calvinia on R355 to Loeriesfontein (3119BC), on red, sandy clay flats, among low scrub, 4 September 2007, *Harrower 3594* (NBG, photo); Akkerendam Nature Reserve, Calvinia (3119BD), stony clay flats below Hantam Mountains, among low scrub, 26 August 2007, *Avenant s.n.* (NBG, photo); Farm Hangnes, Leliekrantz Road NW of Middelpos (3120CC), scattered solitary plants on flats among low scrub, in deep red sand, in full sun or light shade of bushes, elev. 1189 m, 24 September 2015, *Duncan 666* (NBG).

Notes:—The first collection of *L. inflata* was made by W.F. Barker within the Akkerendam Nature Reserve at the base of the Hantam Mountains at Calvinia on 22 July 1961. On 22 September 2006, G.C. Summerfield recorded it on the farm Hangnes northwest of Middelpos in the Roggeveld, and he was the first to note that the oblong seeds of this species differed markedly from the globose ones of *L. mutabilis*, and thus suspected it to represent an undescribed species. In August 2007, F. Avenant photographed it in flower at Akkerendam Nature Reserve, and in September 2007, A.D. Harrower recorded it in flower just west of

Calvinia. During a visit to Hangnes on 24 September 2015, the plant was sampled and studied in habitat by G.D. Duncan.

Diagnostic features and affinities:—*Lachenalia inflata* is recognised in flower by an erect or suberect spike of urceolate, bright yellow or greenish yellow, slightly cernuous flowers carried on a strongly inflated, maroon, or dull mauve-flushed peduncle which is marked with purple or maroon blotches (Fig. 11D, E). It has shallowly cup-shaped perianth tubes, the outer perianth segments have a dark yellow, brown or greenish brown apical gibbosity, and the protruding inner perianth segments have a dark yellow, dull brown or brownish green median keel. The flowers have included, declinate stamens, and bracts that are cup-shaped at the base of the inflorescence and lanceolate above. The plant usually has a solitary leathery lanceolate leaf (rarely 2) which is conduplicate or deeply channelled, and the margins are thickened/cartilaginous and slightly to strongly undulate or sometimes crisped. The ripe fruit is an ellipsoid capsule containing oblong, glossy black seeds with a smooth, black strophiole and an inflated raphe. *Lachenalia inflata* is included within subsect. *Oncoraphae* of sect. *Lachenalia* (Duncan et al. 2022). It was initially considered to be merely a yellow-flowered form of *L. mutabilis*, but in phylogenetic analysis it was retrieved as sister to the sister pair *L. bulbifera* (Cirillo) Engl. (Engler 1899) and *L. punctata* Jacq. (Jacquin 1789), two widespread, long-tubed, tubular-flowered lachenalias mainly from coastal parts of the Western and Northern Cape. These three taxa constitute the only members of subsect. *Oncoraphae* and are united by the possession of seeds with an inflated raphe (Duncan et al. 2022). *L. mutabilis* differs from *L. inflata* in having a light blue perianth tube, greenish-brown, light to deep mauve outer perianth segments with prominent brown apical gibbosities, and inner perianth segments that are yellowish-green or translucent white, with brown or green median keels, with the lower inner perianth segment slightly longer than the upper inner perianth segments. It differs further in having longer filaments 7–9 mm long (versus 5–6 mm long), globose seeds (versus oblong) with a longer length range of 1.2–1.4 mm (versus 1.2 mm) and are wider (1.3–1.4 mm, versus 0.9 mm), and have no inflated raphe.

Distribution and habitat:—*Lachenalia inflata* occurs within the Succulent Karoo Biome and is currently known from only three locations (Fig. 12). It frequents dwarf shrubland in Hantam Karoo vegetation on stony red clay flats and lower slopes of the Hantamsberg within the Akkerendam Nature Reserve at Calvinia, as well as on clay flats to the west of this town, whereas to the northwest of Middelpos (southeast of Calvinia), it occurs in Roggeveld Karoo

vegetation in deep red sand (Mucina and Rutherford 2006) (Fig. 11F). Plants occur as scattered solitary individuals or in groups of two, in open aspects or within the protection of low karroid scrub, and flowering takes place from late winter to spring (late July to late September).

3.3.3. *Lachenalia granitica*

Lachenalia granitica G.D.Duncan, *Phytotaxa* 585(4): 266 (2023). (Figs. 11, 12)

Type:—SOUTH AFRICA. Western Cape: Vredenburg (3217 DD), among granite rocks immediately south of town, elev. 167 m, 6 August, 1962, *Barker 9680* (holotype NBG!).

This new species differs from *L. longibracteata* E.Phillips (Phillips 1931) in having a spicate inflorescence with longer, metallic blue and often blue-spotted outer perianth segments (9–10 mm long), translucent white inner perianth segments, longer filaments (9–11 mm long), a shorter style (9–10 mm long), a leaf upper surface that is often shallowly pustulate, a smaller globose seed ($0.9\text{--}1.0 \times 1.0$ mm) with a shorter inflated strophiole (0.8 mm long), and a flowering period that begins much earlier in midwinter (late June).

Deciduous, winter-green geophyte 90–330 mm high. Bulb globose, 10–25 mm in diam., solitary; tunic multilayered, outer tunics spongy, dark brown, inner tunics membranous; cataphyll $10\text{--}32 \times 10\text{--}15$ mm, translucent white, apex obtuse. Leaf 1 or 2, lanceolate, $35\text{--}190 \times 8\text{--}28$ mm, suberect, dull green, upper surface usually smooth, rarely covered with flattened pustules; margins coriaceous; leaf bases clasping, 20–40 mm long, light green; primary seedling leaf prostrate, flat. Inflorescence spicate, 7–50-flowered, dense, peduncle erect or suberect, glaucous, plain or lightly green-spotted; bracts ovate at base of inflorescence, becoming lanceolate above, $2\text{--}25 \times 3\text{--}5$ mm, translucent white. Perianth zygomorphic, oblong-campanulate, suberect, sweet-scented; tube cup-shaped, 4–5 mm long, metallic blue or blue-green, plain or darker blue-spotted; perianth segments light metallic blue, plain or darker blue-spotted; outer perianth segments narrowly ovate, $9\text{--}10 \times 3\text{--}4$ mm, apical gibbosity brown or greenish brown; inner perianth segments obovate, $10\text{--}11 \times 4\text{--}5$ mm, translucent white, fading to brownish maroon, apices obtuse, slightly recurved, median keels bright green. Stamens included or shortly exserted; filaments declinate, 9–11 mm long, white; anthers oblong, 1 mm long, pollen yellow. Ovary ellipsoid, 3×2 mm, dull green; style declinate, 9–10 mm long, white, becoming strongly exserted in fruit; stigma minutely capitate. Capsule ellipsoid, $8\text{--}10 \times 5\text{--}6$ mm. Seed globose, $0.9\text{--}1.0 \times 1.0$ mm, matt, black,

secondary sculpturing rugose; strophiole inflated, 0.8 mm long, smooth, glossy black.

Flowering time: June to September.

Etymology:—The specific epithet *granitica* refers to the granite rocky outcrops and granitic flats of the Cape West Coast, to which this species is endemic.

Other material examined:—SOUTH AFRICA. Western Cape: Witklip south of Vredenburg (3217DD), 9 August 1984, *Barker 10250* (NBG); granite outcrop south of Vredenburg, off R399 (3217DD), among houses, in loamy clay soil, westerly aspect, 20 June 2006, elevation 167 m, *Cowell & Nurrish 537* (NBG); Stompneus, northwest of St. Helena Bay (3217DD), 27 June 1967, *Hall 3139* (NBG); above Stompneus (3217DD), 17 June 1965, *Barker 10567* (NBG). Nooitgedacht Farm near St Helena Bay (3218CC), 28 June 1972, *Barker 10228* (NBG). Entrance to Saldanha off R399, on granite hill above town (3317BB), occasional in loamy clay on west-facing rocky slope, 9 October 1983, *Duncan 119* (NBG). Langebaan (3318AA), 21 July 1938, *Lewis s.n.* sub. NBG 222/32 (NBG). Darling (3318AD), 23 August 1947, *Barker 4599* (NBG); Waylands Farm Flower Reserve east of Darling (3318AD), 8 September 1974, *Barker s.n.* (NBG, photo.).

Notes:—The earliest known collection of *L. granitica* is that of G.J. Lewis who recorded it in flower at Langebaan on the Cape West Coast on 21 July, 1938. Most collections have been made in the vicinity of Vredenburg to the north. The species is becoming increasingly threatened by coastal housing development.

Diagnostic features and affinities:—*Lachenalia granitica* is recognised in flower by a dense spike of suberect, oblong-campanulate, sweet-scented flowers with metallic blue or blue-green perianth tubes, light metallic blue outer perianth segments which may be plain or darker blue-spotted, and protruding, translucent white inner perianth segments with obtuse, slightly recurved apices. The outer perianth segments have a prominent brown or greenish brown apical gibbosity, and the inner perianth segments have bright green median keels and fade to brownish maroon (Fig. 11G, H, I). Each flower is subtended by a prominent lanceolate, translucent white bract, and in the upper part of the inflorescence the bracts may be up to 25 mm long (Fig. 11G). The plant produces one or two lanceolate, dull green, suberect leaves with coriaceous margins, mostly with a smooth surface, or rarely with sporadic flattened pustules. The ripe fruit is an ellipsoid capsule containing globose, matt, black seeds with an inflated, smooth, glossy strophiole.

Lachenalia granitica is a member of sect. *Lachenalia*, subsect. *Lachenalia* (Duncan et al. 2022). Due to its prominent floral bracts, and similar oblong-campanulate flowers, it has previously been regarded as a blue-flowered form of *L. longibracteata* (Duncan 2012a), however in phylogenetic analysis it was retrieved as sister to the turquoise, tubular-flowered *L. viridiflora* W.F.Barker (Barker 1972) (with which it occurs sympatrically on granite outcrops in northern parts of its Cape West Coast range), within the uppermost clade of subsect. *Lachenalia* (Duncan et al. 2022). *L. longibracteata* differs from *L. granitica* in having a racemose inflorescence with shorter, greenish yellow outer perianth segments (6–9 mm long), shorter filaments (7–9 mm long), a longer style (11–14 mm long), a leaf upper surface that is always smooth, a larger globose seed (1.1 × 0.9 mm) with a longer inflated strophiole (1.0 mm long) and a later flowering period from early to late spring (late August to mid-October).

Distribution and habitat:—*Lachenalia granitica* occurs in the Fynbos Biome and is confined to the Cape West Coast, extending from Darling in the south to St. Helena Bay in the north (Fig. 12). It is endemic to granite outcrops and flats, occurring among low scrub in partially shaded aspects within boulder crevices in dark brown, loamy soils in Saldanha Granite Strandveld, and in open aspects on granite flats, in Swartland Granite Renosterveld (Mucina and Rutherford 2006). The plants occur as scattered solitary individuals or in small groups, and when in flower, are inconspicuous among surrounding vegetation. Flowering commences in mid-winter and ends in early spring (late June to early September). Near Vredenburg, the flowerheads are grazed by steenbuck (*Raphicerus campestris*) and in winter the bulbs are excavated and eaten by Cape porcupines (*Hystrix africaeaustralis*) (Duncan 2012a).

3.3.4. *Lachenalia filamentosa*

Lachenalia filamentosa G.D.Duncan, *Phytotaxa* 585(4): 267 (2023). (Figs. 11, 12)

Type:—SOUTH AFRICA. Western Cape: Die Poort, 5 miles [8 km] south of Bredasdorp (3420CA), in [limestone] sand, elev. 84 m, 3 September 1943, *Barker 2500* (holotype NBG!).

This new species differs from *L. contaminata* Aiton (Aiton 1789) in having well-exserted stamens (8–11 mm long), longer outer perianth segments (6–7 mm long), longer inner perianth segments (7–8 mm long) and a larger globose seed (1.1–1.2 × 1.0 mm).

Deciduous, winter-green geophyte 110–150 mm high. Bulb subglobose, 15–20 mm in diam., offset-forming (1–3 offsets formed per bulb, but not necessarily every year); tunic multi-layered, outer tunics spongy, dark brown, inner tunics membranous, light brown; cataphyll translucent white, not adhering to leaf bases, apex obtuse. Leaves 3–6, linear, 120–155 × 1.5–5.0 mm, fleshy, glaucous-green, upper surface shallowly channelled in lower half, terete in upper half, lower surface glaucous-green or maroon-flushed, apices acute; primary seedling leaf erect, terete. Inflorescence racemose, 8–30-flowered; peduncle erect or suberect, light green, plain or purple-blotched in lower third, shading to dull maroon above; pedicels 2–3 mm long, suberect in flower, white or light magenta, becoming erect in fruit; bracts cup-shaped, green at base of inflorescence, shading to white above, 1–3 × 1–4 mm; perianth narrowly campanulate, suberect, spice-scented. Perianth tube cup-shaped, 2 mm long, white; outer perianth segments narrowly ovate, 6–7 × 3–4 mm, white, apical gibbosity narrow or prominent, magenta, median keel narrow or broad, magenta; inner perianth segments narrowly obovate, 7–8 × 3–4 mm, protruding 1 mm beyond outer tepals, white, median keel narrow or broad, magenta. Stamens well exserted, narrowly spreading; filaments white, inserted at distinctly different levels; filaments at base of outer perianth segments 8–9 mm long, filaments at base of inner perianth segments 10–11 mm long; anthers oblong, 1 mm long, maroon prior to anthesis, pollen yellow. Ovary ellipsoid, 2.0 × 1.8 mm, dull green, style straight, 10–11 mm long, becoming well exserted 7–8 mm beyond perianth segments as ovary enlarges, stigma capitate. Capsule ellipsoid, 7–8 × 4–5 mm, suberect. Seed globose, 1.1 × 1.0 mm, glossy, black; strophiole rudimentary, 0.2 mm long, ridged. Flowering time: August to September.

Etymology:—The specific epithet *filamentosa* refers to the thread-like, well-exserted filaments.

Other material examined:—SOUTH AFRICA. Western Cape: southeast of Ludrift Farm, near Lambert's Bay, wetland adjacent to sandveld fynbos (3218AB), 27 September 2002, *Low* 7682 (NBG). Farm 'Uitkyk', 10 km N of Porterville, on clay-shale (3218DD), 24 September 2002, *Helme* 2754 (NBG). Citrusdal, 3 miles (5 km) north of town, 31 August 1938 (3219CA), *Salter* 7492 (NBG). Eensaamheid Nature Reserve, 10 km west of Paarl, in waterlogged shale (3318 DD), 26 September 2002, *Helme* 2755, (NBG). Tulbagh, Voëlvllei Tortoise Reserve, in red-brown stony clay (3319AC), 5 October 1989, *Solomon* 100 (NBG); Rawsonville, between old and new N1, on sandstone (3319CB), 27 August 2007, *Helme*

4937 (NBG). Farm Boesmansrivier at foot of Riviersonderend Mountains, in white Table Mountain Sandstone, in fruit (3419BB), 10 October 1979, *Malan 117* (NBG); between Heuningrug and Wiesduif, on limestone koppie (3419DB), 29 August 1995, *Paterson-Jones 573* (NBG). De Hoop Nature Reserve, behind Manager's house, in limestone fynbos (3420AD), 4 December, 2007, *Duncan, Pekeur & Bennett 3596* (NBG); De Hoop Nature Reserve, in light brown loam (3420AD), 21 August 1984, *Scott 450* (NBG); De Hoop Nature Reserve, Melkkamer, in sand (3420AD), 20 September 1984, *van Wyk 1963* (NBG); Bredasdorp (3420CA), 4 September 1929, *Barnes s.n.* (BOL); Die Poort (3420CA), 3 August 1940, *Compton 9006* (NBG); Die Poort, 5 miles [8 km] south of Bredasdorp, on limestone (3420CA), 19 September 1962, *Nordenstam 1504* (NBG).

Notes:—The earliest collections of *L. filamentosa* were made by P. Barnes near Bredasdorp on 4 September, 1929 (BOL), and T.M. Salter north of Citrusdal on 31 August, 1938 (NBG). The species has been recorded numerous times in the vicinity of Bredasdorp and the adjacent De Hoop Nature Reserve.

Diagnostic features and affinities:—*Lachenalia filamentosa* is recognised in flower by dense racemes of narrowly campanulate, suberect white flowers, the outer perianth segments with a narrow or prominent, magenta apical gibbosity, and the outer and protruding inner perianth segments with a narrow or broad, magenta median keel. The stamens are well-exserted and narrowly spreading, with prominent maroon unripe anthers, and yellow ripe pollen (Fig. 11J–L). During the fruiting stage, the pedicels change orientation from suberect to erect (Fig. 11L). The 3–6 linear leaves have acute apices and are terete in the upper half, and shallowly channelled and considerably wider (up to 5 mm wide) in the lower half.

Lachenalia filamentosa is a member of sect. *Lachenalia*, subsect. *Angustae* (Duncan et al. 2022). It was previously considered to be merely a form of *L. contaminata* with well-exserted stamens (Duncan 2012a), however in phylogenetic analysis it was retrieved as sister to a typical form of *L. contaminata*, despite the fact that a different accession of a typical form of *L. contaminata* was retrieved in a phylogenetically isolated part of the 'subsect. *Angustae*' clade (Duncan et al. 2022). *L. contaminata* differs from *L. filamentosa* in having included stamens (5 mm long), shorter outer perianth segments (5 mm long), shorter inner perianth segments (6–7 mm long) and a smaller globose seed ($0.9\text{--}1.0 \times 0.8\text{--}0.9$ mm).

Distribution and habitat:—*Lachenalia filamentosa* is fairly widely distributed in the western, southwestern and southern parts of the Western Cape, from De Hoop Nature

Reserve in the south to Lambert's Bay in the north (Fig. 12). It traverses a range of habitats and vegetation types, including limestone flats and hills in De Hoop Limestone Fynbos (Mucina and Rutherford 2006) (Fig. 11L), sandstone mountain slopes in North Sonderend Sandstone Fynbos near Riviersonderend, and clay-shale flats and hills in Breede Shale Renosterveld near Tulbagh, and usually occurs in large colonies. Elevation ranges from 15 m on the south coast to 900 m in the northern foothills of the Riviersonderend Mountains. Flowering takes place from early to late spring (early August to late September).

3.4. Taxonomic adjustments

The statuses of three *Lachenalia* taxa are adjusted here (Fig. 13). Firstly, the plant originally regarded as a variety of *L. unifolia* Jacq. (Jacquin 1797b), then as a variety of *L. mediana* Jacq. (Jacquin 1791), and then as a subspecies of *L. mediana*, is elevated to species level. Secondly, the plant originally published as a good species, then regarded as a variety of *L. orchioides* (L.) Aiton (Aiton 1789), then elevated as a subspecies of *L. orchioides*, is returned to species level. Thirdly, the plant originally described as a variety of *L. glaucina* Jacq. (Jacquin 1797a), then published as a subspecies of *L. orchioides*, is elevated to species level.

3.4.1. *Lachenalia rogersii*

Lachenalia rogersii (Baker) G.D.Duncan, *comb. nov. & stat. nov.* $\equiv$ *Lachenalia unifolia* var. *rogersii* Baker (Baker 1897: 431). *Lachenalia mediana* var. *rogersii* (Baker) W.F.Barker (Barker 1989: 642) $\equiv$ *Lachenalia mediana* subsp. *rogersii* (Baker) G.D.Duncan (Duncan 2012a: 195), *syn. nov.*

Syntypes:—SOUTH AFRICA. Cape: precise locality and date unknown, *Rogers s.n.* (lectotype K!, designated by W.F. Barker (Barker 1989: 642); Tulbagh, precise date unknown, *Pappe s.n.* (lectotype K!, designated by W.F. Barker (Barker 1989: 642); isolectotype SAM 23131!, designated by W.F. Barker (Barker 1989: 642).

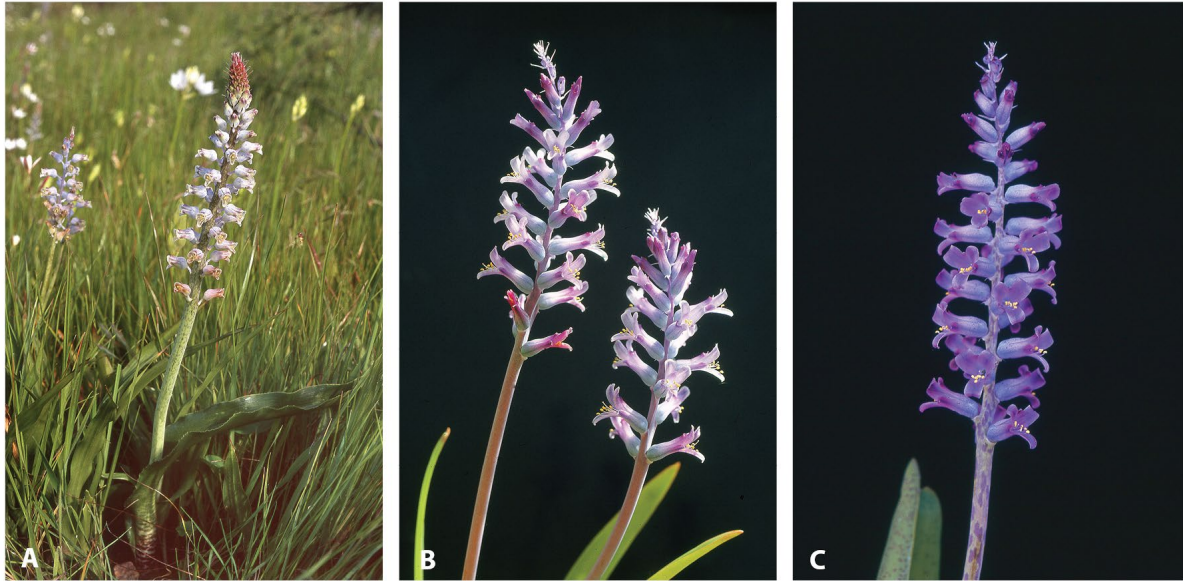


Figure 13. *Lachenalia* taxa elevated from subspecific to species status. *L. rogersii* (previously *L. mediana* subsp. *rogersii*) (A); *L. glaucina* (previously *L. orchiodes* subsp. *glaucina*) (B); *L. parviflora* (previously *L. orchiodes* subsp. *parviflora*) (C).

Deciduous, winter-green geophyte 150–330 mm high. Bulb globose, 12–15 mm in diam., offset-forming (1 offset formed per bulb, but not necessarily every year); tunic multi-layered, outer layers spongy, dark brown; inner cataphyll translucent white, apex obtuse. Leaves 1 or 2, lanceolate, 100–140 × 15–25 mm, lower surface with sporadic purplish-brown blotches; leaf margins undulate and crisped; leaf bases 20–40 mm long, heavily marked with transverse magenta bands; primary seedling leaf flat, prostrate. Inflorescence racemose, 15–40-flowered; peduncle erect or suberect, light green, plain, or spotted or blotched with dark maroon or brown; pedicels 2–10 mm long; bracts ovate at base of inflorescence, becoming lanceolate above, 2–6 × 1–3 mm. Perianth zygomorphic, oblong campanulate, slightly cernuous in flower, becoming erect in fruit; outer perianth segments ovate, 6–7 × 4–5 mm, light blue or light pink, apical gibbosities green; inner perianth segments obovate, 9–10 × 3–4 mm, median keels light blue near apex. Stamens included; filaments declinate, 9–11 mm long, white. Ovary ellipsoid, 3–4 × 2–3 mm, light green; style included, 8–9 mm long, white. Capsule ellipsoid, 7–9 × 5–6 mm. Seed globose, 1.2 × 1.3 mm, glossy, black; strophiole inflated, 1.1 mm long, smooth. Flowering time: August to September.

Lachenalia unifolia var. *rogersii* was described from syntype material collected at an unrecorded locality at the Cape by the Rev. W.M. Rogers, possibly in April 1880, as well as by C.W.L. Pappe at Tulbagh on an unrecorded date (Baker 1897). On the basis of seed

morphology, Barker (1989) considered the plant to be more appropriately regarded as a variety of *L. mediana* and the new combination *L. mediana* var. *rogersii* was made. The taxon was subsequently elevated to subspecific level as *L. mediana* subsp. *rogersii* since it differs morphologically from var. *mediana* and breeds true in allopatric populations (Duncan 2012a). In a recent phylogenetic hypothesis of *Lachenalia*, *L. mediana* subsp. *mediana* and *L. mediana* subsp. *rogersii* were retrieved as sister taxa (Duncan et al. 2022). These taxa are now considered worthy of elevation to species level, based on differences in diploid chromosome number ($2n = 14$ in subsp. *mediana*: Johnson and Brandham 1997, versus $2n = 26$ in subsp. *rogersii*: Spies et al. 2008) and differences in perianth morphology and habitat preference. *L. rogersii* differs from *L. mediana* in having a slightly cernuous perianth (versus spreading or suberect), and in having light pink or light blue outer perianth segments (versus grey) that are shorter (6–7 mm long versus 7–8 mm long) and more contracted towards the mouth (versus flared), with the filaments always included (versus usually shortly exserted) (Fig. 13A). Historically, *L. rogersii* has always been confined to locations in the Hermon, Saron, Tulbagh and Porterville districts in the southwestern part of the Western Cape. Its habitat is seasonally waterlogged, clay soils, in Breede- and Swartland Shale Renosterveld (Mucina and Rutherford 2006), the plants sometimes occurring along swamp and stream margins, versus relatively well-drained, drier clay soils in *L. mediana*, which has a wider distribution from Darling on the Cape West Coast to Caledon in the Overberg. Populations of *L. rogersii* are now highly fragmented as a result of wheat cultivation, overgrazing and the smothering effect of invasive alien plants (Duncan 2012a).

3.4.2. *Lachenalia glaucina*

Lachenalia glaucina Jacq. (1797a: 59) $\equiv$ *Orchiastrum glaucinum* (Jacq.) Lem. (Lemaire 1855: 100), *nom. illeg.* $\equiv$ *Lachenalia orchioides* var. *glaucina* (Jacq.) W.F.Barker (1989: 640) $\equiv$ *Lachenalia orchioides* subsp. *glaucina* (Jacq.) G.D.Duncan (Duncan 2012a: 118), *syn. nov.*

Type:—SOUTH AFRICA. Cape: collector and precise locality unknown, figure in Jacquin (1793–1794) (neotype t. 391, designated by Duncan 2012a: 118).

Deciduous, winter-green geophyte 150–400 mm high. Bulb globose, 10–20 mm in diam., offset-forming (1 or 2 offsets formed per bulb, but not necessarily every year); tunic multi-layered, outer layers spongy, light to dark brown; inner cataphyll translucent white, apex

obtuse. Leaves 1 or 2, lanceolate, 85–150 × 15–30 mm, spreading or suberect, light to dark green, upper surfaces plain or heavily marked with dark green, brown or brownish-maroon spots; leaf margins coriaceous; leaf bases clasping, 20–40 mm long; primary seedling leaf flat, prostrate. Inflorescence spicate, 15–40-flowered; peduncle 100–150 mm long; bracts ovate at base of inflorescence, becoming lanceolate above, 2–8 × 1–4 mm. Perianth zygomorphic, tubular, suberect; perianth tube 6 mm long, turquoise; outer perianth segments oblong, 8–9 × 4–5 mm, light blue to purplish blue, apical gibbositities dark blue or dark purplish blue; inner perianth segments oblong-obovate, 13–15 × 4–5 mm, translucent white, light blue or light purplish blue, median keels dark blue. Stamens included, more or less straight; filaments 10–11 mm long, white. Ovary ellipsoid, 4–5 × 3–4 mm, light green; style included, 11–12 mm long, white. Capsule ellipsoid, 9–12 × 4–5 mm. Seed globose, 0.9–1.0 × 0.8–0.9 mm, glossy, black; strophiole inflated, 0.7–0.8 mm long, smooth. Flowering time: September to October.

Lachenalia glaucina was described by N.J. Jacquin in volume 5 of *Collectanea* in 1797 from material gathered at the ‘Cape of Good Hope’ by unspecified collectors (probably by Schönbrunn gardeners Francis Boos and George Scholl) that was cultivated at the Imperial Gardens at Schönbrunn, Vienna. It had previously been illustrated by means of a hand-coloured engraving by N.J. Jacquin in 1793–1794 (year uncertain) on plate 391 of his *Icones Plantarum Rariorum*, and prior to that, in an engraving (Figure 1 on plate XI) in Jakob and Johann Philipp Breyne’s *Prodromi Fasciculi Rariorum Plantarum*, in 1739. Other than its blue perianth, the plant resembles *L. orchoides* and the new combination *Lachenalia orchoides* var. *glaucina* was made (Barker 1989). The taxon was subsequently elevated to subspecific level as *L. orchoides* subsp. *glaucina* (Duncan 2012a). Due to the destruction by fire of the holotype material in Vienna during World War 2, Jacquin’s plate 391 was designated as the neotype (Duncan 2012a). Historically, the distribution of *L. glaucina* has always been confined to the eastern slopes of Table Mountain, and it is currently recorded from four small, isolated populations, one in natural vegetation within the grounds of Kirstenbosch National Botanical Garden in Newlands, one within the adjacent Cecilia Forest in Constantia, one at Zonnestraal Farm in Wynberg, and another in Wynberg Park. It is confined to Peninsula Granite Fynbos (Mucina and Rutherford 2006), growing as scattered solitary individuals or in small colonies on lower mountain and hill slopes in stony loam, in open aspects, in light shade of surrounding scrubby growth, or in deep shade of trees (Duncan 2012a). In a recent phylogenetic hypothesis of *Lachenalia*, *L. orchoides* subsp. *glaucina* was

retrieved within subsect. *Lachenalia* of sect. *Lachenalia*, in a phylogenetically isolated position from *L. orchioides* subsp. *orchioides*, within subsect. *Lachenalia*, indicating recognition at the species level (Duncan et al. 2022). Morphologically, *L. orchioides* and *L. glaucina* are similar with respect to perianth, leaf and seed shape, *L. glaucina* differing most obviously in having translucent white, light blue or purplish-blue inner perianth segments, and light blue or purplish blue outer perianth segments, the latter with prominent deep blue or purplish blue apical gibbosities (Fig. 13B). Perianth tube and perianth segment length and width dimensions of *L. glaucina* fall within the range for *L. orchioides*, but *L. glaucina* has slightly narrower seeds (0.8–0.9 mm) versus 0.9–1.1 mm in *L. orchioides*. *L. orchioides* has a much wider distribution from the Nardousberg Mountains near Klawer in the northwestern Western Cape to the Kouga Mountains north of Haarlem in the southwestern Eastern Cape and inland to Ceres, Montagu and Vanwyksdorp in the Western Cape. The two species always occur as separate populations, and *L. glaucina* has a generally later flowering period from late September to late October, with a peak in early October, versus late June to late October in *L. orchioides*, with a peak in mid-September (Duncan 2012a).

3.4.3. *Lachenalia parviflora*

Lachenalia parviflora (W.F.Barker) G.D.Duncan, *comb. nov. & stat. nov.* $\equiv$ *Lachenalia glaucina* var. *parviflora* W.F.Barker (Barker 1949: 39) $\equiv$ *Lachenalia orchioides* subsp. *parviflora* (W.F.Barker) G.D.Duncan (Duncan 2012a: 121), *syn. nov.* Type:—SOUTH AFRICA. Western Cape: fire guard near Mostert's Ravine, September 1895, *Wolley Dod 380* (holotype BOL!; isotype K!).

Deciduous, winter-green geophyte 130–200 mm high. Bulb globose, 15–20 mm in diam., offset-forming (1 offset formed per bulb, but not necessarily every year); tunic multi-layered, outer layers dark brown, spongy, inner layers membranous, light brown; inner cataphyll translucent white, apex obtuse. Leaves 1 or 2, lanceolate, 60–100 $\times$ 10–15 mm, spreading or suberect, light to dark green, upper and lower surfaces plain or with brownish-maroon spots; leaf margins coriaceous; leaf bases clasping, 20–40 mm long, lower portion white, upper portion green or suffused with brownish maroon; primary seedling leaf flat, prostrate. Inflorescence spicate, 12–30-flowered; peduncle 80–100 mm long, light green, plain or marked with darker green or brownish maroon speckles; perianth zygomorphic, tubular, spreading or weakly suberect; tube 6 mm long, blue with darker blue speckles; outer perianth segments oblong, 5–6 $\times$ 4–5 mm, violet, apical gibbosities deep bluish-purple; inner perianth

segments obovate, $6-7 \times 5-6$ mm, violet, median keels deep bluish-purple, apices recurved. Stamens included, more or less straight; filaments 8–9 mm long, white. Ovary ellipsoid, 3×2 mm, light green; style included, 5–6 mm long. Capsule ellipsoid, $7-8 \times 4-5$ mm. Seed globose, 0.9×0.8 mm, glossy, black; strophiole inflated, 0.7–0.8 mm long, smooth. Flowering time: August to October.

Lachenalia glaucina var. *parviflora* was described from type material collected by A.H. Wolley Dod in September 1895 near Mostert's Ravine, on the lower northeastern slopes of Devil's Peak, in the northeastern Cape Peninsula, and published by Barker (1949). When Barker (1989) re-assessed *L. glaucina* (var. *glaucina*) as *L. orchioides* var. *glaucina*, no reference was made to the status of *L. glaucina* var. *parviflora*, and the latter taxon was subsequently re-assessed by Duncan (2012a) as *L. orchioides* subsp. *parviflora*.

In a recent phylogenetic hypothesis of *Lachenalia*, *L. orchioides* subsp. *parviflora* was placed within subsect. *Lachenalia* of sect. *Lachenalia* and retrieved as sister to *L. fistulosa* Baker (1884, indicating recognition at the species level (Duncan et al. 2022). *L. fistulosa* differs from *L. parviflora* in having narrower, urceolate, white, cream, yellow, light blue, lilac or violet flowers with a shorter perianth tube 2–3 mm long (versus 6 mm long), longer, ovate outer perianth segments 7–8 mm long (versus 5–6 mm long), longer inner perianth segments 8–9 mm long (versus 6–7 mm long), shorter filaments 5–7 mm long (versus 8–9 mm long) and a peculiar heavy, sweet scent (Duncan 2012a).

Lachenalia parviflora is superficially similar to *L. glaucina* in its violet-blue, sessile flowers, but differs in having shorter outer perianth segments 5–6 mm long (versus 8 mm long), shorter inner perianth segments 6–7 mm long (versus 13–15 mm long) that are strongly recurved (versus slightly recurved) and in its shorter stature 130–200 mm high in flower (versus 150–400 mm high) (Fig. 13C).

Lachenalia parviflora has historically always been confined to the lower northeastern slopes of Table Mountain below the backdrop of Devil's Peak, including the uppermost parts of the suburbs of Woodstock and Devil's Peak Estate, and to the suburb of Observatory. It is usually a dwarf species when encountered in full sun, but relatively tall specimens up to 200 mm high have been recorded within shade of exotic trees in the suburb of Observatory. Plants occur in colonies in acidic clay in Cape Winelands Shale Fynbos (Mucina and Rutherford 2006) and flower in profusion in the spring season immediately following summer bush fires (Duncan 2012a).

3.5. Acknowledgements

I thank G.A. Verboom for critically reading the manuscript and providing valuable advice, G.C. Summerfield for much co-operation during field trips, P.V. Bruyns for much appreciated assistance with the distribution map, and A.D. Harrower and D. Gwynne-Evans for the use of photographs. This research was supported by a South African National Biodiversity Institute student grant to the author.

3.6. Addendum

Following the publication of the above paper (<https://doi.org/10.11646/phytotaxa.585.4.2>) it became apparent that an error in typification had occurred, however, correcting this error in the thesis before doing so in the published literature risks generating taxonomic confusion. I therefore undertake to publish a paper, as soon as possible, in which this error can be formally addressed in a taxonomic journal. The error is listed as follows:

(a) Addressing the typification *Lachenalia rogersii*, which is currently typified by two syntypes, but which would be better typified by the selection of a single lectotype.

CHAPTER 4. REPRODUCTIVE SAFEGUARDING

Published paper: Strong habitat and seasonal phenology effects on the evolution of self-compatibility, clonality and pollinator shifts in *Lachenalia* (Asparagaceae: Scilloideae). *New Phytologist* 244: 307–317 (2024). <https://doi.org/10.1111/nph.19786>

Summary

Plants employ a diversity of reproductive safeguarding strategies to circumvent the challenge of pollen limitation. Focusing on southern African *Lachenalia* (Asparagaceae: Scilloideae), we test the hypothesis that the evolution of reproductive safeguarding traits (self-compatibility, autonomous selfing, bird pollination and clonal propagation) is favoured in species occupying conditions of low insect abundance imposed by critically infertile fynbos heathland vegetation and by flowering outside of the austral spring insect abundance peak. We trace the evolution of these traits and selective regimes on a dated, multi-locus phylogeny of *Lachenalia* and assess their evolutionary associations using ordinary and phylogenetic regression. Ancestral state reconstructions identify an association with non-fynbos vegetation and spring flowering as ancestral in *Lachenalia*, the transition to fynbos vegetation and non-spring flowering taking place multiple times. They also show that self-compatibility, autofertility, bird pollination, and production of multiple clonal offsets have evolved repeatedly. Regression models suggest that bird pollination and self-compatibility are selected for in fynbos and in non-spring flowering lineages, with autofertility being positively associated with non-spring flowering. These patterns support interpretation of these traits as reproductive safeguarding adaptations under reduced insect pollinator abundance. We find no evidence to support interpretation of clonal propagation as a reproductive safeguarding strategy.

Keywords: bird pollination; fynbos; Greater Cape Floristic Region; *Lachenalia*; reproductive safeguarding; self-incompatibility; selfing

4.1. Introduction

Pollen limitation is the reduction in reproductive success resulting from insufficient and/or inferior pollen arriving on a plant's stigmas (Ashman et al. 2004; Aizen and Harder 2007). It is commonly a consequence of low local pollinator abundance (Buchman and Nabham 1996),

which is in turn influenced by environmental factors that constrain the growth and reproductive success of pollinator species. In both Eurasian *Aconitum lycoctonum* (Ranunculaceae) and Himalayan *Saussurea* (Asteraceae); for example, insect pollinator abundance and pollination efficacy are reduced at high elevation, presumably because low temperatures, high wind speeds, and a shorter growing season compromise insect performance (Utelli and Roy 2000; Law et al. 2010). The negative impact of increased elevation on pollinator abundance varies between pollinator groups, however, affecting bees and butterflies more strongly than flies, with the result that the latter are generally more important pollinators at high elevations, especially in temperate zones (Kalin-Arroyo et al. 1982; Hoiss et al. 2012; Pellissier et al. 2012; Classen et al. 2015; Lefebvre et al. 2018; McCabe et al. 2019; McCabe and Cobb 2021). Endothermic vertebrates (i.e., birds, mammals) are probably also less sensitive to low temperatures than invertebrates, thus enhancing their effectiveness as high-elevation pollinators (Cruden 1972) and explaining the frequent transition to vertebrate pollination observed in neotropical plants at high elevations (Lagomarsino et al. 2017; Dellinger et al. 2021). Pollinator abundance also fluctuates over time, both seasonally and interannually, in response to fluctuations in the availability of key resources including temperature, moisture, and floral hosts (e.g., Pinheiro et al. 2002; Bashir et al. 2015; Rabeling et al. 2019; Rohde and Pilliod 2021). In Mediterranean ecosystems, for example, pollinator abundance peaks in spring, when the temperature-moisture balance is optimized, and flowering is at its peak (Herrera 1988; Bosch et al. 1997). Variation in the seasonality of abundance patterns between pollinator groups, however, remains poorly studied (but see Abrahamczyk et al. 2011).

Plants employ a diversity of reproductive safeguarding strategies to circumvent the challenges presented by pollen limitation arising from reduced pollinator abundance. These include classic reproductive assurance (Lloyd 1979) involving the evolution of self-compatibility in conjunction with autonomous pollen deposition (i.e., selfing). This addresses the problem of pollinator limitation by removing the need for any pollination agent (Larson and Barrett 2000; Goodwillie 2001; Hill et al. 2008) but comes at the cost of pollen discounting, inbreeding depression, reduced population genetic diversity, and increased long-term extinction risk (Barrett et al. 2014). Clonality represents an alternative reproductive safeguarding strategy, one which works for self-incompatible species and facilitates population persistence in the absence of pollinators (Bond 1994; Vallejo-Marin and O'Brien 2007; Pauw and Bond 2011). Owing to direct investment trade-offs between sexual and

clonal propagation, however, and because the spatial extent of clonal organisms commonly results in increased rates of geitonogamous pollen transfer, the transition to clonal reproduction incurs costs akin to those incurred by autonomous selfing (Vallejo-Marin et al. 2010). A third solution to pollen limitation arising from low pollinator abundance is the use of a different, more locally abundant pollinator. Wind pollination, for example, is believed to have evolved as a response to scarce and/or unreliable animal pollination (Goodwillie 1999; Friedman and Barrett 2009; Hesse and Pannell 2011), and the evolution of fly or vertebrate pollination at high elevations is commonly interpreted as a selective response to the limited availability of other kinds of pollinators (e.g., Lagomarsino et al. 2017; Dellinger et al. 2021; McCabe and Cobb 2021). Pollinator shifts introduce their own constraints, however. The efficacy of wind pollination, for example, is density-dependent and so may require the maintenance of high population densities (Knapp et al. 2001; Davis et al. 2004) and concerted flowering across individuals in a population (Kelly and Sork 2002; Moreira et al. 2014). Given that autofertility, clonality and pollinator shifts (which we refer to as reproductive safeguarding strategies throughout) all have constraints or incur costs, plants might be expected to avoid using multiple strategies unnecessarily, leading to the expectation of a negative association or trade-off between these strategies.

Pollen limitation may be an important constraint on the reproductive biology of plants growing in the Cape Floristic Region (CFR) of South Africa (Goldblatt 1978), with several authors commenting on the apparent scarcity of insects in the region (e.g., Marloth 1908; Johnson 1992, 2004). In addition, McCall and Primack (1992) reported lower rates of flower visitation by insects in a Cape fynbos community than in deciduous woodland-meadow and alpine tundra communities of the north-eastern United States. The CFR is, however, physically heterogeneous, its rugged relief being associated with steep edaphic and climatic gradients and associated vegetation, floristic, and faunal turnovers (Mucina and Rutherford 2006; Manning and Goldblatt 2012; Bergh et al. 2014). In that context, the suggestion that insects are scarce in the Cape is probably an oversimplification, with insect pollinator abundance and pollen limitation probably varying across the landscape, and between different pollinator groups. This likely explains the failure of Rodger and Ellis (2016) to detect a difference in pollinator limitation between the Greater Cape Floristic Region (GCFR, comprising the CFR plus the succulent karoo region) and elsewhere. Consistent with this perspective, data presented by Adedija et al. (2018, 2020) show that pollinator abundance on the Jonaskop Mountain in the CFR varies with elevation, with overall insect abundance being

greatest at low to mid elevations, but with flies maintaining a uniform abundance across all four elevation bands studied. As in other systems, the overall decline in insect abundance at high elevations appears to be linked to declines in air temperature and increased windspeeds (Lee and Barnard 2015). In addition, the mountains of the CFR are formed of quartzitic rocks which yield critically infertile, sandy soils that support a sclerophyllous, evergreen heathland vegetation (fynbos) whose low nutritional quality almost certainly limits the abundance of phytophagous insects (e.g., Lepidoptera; see Cottrell 1985) and possibly that of animals generally (cf., Radloff 2008; Anderson et al. 2014). This may explain the comparatively high incidence of wind pollination in dioecious fynbos plants (Steiner 1988), wind pollination having evolved repeatedly from insect-pollinated ancestors in this system, as well as the comparatively frequent use of birds and rodents as pollen vectors (Rebelo 1987; Johnson 1992; Le Maitre and Midgley 1992). At low elevations improved soil fertility supports vegetation (renosterveld and succulent karoo) which is more nutritious and palatable (Radloff 2008). Strong precipitation seasonality, however, results in plant growth and flowering in these low elevation systems being much more seasonal (spring peak) than in fynbos, especially in the western CFR which experiences a distinct Mediterranean-type climate (Manning and Goldblatt 2012; Rundel et al. 2016). As in other Mediterranean-type ecosystems (e.g., Herrera 1988; Bosch et al. 1997) this may result in a drop in the abundance of insect pollinators outside the spring flowering season (Whitehead et al. 1987; Hepburn and Jacot Guillarmod 1991).

In this paper, we test the hypothesis that the evolution of reproductive safeguarding traits is favoured in situations in which insect-modulated pollen limitation limits reproduction. Specifically, we test the expectation that the evolution of self-compatibility, autonomous selfing, bird pollination, and clonal propagation is associated with critically infertile, insect-depauperate fynbos vegetation, relative to more fertile habitats, and with flowering phenologies that do not overlap the spring seasonal peak in insect abundance. Finally, we assess whether there is evidence of the expected trade-off (Vallejo-Marin et al. 2010) between alternative strategies for circumventing pollen limitation. To test these ideas, we trace the evolution of these traits and selective regimes on a dated, multi-locus phylogeny of *Lachenalia* (Asparagaceae: Scilloideae) and assess their evolutionary associations using phylogenetic regression. *Lachenalia* is a species-rich genus of geophytes largely endemic to the GCFR which is appropriate for testing these hypotheses because: (i) it occupies a range of vegetation types in the GCFR, including fynbos, renosterveld, and succulent karoo; (ii)

although most species flower in spring (August-October), some species flower outside this period, in summer, autumn or winter; (iii) while most species of *Lachenalia* are insect and predominantly bee-pollinated, a small proportion are bird-pollinated; (iv) although all species produce bulbs, there is variation in the degree of clonal propagation, with species varying in the number of offsets or bulbils they produce; and (v) preliminary data reveal that several species of *Lachenalia* are self-compatible and others not, with some species also being capable of autonomous selfing (Duncan 2012a; Rink 2013; Rodger et al. 2021).

4.2. Materials and Methods

4.2.1. Pollination experiment

To quantify self-compatibility and selfing ability in species of *Lachenalia* J.Jacq. ex Murray, a pollination experiment was conducted using vouchered, wild-collected plants, in cultivation at Kirstenbosch National Botanical Garden. Plants representing a total of 89 species (63% of 142 total species) were available for study (Appendix 4). Prior to initiation of the experiment, the study plants had been cultivated in pots placed on raised benches in a polycarbonate-roofed glasshouse lined with a fine netting to exclude pollinators. Towards the end of summer (February) 2021, dormant bulbs were removed from their pots, and 2–10 of the largest bulbs, each from a different clone, replanted into new soil. In mid-autumn (April) the bulbs were given an initial heavy watering and, following shoot emergence, watered weekly until the leaves began to show signs of senescence (typically in spring). Once the leaves were sufficiently extended, a non-toxic, xylene-free, Pentel Maxiflo permanent marker was used to number each study plant on one of its leaves. Although the bulb glasshouse at Kirstenbosch is lined with a fine netting, an additional net covering, mounted on a wire frame, was inserted onto each pot as soon as flower buds began to appear, to eliminate the possibility of insect pollination.

The number of plants used to assess self-compatibility and selfing ability ranged from 2 to 10 per species, with a mean of 6.4 (Appendix 4). Three flowers on each plant were marked, by tying a loop of coloured cotton around the pedicel or flower base, for application of one of three treatments: (i) autonomous self-pollination; (ii) geitonogamous self-pollination; and (iii) outcrossing. Since the three available *L. calcicola* plants produced two flowers each, however, only autonomous self-pollination and outcrossing treatments were applied to this species. Flowers selected for autonomous self-pollination were left untouched, while for

geitonogamous self-pollination and outcrossing treatments fine-tipped forceps and fine watercolour paint brushes were used to transfer pollen, these implements being washed in 70% ethanol between pollen applications. Geitonogamous self-pollination and outcrossing treatments were applied only once all the stamens in the treatment flower had dehisced, since typically the stigma only becomes receptive 2–7 days thereafter (Duncan 2012a). In the case of geitonogamous self-pollination, pollen transfer was achieved by using forceps to remove a stamen from one flower on the treatment plant and repeatedly using this to dust pollen onto the receptive stigma of the flower marked for geitonogamous self-pollination. In all species, a single stamen contains many more pollen grains than there are ovules in an ovary, and a typical ovary in *Lachenalia* contains 21 ovules. For outcrossing, a water-paint brush was used to gather pollen from three or more flowers on a different plant and to dust this pollen onto the stigmas of flowers marked for outcrossing. Treated flowers were observed for signs of ovary swelling, and seed capsules were harvested as soon as they had ripened, just prior to dehiscence, and stored until seed extraction and counting took place.

4.2.2. Selective regime and trait scoring

Vegetation association and flowering seasonality – To quantify the strength of association of each *Lachenalia* species with fynbos vegetation, we determined what proportion of records in the Botanical Database of Southern Africa (BODATSA: <https://newposa.sanbi.org>) and in the Bolus Herbarium (BOL) were from fynbos sites (*sensu* Bergh et al. 2014). This was done using locality and habitat data associated with the record. The result is a fynbos-occurrence index ranging from 0 (entirely absent from fynbos; probably a succulent karoo or renosterveld species) to 1 (only occurs in fynbos). Flowering seasonality was quantified in a similar manner, using the proportion of records collected outside of the spring season (August to October) as a measure of non-spring flowering. The result is a non-spring flowering index ranging from 0 (flowering restricted to the spring period) to 1 (flowering exclusively outside the spring period). The number of specimens used to score each species for habitat association ranged from 1 to 384 (mean = 31.7), and the number of flowering records per species used for scoring flowering seasonality ranged from 1 to 362, with a mean of 30.2 (Appendix 4). To facilitate selected analyses, and consistent with the bimodal distribution of their values, both fynbos association and non-spring flowering were converted into binary form using a threshold of 0.5. Here, a score of 0 indicates that a species is largely absent from

fynbos or flowers mostly in spring, and a score of 1 that it associates predominantly with fynbos or flowers mainly outside of spring.

Pollination mode – Species of *Lachenalia* were scored as either insect (0) or bird pollinated (1) based on observations of floral visitors and descriptions of floral morphology as reported in Duncan (2012a). On this basis, the vast majority of *Lachenalia* species were scored as insect pollinated, with bees identified as the principal floral visitors, and 14 species as bird pollinated. In addition to exhibiting many characteristics of the bird pollination syndrome (flowers pendulous or suberect; yellow, orange, or red colouration; long-tubular perianth; copious nectar; unscented; and stamens inserted in tube) most of the putatively bird pollinated species had been observed in the field to be visited by birds, particularly orange-breasted, southern double-collared and malachite sunbirds (*Anthobaphes violacea*, *Cinnyris chalybeus* and *Nectarinia famosa*).

Vegetative propagation capacity – Although all *Lachenalia* species are capable of vegetative persistence on account of their bulbous habit, species vary widely in their ability to propagate via the production of vegetative bulb offsets (bulbils). Where most species produce no offsets or a single offset annually, others are more vegetatively prolific, producing multiple offsets each year. Several years' experience of propagating *Lachenalia* (G. Duncan, pers. obs.) reveals that offset production is a consistent species-level feature which is not influenced by abiotic factors such as moisture or light level. Between-species variation in offset production was scored qualitatively over many years of propagating the *Lachenalia* collection at Kirstenbosch, which involves lifting bulbs every summer. All species in the phylogeny were scored as producing many, one or no offsets. The reliability of these long-term qualitative observations was confirmed by counting the number of offsets produced on plants used for the pollination experiments. To facilitate analysis, offset production of all species was scored binarily, with species scored as producing one or none (0) or two or more offsets each year (1).

Self-compatibility and autofertility – Estimates of self-compatibility and autofertility were based on the results of the pollination experiment. Where self-compatibility was determined as the average number of seeds produced by geitonogamous self-pollination plants divided by that produced by outcrossing plants, autofertility was determined as the average number of seeds produced by autonomous self-pollination plants divided by that produced by outcrossing plants. Both variables yield an index that typically varies from 0 (no self-

compatibility or autofertility) to 1 (full self-compatibility or autofertility), although scores > 1 were recorded for some species, indicating greater seed yield under the autonomous or geitonogamous self-fertility treatments than under the outcrossing treatment. Although self-compatibility and autofertility are fundamentally continuous, we elected to binarize them because this is standard practice (given the strong bimodality of these traits across angiosperms), and necessary for derivation of our reproductive safeguarding index (below). For both traits, we used a threshold of 0.2 (i.e., > 0.2 indicates self-compatibility or autofertility, consistent with common practice (e.g. Bawa 1974; Raduski et al. 2012)).

Reproductive safeguarding – Based on whether they are bird-pollinated and/or produce multiple offsets and/or are autofertile, species were scored as either lacking (0) or possessing (1) reproductive safeguarding against low insect pollinator availability. Species lacking reproductive safeguarding are those possessing none of these attributes.

4.2.3. Dated phylogenetic tree

To generate a time-calibrated phylogenetic tree, we applied the penalized likelihood approach (Sanderson 2002), as implemented in the program treePL (Smith and O'Meara 2012), to a set of 1000 bootstrap trees resulting from the concatenated, partitioned, phylogenetic analysis of all genes obtained from Duncan et al. (2022). Based on 378 loci sampled by target enrichment, these trees had been inferred using maximum likelihood, as implemented in RAxML (v.8.2.12; Stamatakis 2006). Whereas a GTR model was applied to each locus for the inference of phylogenetic relationships (Chapter 2), the options available in treePL necessitated the use of GTRCAT models for the inference of node ages. The best maximum likelihood tree resulting from this analysis, including branch support (bootstrap), is provided in Figure 14.

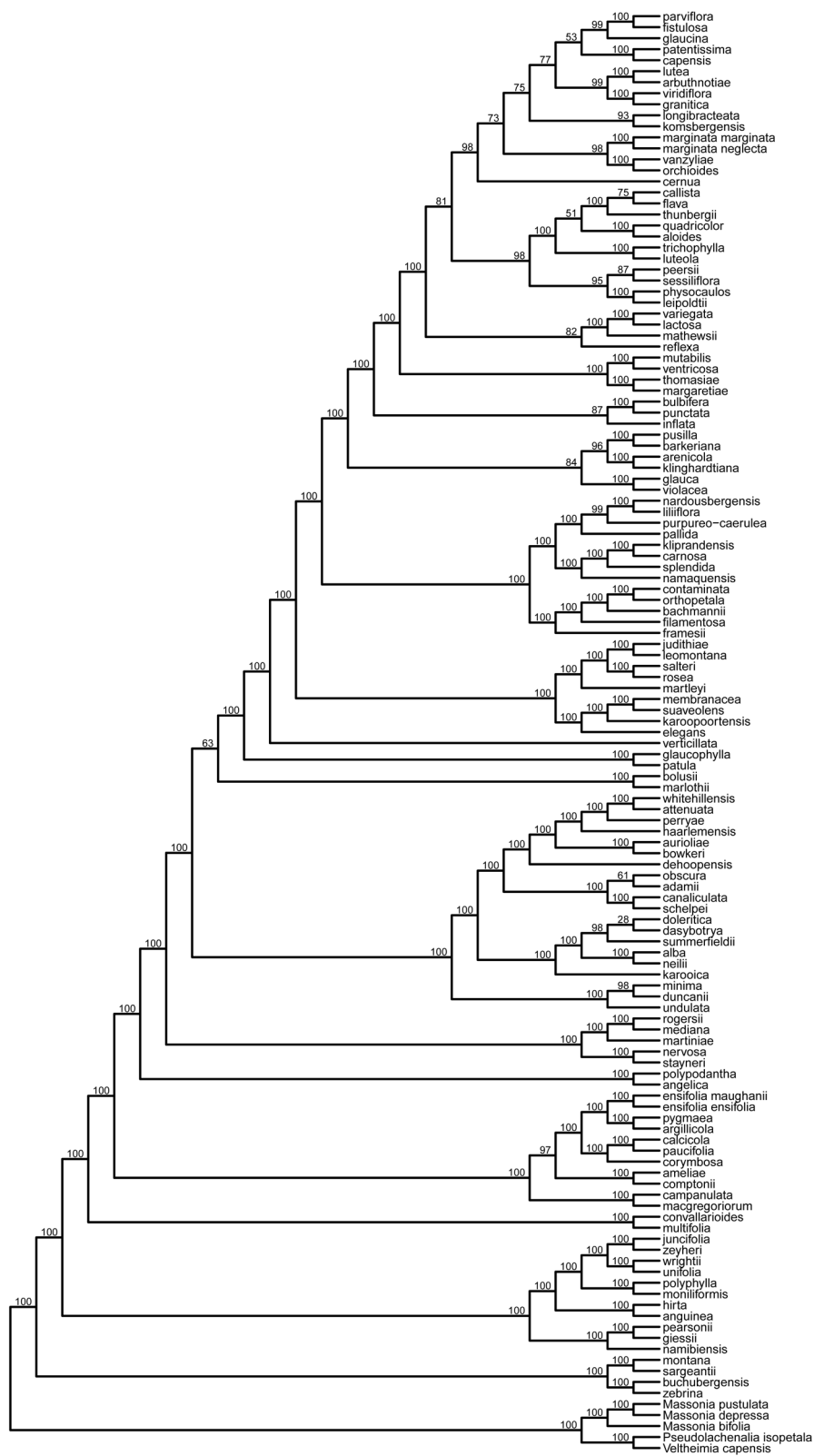


Figure 14. Best maximum likelihood tree of *Lachenalia* resulting from the phylogenetic analysis of the 378 concatenated loci obtained from Duncan et al. (2022) performed in RAxML (version 8.2.12; Stamatakis 2006). Bootstrap support values are indicated above branches.

We conducted a prime run followed by a cross-validation analysis in treePL to establish the best optimization parameters and to identify the most appropriate smoothing parameter, which here was 0.01. Although the smoothing parameter would ideally be estimated separately for each of the 1000 bootstrap trees this is impracticable and, besides, our experience suggests that the choice of tree does not massively impact the smoothing parameter estimate. Therefore, a single smoothing parameter was estimated using the best tree from the RAxML analysis and applied to all the bootstrap trees. Due to a lack of fossils assignable to *Lachenalia*, we used a secondary calibration (minimum age = 24.8 Ma and maximum age = 38.5 Ma) obtained from Buerki et al. (2012), assigning this to the node comprising *Lachenalia* and the outgroup taxa from genera *Massonia*, *Veltheimia* and *Pseudolachenalia*. The resulting ultrametric trees were then summarized in TreeAnnotator (Suchard et al. 2018) to obtain a median divergence time estimate for each node in the best RAxML tree (Figure 15; see also Appendix 5).

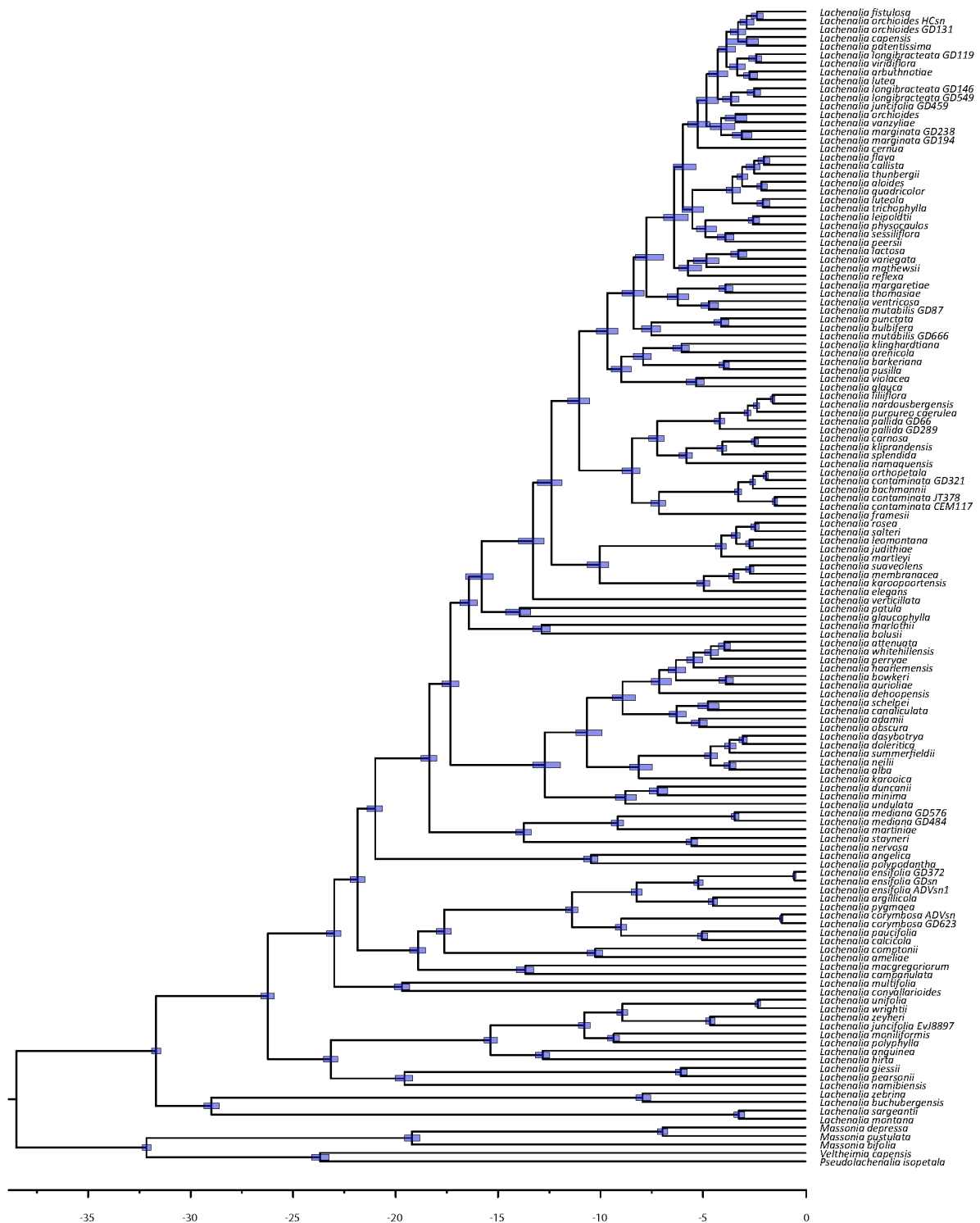


Figure 15. Dated phylogenetic tree of *Lachenalia* used for phylogenetic comparative analyses. Divergence times are in million years ago (Ma) as indicated by the time scale below the tree. Bars on each node indicate the 95% confidence interval on the estimates obtained from 1000 bootstrap trees.

4.2.4. Trait evolution and phylogenetic signal

To reconstruct historical changes in the traits and selective regimes of interest, we elected to use binarily scored variables and a discrete evolutionary model because the resulting reconstructions are easier to interpret. For traits having a continuous basis, however, reconstructions done in this way do not differ qualitatively from those generated under a continuous model. Reconstructions were done on the dated phylogenetic tree using maximum likelihood under the Mk1 model, which was shown by likelihood ratio tests to fit the data better than the Mk2 model. This was done using the *ace* function of the *ape* package v.5.6-2 (Paradis et al. 2004; Paradis and Schliep 2019), using R v.4.2.1 (R Core Team 2022).

To be consistent with the reconstructions, tests of phylogenetic signal were done principally using the binarily scored traits. For this purpose, we used a tip permutation procedure (Laurin 2004) in which the consistency index of a trait on the observed tree is assessed against a null distribution generated by randomly shuffling the tip values 999 times and each time determining the consistency index. For self-compatibility, autofertility, fynbos-occurrence, and non-spring flowering, however, we also applied a continuous test of phylogenetic signal in which the observed variance of the standardized contrasts shown by a trait is compared against a null generated by randomly shuffling the tip values (Blomberg et al. 2003). This was done using the *phylosignal* function in the *picante* package v.1.8.2 (Kembel et al. 2010).

4.2.5. Tests of trait/selective regime association

Ordinary and phylogenetic generalized linear models (GLMs), with a logistic link function, were employed to assess the dependence of reproductive safeguarding traits (pollination mode, vegetative propagation capacity, self-compatibility, autofertility, and the reproductive safeguarding index) on the two selective regimes fynbos occurrence and non-spring flowering. Although ordinary GLMs do not account for phylogenetic covariance, they avoid some potentially problematic assumptions of phylogenetic GLM, and all tests were therefore conducted using both models. Moreover, while the results of the two tests were often very similar, indicating a lack of phylogenetic covariance, they were never identical.

For the purpose of testing trait/selective regime associations, and consistent with common practice, reproductive safeguarding traits (response variables) were generally treated as binary and the selector regimes (predictors) as continuous, with the GLMs incorporating a logistic link function. Acknowledging their fundamentally continuous basis, however, we

also ran a parallel set of analyses in which self-compatibility and autofertility were treated as continuous. In all instances, the optimal model was identified using backward stepwise model simplification, starting with a model including both predictors and an interaction between them. All analyses were conducted in R, with phylogenetic GLMs estimated using the *phyloglm* function of the *phylolm* package v.2.6.2 (Ho and Ané 2014) with residuals modelled using the dated phylogenetic tree.

Trade-offs between reproductive safeguarding traits were assessed in a pairwise manner, using Pagel's (1994) test for the correlated evolution of two binary traits.

4.3. Results

4.3.1. Trait evolution and phylogenetic signal

Ancestral state reconstructions identify an association with non-fynbos vegetation (i.e., succulent karoo and/or renosterveld) and spring flowering as ancestral in *Lachenalia*, the transition to fynbos vegetation taking place 13 times and that to non-spring flowering 18 times (Fig. 16a, b). One transition to fynbos vegetation from succulent karoo and/or renosterveld has produced a minor radiation, yielding a predominantly fynbos-associated clade comprising 38 taxa (*L. inflata*–*L. fistulosa* clade). Insect pollination and the production of no or one offset annually are also ancestral, with bird pollination evolving seven times (Fig. 16c) and the production of multiple offsets 16 times (Fig. 16d). The evolution of self-compatibility/self-incompatibility in *Lachenalia* is highly homoplasious, which renders the inferred ancestral condition uncertain (Fig. 17a). Given that self-incompatibility is more easily lost than gained and is therefore almost certainly ancestral (Takebayashi and Morell 2001; Igic et al. 2008), it implies that self-compatibility has evolved repeatedly in *Lachenalia*. Autofertility has evolved much less frequently, originating just five times (Fig. 17b). Considered across all studied mechanisms (i.e., bird pollination, multiple offset production, autofertility), reproductive safeguarding has originated 16 times in *Lachenalia* (Fig. 17c). Except for self-compatibility ($P = 0.113$) and autofertility ($P = 0.066$) the evolution of all selective regimes and traits shows significant phylogenetic signal (Table 1).

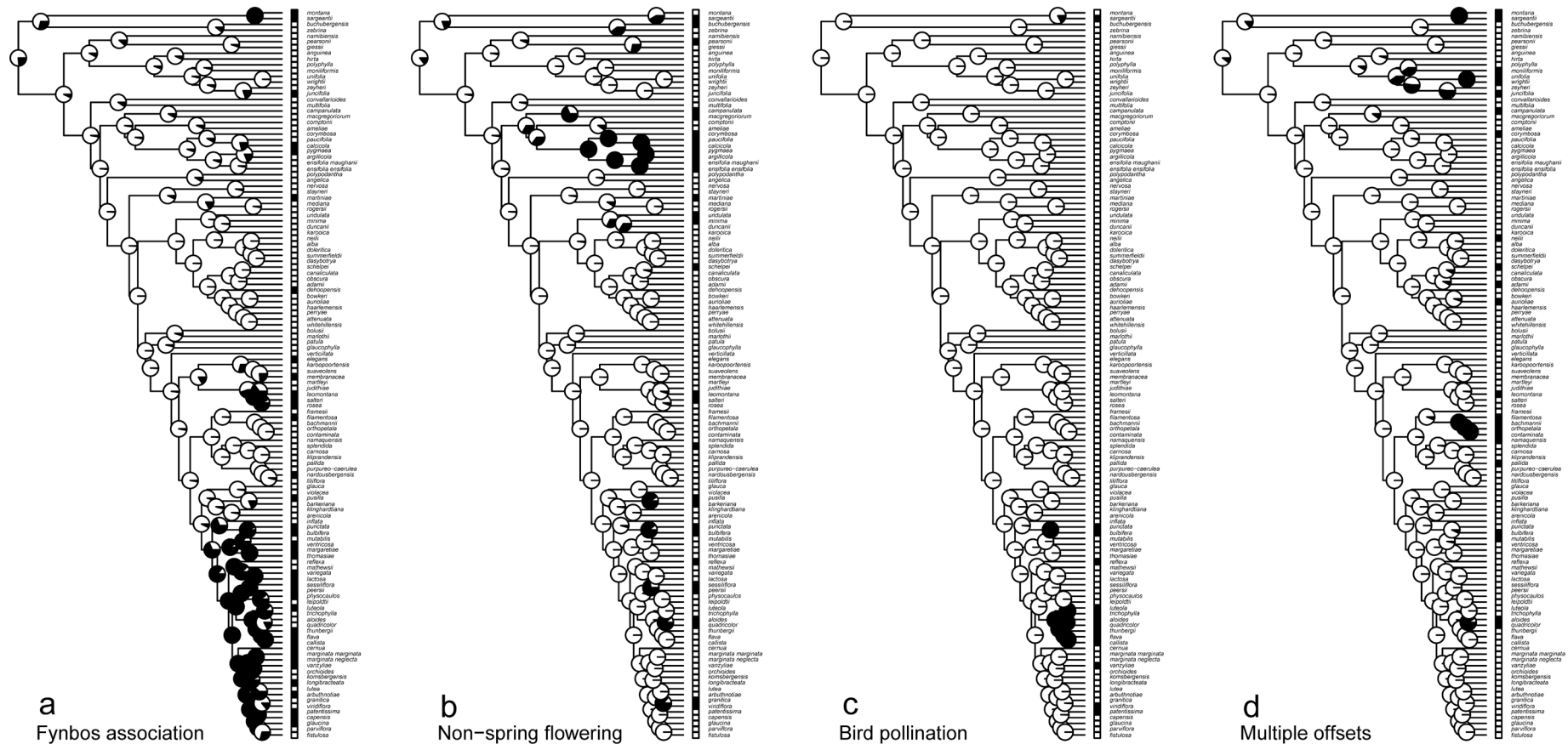


Figure 16. Maximum likelihood ancestral character state reconstructions in *Lachenalia* of (a) vegetation type (white = non-fynbos, black = fynbos), (b) flowering season (white = spring-flowering, black = non-spring flowering), (c) pollination mode (white = insect pollinated, black = bird pollinated), and (d) offset production (white = no or one offset produced annually, black = multiple offsets produced).

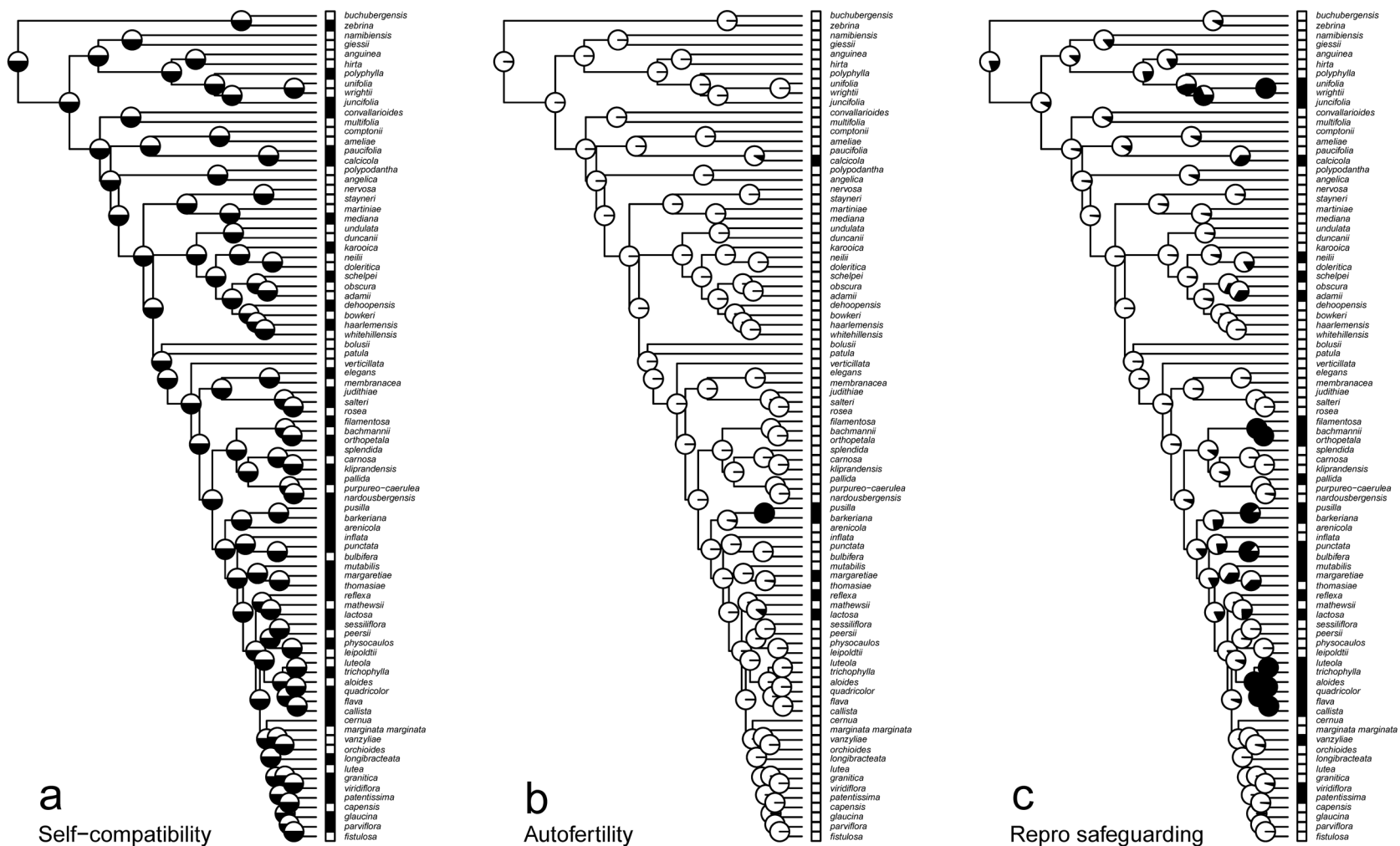


Figure 17. Maximum likelihood ancestral character state reconstructions in *Lachenalia* of (a) self-compatibility (white = self-incompatible, black = self-compatible), (b) autofertility (white = not autofertile, black = autofertile) and (c) reproductive safeguarding (white = absent, black = present).

Table 1. Phylogenetic signal in selective regimes and reproductive safeguarding traits in *Lachenalia*. For each trait/selective regime the following are presented: observed consistency index (CI_{obs}), the expected mean consistency index generated by randomly shuffling the tip values 999 times ($CI_{mean.rand}$), and the probability (P) of obtaining a consistency index $\geq CI_{obs}$ by chance.

Selective regime or trait	CI_{obs}	$CI_{rand.mn}$	P
Vegetation association	0.038	0.031	0.002
Flowering seasonality	0.056	0.037	0.001
Pollination mode	0.125	0.070	0.001
Vegetative propagation capacity	0.059	0.047	0.004
Self-compatibility	0.038	0.035	0.113
Autofertility	0.200	0.171	0.066
Reproductive safeguarding	0.059	0.043	0.004

4.3.2. Tests of trait/selective regime association

An ordinary GLM relating bird pollination to fynbos association and non-spring flowering identifies both factors as having a significant positive effect and, while a negative interaction term is retained in the optimal model, this is marginally non-significant (Fig. 18a-c; interaction term $P = 0.055$). This implies that bird pollination is positively associated with both fynbos vegetation and non-spring flowering, but that the latter effect is more pronounced in non-fynbos vegetation. The optimal phylogenetic GLM is similar but differs in the loss of the interaction term and in the fynbos association effect being marginally non-significant ($P = 0.069$; Fig. 18d).

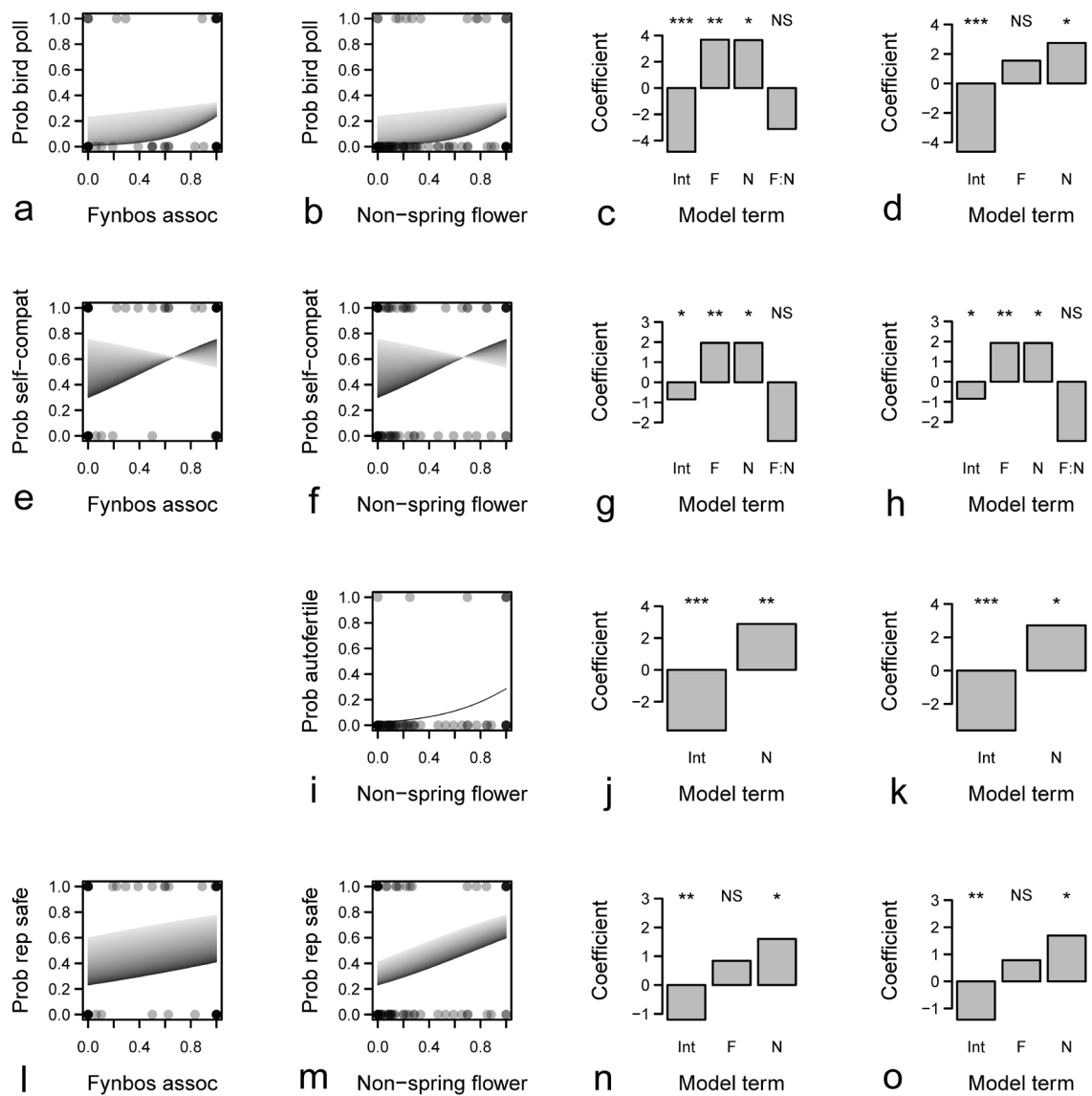


Figure 18. Ordinary (GLM) and phylogenetic (PGLM) generalized linear models describing the relationships in *Lachenalia* of (a-d) bird pollination, (e-h) self-compatibility, (i-k) autofertility, and (l-o) reproductive safeguarding to fynbos association (F), non-spring flowering (N), and the interaction between these variables (F:N). In all cases, the models shown are those identified as optimal by backward stepwise model simplification. The observed values of each response variable are plotted against fynbos occurrence (a, e, f) and non-spring flowering (b, f, i, m), together with the surface described by the optimal GLM for that variable. For each response variable, we also present a summary (coefficients and associated significance (P)) of the optimal GLM (c, g, j, n), and the corresponding PGLM (d, h, k, o). Plotted points are transparent, with overlapping points being darker. In the model summaries, significance is indicated as follows: NS, no significance (but still retained in the optimal model), *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Neither ordinary nor phylogenetic GLMs identify vegetative propagation capacity to be related to either fynbos association or non-spring flowering, although under phylogenetic GLM the effect of non-spring flowering is near-significant and positive ($\beta = 1.144$, $P = 0.061$). This implies a tendency for offset production to be somewhat more profuse in non-spring flowering species.

Ordinary and phylogenetic GLM identify both fynbos-association and non-spring flowering as having a positive effect on binarily-scored self-compatibility, with a significant negative interaction implying that non-spring flowering and self-compatibility associate positively only in non-fynbos environments (Fig. 18e-h). Treating self-compatibility as continuous results in the interaction term being lost under ordinary regression, the retention of fynbos association as the only significant predictor under phylogenetic regression (Table 2a).

In contrast to self-compatibility, only non-spring flowering is identified as a significant predictor of binarily-scored autofertility (Fig. 18i-k). This pattern persists when autofertility is treated as continuous although only under ordinary regression; under phylogenetic regression the effect of non-spring flowering is marginally non-significant ($P = 0.052$; Table 2b). This is unsurprising given the small numbers of autofertile species (six) and evolutionary transitions to autofertility (five) in *Lachenalia*, and their implications for statistical power.

Table 2. AIC-optimized ordinary (GLM) and phylogenetic (PGLM) regression models describing the dependence of (a) self-compatibility and (b) autofertility on fynbos association and non-spring flowering in *Lachenalia*. In contrast to the models presented in Fig. 18e-k, the models presented here treat the response variables (self-compatibility and autofertility) as continuous instead of binary variables. AIC = Akaike Information Criterion; Coeff = coefficient; t = t-test value, used to determine whether there is a statistically significant relationship between the predictor variable and response variable; *P* = significance value.

Predictor	Ordinary regression			Phylogenetic regression		
	Coeff	T	<i>P</i>	Coeff	t	<i>P</i>
a) Self-compatibility						
Intercept	0.477	8.784	<0.001	0.440	1.359	0.178
Fynbos occurrence (F)	0.258	2.942	0.004	0.200	2.503	0.014
Non-spring flowering (N)	0.251	2.244	0.027	-	-	-
Interaction (F:N)	-	-	-	-	-	-
b) Autofertility						
Intercept	0.011	0.032	0.731	-0.004	-0.017	0.987
Fynbos occurrence (F)	-	-	-	0.094	1.702	0.092
Non-spring flowering (N)	0.242	0.076	0.002	0.151	1.971	0.052
Interaction (F:N)	-	-	-	-	-	-

Considered across all studied mechanisms (i.e., bird pollination, multiple offset production, autofertility), reproductive safeguarding is positively associated with non-spring flowering under both ordinary and phylogenetic GLM, with fynbos association showing a marginally non-significant ($P = 0.086$) association under ordinary regression (Fig. 18l-o).

None of the tests of correlated evolution between pairs of reproductive safeguarding traits provide evidence of any trade-off between them (Table 3). None of these trait pairs are significantly correlated and, although autofertility and self-compatibility come close ($P = 0.062$), the association between these traits is positive.

Table 3. Tests of correlated evolution (Pagel 1994) between pairs of reproductive safeguarding traits in *Lachenalia*. For each pair of traits, the first value reported is the log likelihood-ratio comparing a model in which the two traits evolve independently to one in which their evolution is correlated. The second value (in parentheses) is the associated significance (P), a significant result indicating correlated trait evolution.

Predictor	Response		
	Bird pollination	Vegetative propagation	Self-compatibility
Vegetative propagation	−0.468 (1.000)	-	-
Self-compatibility	−1.651 (1.000)	0.875 (0.928)	-
Autofertility	−2.641 (1.000)	2.119 (0.714)	8.971 (0.062)

4.4. Discussion

Notwithstanding power limitation resulting from the infrequent origination of some traits, bird pollination, self-compatibility and autonomous self-fertilization are evolutionarily associated with sclerophyllous fynbos vegetation and/or flowering outside the peak spring season, the latter pattern being especially pronounced in strongly seasonal renosterveld and succulent karoo habitats. While these patterns support interpretation of these attributes as reproductive safeguarding adaptations, this interpretation also assumes that insect pollinators are generally scarce in fynbos and seasonally (autumn and winter) scarce in renosterveld and succulent karoo vegetation.

Beyond anecdotal accounts (Marloth 1908; Johnson 1992, 2004), however, quantitative support for a general scarcity of insects in fynbos is limited. Perhaps the clearest evidence comes from the work of Adedija et al. (2020), who describe a sharp decline in insect abundance from low- and mid-elevation succulent karoo and renosterveld sites to higher-elevation fynbos sites at Jonaskop, and that of McCall and Primack (1992) who report lower rates of insect visitation to flowers in fynbos (Swartboskloof, Jonkershoek) than in three other vegetation types, including northeast American tundra. Information on seasonal fluctuations in pollinating insect abundance in either succulent karoo/renosterveld or fynbos vegetation is even more scant. Given a strong dependence of insect pollinator abundance and diversity on flower abundance (e.g., Blaauw and Isaacs 2014; Schoch et al. 2022; Van Drunen et al. 2022), however, a pattern of concentrated spring flowering (Struck 1994) predicts that insect pollinators should be most abundant in spring (Adedija et al. 2020) and this matches our own field observations.

A shift to pollination by birds offers a solution to pollen limitation resulting from low insect pollinator availability both in the context of fynbos and/or non-spring flowering. Both orange-breasted and southern double-collared sunbirds (*Anthobaphes violacea* and *Cinnyris chalybeus*), the principal visitors to bird-pollinated *Lachenalia* species (Duncan 2012a), are perennially abundant in the CFR, the former associating obligately with fynbos and the latter associating with non-fynbos vegetation types, especially thicket, and the ecotones between fynbos and these vegetation types (Skead 1967; Harrison et al. 1997; Hockey et al. 2005). Significantly, in low elevation fynbos sites of the sort favoured by fynbos-associating *Lachenalia*, sunbird abundance is greatest in winter (Rebelo et al. 1984), which is when most fynbos-associating, bird-pollinated *Lachenalia* species flower and when insects are least

active. In more seasonal vegetation types, such as renosterveld and succulent karoo, the perennial presence of sunbirds, particularly southern double-collared and possibly dusky sunbirds (*Cinnyrus chalybeus* and *C. fuscus*), may similarly offer reproductive safeguarding to species flowering outside the peak spring flowering season, thus explaining the high incidence of bird-pollination in autumn- and winter-flowering *Lachenalia* species.

The combination of self-compatibility and autonomous selfing (or autofertility) represents an alternative reproductive safeguarding mechanism, one that is widely employed by plants to circumvent the challenge of pollinator limitation (e.g., Goodwillie 2001; Busch 2005; Evans et al. 2011; Busch and Delph 2012; Shivanna 2015). This combination of traits, which removes the need for pollinators, is rare in *Lachenalia*, being present in only six species, and representing five independent evolutionary origins. By contrast, self-compatibility is widespread in *Lachenalia* (48% of species) and has evolved frequently in association with transitions to nutrient-poor fynbos habitats or to a non-spring flowering strategy in renosterveld and succulent karoo habitats. While self-compatibility in the absence of autonomous self-pollination does not alleviate the need for pollinators to effect ovule fertilization, it can function as a partial reproductive safeguarding mechanism when the benefits of augmentation of seed output by within-plant pollen transfer outweigh the ovule discounting and inbreeding depression costs of selfing. This is likely when outcross pollen receipt is limiting and the rates of self-pollen transfer are high relative to outcross transfers, as would be the case when pollinators are sparse, and plants are widely spaced and produce large numbers of flowers (Harder and Barrett 1995; Harder et al. 2004). *Lachenalia* possesses these attributes, by virtue of its dense and typically conspicuous, racemose inflorescences and its tendency to produce spatially aggregated patches of clonal plants through vegetative propagation. Increased colonisation potential of self-compatible lineages (i.e., Baker's Law; Baker 1955), paired with reduced extinction rates in environmentally stable fynbos habitat, relative to renosterveld and succulent karoo (Ellis et al. 2014), provides an alternative explanation for the evolutionary association of self-compatibility with fynbos vegetation. This explanation cannot, however, account for the associations of self-compatibility and autofertility with non-spring flowering, nor the association of bird pollination with either selective regime.

Although we hypothesized that clonal propagation via the production of multiple offsets might function as a reproductive safeguarding mechanism in *Lachenalia*, we found no support for this idea and propose that selection on this trait is mediated by factors other than

reproductive safeguarding. For example, corms and bulbs are an important food resource for fossorial mammals in the CFR, including diverse species of mole-rat (e.g., Jarvis et al. 1998; Bennett and Faulkes 2000) which may target larger bulbs (cf., Contreras and Gutierrez 1991), increasing the importance of (smaller) offsets as a guarantor of persistence. In addition, variation in the size of storage organs in geophytes may reflect adaptation to climatic gradients, specifically in the amount and reliability of winter precipitation (Procheş et al. 2005). The possibility that clonality has been influenced by selection for benefits other than reproductive safeguarding is further supported by the lack of evidence for a trade-off between clonality and self-compatibility/autofertility.

Our interpretation of bird pollination, autofertility, and self-compatibility in *Lachenalia* as adaptations for reproductive safeguarding is dependent on insect abundance, particularly bee abundance, being lower in fynbos than in other vegetation types, and in autumn and winter relative to spring and early summer. While evidence for these patterns is limited and our interpretation, therefore, somewhat circumstantial, we are unable to offer an alternative explanation that accounts for the observed evolutionary associations of these traits with both fynbos vegetation and non-spring flowering. Moreover, a paucity of insect pollinators in fynbos helps to account for the repeated evolution of wind pollination in this vegetation type, as well as a high incidence of pollination by rodents. What is needed to test this idea further, however, is additional data documenting spatial and seasonal patterns of insect abundance in the GCFR, as well as studies examining the evolutionary correlations of these traits in other plant lineages.

4.5. Acknowledgements

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4.6. Competing interests

None declared.

4.7. Author contributions

GDD, AGE, FF, and GAV conceived the study, designed the experiments, and analysed the data. GDD undertook the experimental work and performed the data collection. GDD and GAV wrote the manuscript with input from AGE and FF.

4.8. Addendum

Following the publication of the above paper (<https://doi.org/10.1111/nph.19786>) it became apparent that an error had occurred with respect to the assignation of a node in the dating analysis. We therefore undertake to publish a corrigendum, as soon as possible, in which this error can be formally addressed in a journal of plant science. The error is listed as follows:

(a) Although the correct node had been used in the dating analysis (i.e. the values of 24.8-38.5 Ma were assigned to the correct node) and the correct node is assigned in this thesis, the wrong node was mentioned in the paper. In order to avoid confusion, this error will be clarified.

CHAPTER 5. CHROMOSOME EVOLUTION

Completed paper (not submitted): The evolution of ploidy and genome size and their influence on diversification in *Lachenalia* (Asparagaceae: Scilloideae).

Abstract

The extensive variation in genome size across the angiosperms is attributed to both intrinsic mechanisms, such as polyploidization, and extrinsic factors, such as nutrient availability. Owing to its occupation of diverse habitats, that vary in nitrogen and phosphorus availability, the genus *Lachenalia* offers an ideal system for examining the relationships between genome size, polyploidy, and base chromosome number, and their association with habitat fertility. Here, we test four hypotheses related to the genomic evolution of *Lachenalia*: (1) *Lachenalia* displays a dysploidy reduction series from a high ancestral base number; (2) polyploidization yields lineages that are short-lived and recent, consistent with its interpretation as an evolutionary dead-end; (3) changes in chromosome base number (dysploidy) have promoted diversification in *Lachenalia* by facilitating species coexistence; (4) low-nutrient (i.e., fynbos) environments and/or environments experiencing a short growing season select for small genomes and low ploidy. Using novel genome size data for 126 *Lachenalia* taxa and existing chromosome counts we show using phylogenetic regression that polyploidy is the main factor determining genome size variation in *Lachenalia*, with dysploidy change having little impact. A dysploidy reduction series is confirmed for *Lachenalia*, but with higher chromosome base numbers as ancestral in the genus than previously reported, i.e. $x = 11$ or $x = 12$ under time-continuous evolution and $x = 13$ or $x = 15$ under punctuational speciation-linked evolution. The occurrence of polyploidy arises mostly within terminal species, supporting the assertion that it represents an evolutionary dead-end. In contrast, evolutionary changes in chromosome base number are infrequent but persistent, yielding clades of species differing in base number. However, dysploidy did not influence diversification by facilitating coexistence in *Lachenalia*. In contrast to expectation, larger genome size and polyploidization were more frequent in infertile fynbos habitats, possibly because the environmental stability of the fynbos facilitates the persistence of evolutionarily transient polyploids.

5.1. Introduction

Genome size, defined as the quantity of DNA making up a single copy of the complete genome of an organism, varies extensively across the tree of life, especially in land plants where it varies 2440-fold (Bennett and Leitch 2005a, 2011; Schubert and Vu 2016; Hidalgo et al. 2017a, b; Pellicer et al. 2018). Variation in genome size is believed to result from a balance between processes that increase genome size and those that drive genome shrinkage (Leitch and Leitch 2012; Schubert and Vu 2016; Pellicer et al. 2018). Where whole genome duplication (polyploidization) and proliferation of repeat DNA sequences (transposable element amplification) have been identified as the primary agents of genome size increase, chromosomal rearrangements, such as those involving deletion-biased double-strand break (DSB) repair, are inferred to be the key drivers of genome size reduction (Vu et al. 2015; Schubert and Vu 2016; Wang et al. 2021). Since these latter processes may result in chromosome fusion, they have also been identified as the principal mechanisms of chromosome number reduction and genome diploidization following polyploidization (Mandáková and Lysak 2018).

Most eukaryotes, including most land plants, have comparatively small genomes which suggests that large genomes are evolutionarily unstable (Leitch and Leitch 2012; Schubert and Vu 2016; Hidalgo et al. 2017b; Pellicer et al. 2018; Bhadra et al. 2023). Large genomes, particularly those arising from whole genome duplication, confer potential benefits, including the provision of a safeguard against essential gene loss during deletion-biased DSB repair (Schubert and Vu 2016) and enhanced plastic responsiveness in the face of environmental stress (Scholes and Paige 2015; Meyerson et al. 2020). On the other hand, they also incur potential costs, some of which are biophysical. For example, large genomes require larger cells to accommodate them, which can constrain plant physiological processes such as the transpiratory regulation of water use efficiency (Faizullah et al. 2021; Wang et al. 2021; Bhadra et al. 2023). Genome size also influences developmental and growth rates, through its effect on cell cycle duration, with the consequence that plants with short reproductive cycles (e.g., annuals) invariably possess smaller genomes (Bennett 1972; Bennett and Leitch 2005b; Carta et al. 2022, Bhadra et al. 2023). Other constraints on genome size are environmental. For example, owing to the high nitrogen (N) and phosphorus (P) content of DNA, the construction and maintenance of large genomes may present challenges in low-N and/or low-P environments (Šmarda et al. 2013; Kang et al. 2015; Guignard et al. 2016; Walczyk et al. 2019; Wang et al. 2021).

Whole genome duplication, or polyploidization, is a powerful determinant of genome size, with repeated cycles of polyploidization thought to underpin the striking variation in genome size observed in land plants (Leitch and Bennett 1997; Soltis et al. 2003, 2009; Mandáková and Lysak 2018; Carta et al. 2020). By contrast, the impact on genome size of single chromosome gains and losses arising from chromosomal rearrangements (dysploidy) is more modest, possibly because the latter commonly arise via chromosome fusion involving little loss or gain of genomic material (Mayrose and Lysak 2021). Notwithstanding their prominence and potential importance in the historical diversification of land plants (Weiss-Schneeweiss et al. 2013; Zhan et al. 2016; Landis et al. 2018), there is evidence to suggest that the vast majority of polyploidization events give rise to lineages which are short-lived and have lower rates of diversification than their diploid counterparts (Wood et al. 2009; Beck et al. 2011; Mayrose et al. 2011; Escudero et al. 2014; Sader et al. 2019). On this basis it has been suggested, not without controversy (Soltis et al. 2014; Mayrose et al. 2015), that polyploid lineages generally represent evolutionary dead-ends (Mayrose et al. 2011). Dysploid transitions, by contrast, appear to yield lineages which are longer-lived and possess substantial diversification potential (Escudero et al. 2014). Indeed, dysploidy may contribute directly to diversification by limiting gene flow between recently differentiated species, thereby facilitating their coexistence (Sader et al 2019; Valdés-Florido et al. 2023).

The genus *Lachenalia* (Asparagaceae: Scilloideae), by virtue of its variable chromosome number, and its occupation of diverse habitats, including those that vary in the availability of N and P, provides an ideal system for examining the relationships between variation in genome size, polyploidy, and dysploid chromosome number, and their interactions with habitat fertility. *Lachenalia* is a large genus of 142 species (Duncan et al. 2022) of winter-growing bulbous geophytes occurring almost exclusively in the winter-rainfall western and southwestern zones of South Africa and Namibia (i.e., Greater Cape Floristic Region or GCFR *sensu* Born et al. 2007). Here, it straddles a strong precipitation gradient, ranging from areas experiencing an extremely short growing season and receiving <100 mm of precipitation annually, to areas with a long growing season which receive >1500 mm of precipitation per year. This has potential consequences for growth and development rates. *Lachenalia* also inhabits a diversity of vegetation types including sclerophyll heathlands (fynbos) growing on quartzite-derived soils that are critically deficient in important plant nutrients, including N and P (Cramer et al. 2014), and seasonally arid to semi-arid shrublands

(succulent karoo and renosterveld) growing on more fertile clay- and granite-derived soils (Bergh et al. 2014).

Chromosome base numbers in *Lachenalia* ranges from $x = 7$ to $x = 15$ (Spies et al. 2011), a pattern interpreted by Goldblatt et al. (2012) as the product of an extensive dysploidy reduction series based on the closest relatives of *Lachenalia* having comparatively high base numbers (i.e., *Eucomis* $x = 15$; *Massonia* $x = 11-20$; *Pseudolachenalia* $x = 15$; *Veltheimia* $x = 20$). In addition, polyploidy is frequent in *Lachenalia*, with 23% of the 89 species studied by Kleynhans et al. (2012) judged to contain polyploidy, with ploidy reportedly ranging from diploid to octoploid (Kleynhans and Spies 1999; Spies et al. 2011; Kleynhans et al. 2012). That most species of *Lachenalia* are capable of vegetative propagation via bulb offsets, may have facilitated the accumulation of the karyotypic variation observed in the genus (Duncan et al. 2022). In addition, there is anecdotal evidence to suggest that differences in base chromosome number serve as breeding barriers between *Lachenalia* species which occur sympatrically and have overlapping flowering periods (Duncan 2012a). Thus, it is conceivable that chromosome base number variation has facilitated the diversification of *Lachenalia* by enabling species coexistence.

The species tree presented in Chapter 2 of this thesis is regarded as reliable for two main reasons: (1) the coalescent species tree and total-evidence maximum likelihood tree are largely identical with regard to topology and branch support, with most nodes being strongly supported in both analyses (maximum likelihood bootstrap, $BS \geq 90$; ASTRAL quartet support, $QS > 75$); and (2) although the *Lachenalia* clade is topologically highly imbalanced, the monophyly of the ten clades (nodes A to J) recognized as formal sections are in many instances corroborated by diagnostic morphological synapomorphies.

In this paper, we report novel genome size data for 124 *Lachenalia* species and reconcile these with existing karyotype data reported in the literature (e.g., Hamatani and Tagashira 2019; Hamatani et al. 1998; 2004). In the context of our multilocus DNA phylogenetic analysis of the genus (Duncan et al. 2022), these data are then used to show that genome size variation in *Lachenalia* is largely a product of polyploidization, and to test the hypotheses that: (i) *Lachenalia* describes a dysploid reduction series starting from a high ancestral base number (e.g., $x = 15$); (ii) polyploidization yields lineages that are short-lived and recent, consistent with its interpretation as an evolutionary dead-end; (iii) changes in chromosome base number (dysploidy) have promoted diversification in *Lachenalia*, probably by

facilitating species coexistence; and (iv) low-nutrient (i.e., fynbos) environments and/or environments experiencing a short growing season select for small genomes and low ploidy.

5.2. Materials and Methods

5.2.1. Genome size

The genome sizes of 126 *Lachenalia* accessions (Appendix 6), representing 124 species (87% of all species) plus two subspecies, were estimated via flow cytometry following the best practice recommendations of Sliwinska et al. (2022). Actively growing leaf and/or ovary material was sampled from vouchered (lodged at NBG), wild-collected plants in cultivation at Kirstenbosch National Botanical Garden. For each plant, one young, intact leaf, approximately 1 cm in length, was chopped up along with an appropriate amount of an internal reference standard using a new razor blade in a petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20; Otto 1990). For some species the leaf could not be used due to the presence of secondary metabolites which made the analysis unreliable, and instead the flower, ovary, or seeds were sampled. This was justified by the observation that source material did not affect data quality. The resulting suspension was filtered through a 42 µm nylon mesh and incubated at room temperature for at least 5 min. Following incubation, the suspension was stained by using 1 ml of Otto II buffer (0.4 M Na₂HPO₄ · 12 H₂O; Otto 1990) supplemented with the intercalating fluorescent dye propidium iodide (PI), RNAase IIA (both at the final concentrations of 50 µg ml⁻¹) and β-mercaptoethanol (2 µl ml⁻¹). The samples were stained for 5 min at room temperature and analysed using a Partec CyFlow cytometer (Partec GmbH., Münster, Germany) equipped with a 532 nm diode-pumped solid-state laser Cobolt Samba (Cobolt AB, Solna, Sweden) as the source of excitation light. Fluorescence intensity of 5000 particles was recorded. The resulting data were analysed using FloMax Software v.2.4d (Partec GmbH.).

The analyses were conducted using standards that show no signal overlap with any of the samples. *Solanum pseudocapsicum* (1C = 1.295 pg; Temsch *et al.* 2010) served as the primary standard, with *Pisum sativum* cv. ‘Ctirad’ (1C = 4.254 pg) serving as an alternative standard. The genome size of the secondary standard was estimated based on recalculation against the primary standard in simultaneous analyses that were repeated at least ten times on different days.

5.2.2. Chromosome base number and ploidy

Chromosome base number and ploidy were scored using published chromosome counts and base number inferences (Johnson and Brandham 1997; Spies et al. 2008; 2009; 2011; Hamatani and Tagashira 2019; Hamatani et al. 2004; 2007). Consequently, the accessions used to score chromosome base number and ploidy generally differed from those used to determine genome size. For a set of 30 taxa (Appendix 6, marked with *), however, the same accessions were used for both, and these could therefore be used to evaluate the influence of ploidy and base number on genome size. For downstream analysis, the karyotype of each species was scored as two variables (Appendix 6), i.e., chromosome base number ($x = 7, 8, 9, 10, 11, 12, 13, 15$), scored as an additive, multistate character, and the incidence of polyploidy (i.e., whether polyploidy has been recorded in the taxon), scored as a binary character.

5.2.3. Environmental variables

Vegetation association was used as a proxy measure of habitat fertility, fynbos principally inhabiting quartzite-derived soils that are critically deficient in important plant nutrients, including N and P (Cramer et al. 2014), and succulent karoo and renosterveld shrublands growing on more fertile clay- and granite-derived soils (Bergh et al. 2014). The contrasting fertility of the soils under these different vegetation types is well established (Specht and Moll 1983; Campbell 1986; Cramer et al. 2014, 2019). Accordingly, each species was assigned a fynbos-occurrence index which reflects the proportion of records of that species in the Botanical Database of Southern Africa (BODATSA: <https://newposa.sanbi.org>) and in the Bolus Herbarium (BOL) which are from fynbos sites. This index ranges from 0 (never recorded in fynbos) to 1 (only recorded in fynbos).

To quantify variation in growing season duration across species we approximated, for each species, the median duration of the moisture growing season (MGS). MGS is conceptualized as the period of the year in which the availability of moisture is sufficient for crop growth and is defined operationally as the period in which monthly precipitation equals or exceeds 30% of the median monthly A-pan evaporation (FAO 1978). To score this variable, each species' distribution was compared to the MGS duration map of Schulze (1997) to determine its MGS duration range, and the median MGS duration inferred as the midpoint of that range.

5.2.4. Phylogenetic signal and reconstruction of traits and variables

Phylogenetic signal in each trait or environmental variable was assessed by comparing either the variance of standardized contrasts (continuous variables; Blomberg et al. 2003) or its consistency index (discrete traits; cf. Laurin 2004) to a null distribution generated by randomly shuffling the tip values 999 times and each time determining the relevant statistic. This was done using functions implemented in the R packages *ape* v.5.6-2 (Paradis et al. 2004; Paradis and Schliep 2019) and *picante* v.1.8.2 for R (Kembel et al. 2010). In addition, the evolutionary histories of genome size, polyploidy incidence and basic chromosome number were reconstructed using likelihood functions in *ape* v.5.6-2 (Paradis et al. 2004; Paradis and Schliep 2019), *phytools* version 1.9-16 (Revell 2012), and *geiger* v.2.0 (Pennell et al. 2014). In the case of the latter character, this was done using both time-continuous and speciation-linked evolutionary models, the first representing a more conventional approach but the latter better reflecting the coincidence of chromosome base number change with speciation. These alternative models were implemented using the κ branch length transformation, with branch lengths either being untransformed (time-continuous) or made equal by setting $\kappa = 0$. As noted above, chromosome base number was scored as a multistate categorical character (i.e., from $x = 7$ [state = 1] to $x = 15$ [state = 8]), with the transition matrix specified to ensure that transitions between ploidy levels were required to pass through intervening levels (i.e., additive or ordered coding). This was considered reasonable given that multiple chromosome fusions are unlikely to occur simultaneously. Both sets of analyses made use of the dated phylogenetic tree presented in Chapter 4 of this thesis.

5.2.5. Evaluation of trait-trait and trait-environment relationships

The dependence of genome size on chromosome base number and ploidy was assessed using only data from those accessions which had been scored for all three variables. Relationships were assessed using both ordinary and phylogenetic regression, with the optimal model in both instances being identified using AIC-based, backward stepwise regression. The starting point for this was a full model including as predictors, base number, ploidy incidence, and the interaction between them. In addition, we tested whether variation in chromosome base number is associated with variation in mean chromosome size, as one might expect if dysploid reduction occurs via chromosome fusion.

Ordinary and phylogenetic regressions were also used to assess the influence of MGS duration and habitat fertility on genome size and polyploidy, habitat fertility being quantified

as a fynbos occurrence ranging from 0 to 1 (see above). As before, the optimal model was in each case identified using AIC-based, backward stepwise regression, starting from a full model including MGS duration and fynbos occurrence, and an interaction term, as predictors.

5.2.6. Dysploidy and diversification

To assess whether changes in chromosome base number have facilitated diversification in *Lachenalia*, we first tested whether tip (i.e. species-specific) diversification rates are related to base number. This was done using both ordinary and phylogenetic regressions, with tip diversification rates being quantified using the DR statistic (Jetz et al. 2012), as determined using the *epm* package v.1.1.1 (Title et al. 2022). To assess whether base number shifts promote diversification by facilitating coexistence, we also tested for an association between (pairwise) broad range overlap, estimated from species distribution maps, and base number. For this purpose, we generated two binarily scored distance matrices, the first recording, for each pairwise species comparison, whether (1) or not (0) they show range overlap, and a second recording whether (1) or not (0) they have identical chromosome base numbers. We then used a chi-square test to assess whether these variables are associated, with significance evaluated against a null chi-square distribution generated by reshuffling the rows and columns of the base number matrix 999 times, and each time recording the chi-squared statistic. This procedure is necessitated by the non-independence of values in distance matrices.

5.3. Results

5.3.1. Interrelationship of genome size, ploidy, and base number

The distribution of genome size variation in *Lachenalia* appears somewhat multimodal, with modes at 3.13 and 5.88 pg, and three outlying individuals (8.89, 12.34, and 13.25 pg) potentially representing additional mode(s). While it is tempting to interpret these peaks as corresponding to ploidy differences, superimposing the genome size distribution of individuals of known ploidy reveals this correspondence to be imperfect (Fig. 19).

Specifically, where the 3.13 pg peak contains only diploids, the 5.88 pg peak comprises a mix of diploids and tetraploids, with the 8.89 pg individual also being diploid. Given the poor correspondence between genome size and ploidy, it is not feasible to use the former to score the latter.

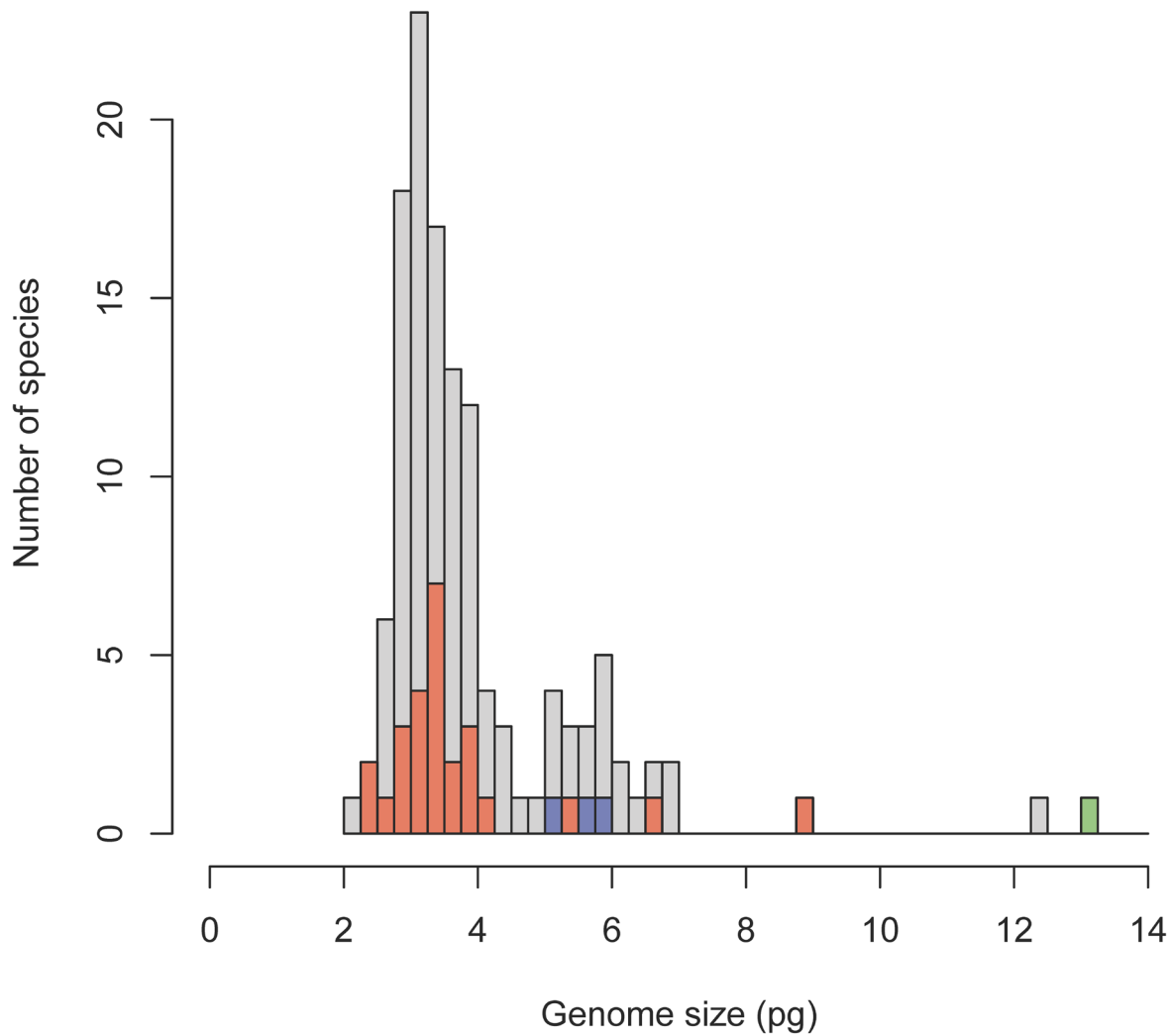


Figure 19. Histogram showing the distribution of genome size across all 126 *Lachenalia* accessions, corresponding to 126 taxa, is indicated in pale grey. Superimposed on this, the distribution of genome size of accessions of known ploidy is shown in colour. Diploids are indicated in red, tetraploids in blue, and hexaploids in green.

Using only accessions whose genome size, chromosome base number, and ploidy are all known, both ordinary and phylogenetic regressions identify ploidy but not chromosome base number as influencing genome size (Fig. 20a, b). A possible exception is the two $x = 15$ species, *L. convallarioides* and *L. zebrina*, whose genome sizes are unusually large (6.54 and 8.89 pg, respectively) relative to other diploids, and chromosome base number accounts for little of the residual variance in genome size (Fig. 20a, b). This suggests that dysploid transitions generally involve little loss or gain of genomic material, an inference corroborated by the negative relationship between mean chromosome size and base number when *L. convallarioides* and *L. zebrina* are excluded (Fig. 20c).

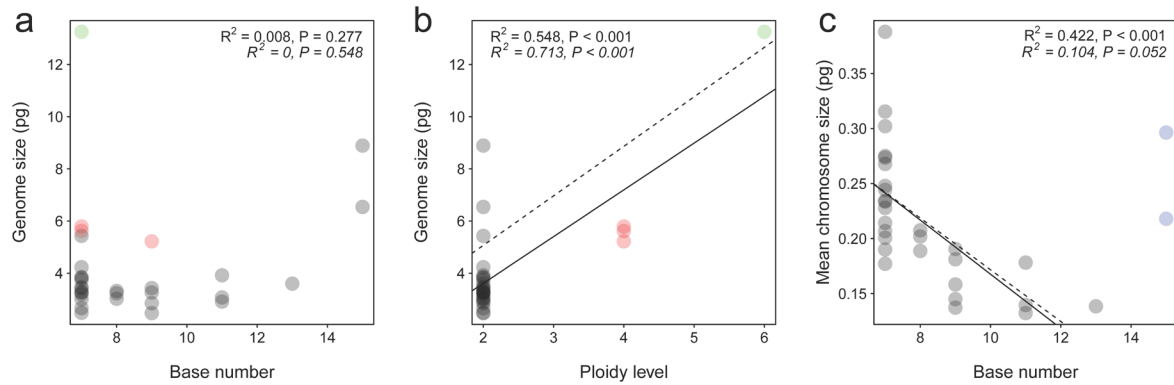


Figure 20. Scatter plots relating (a) genome size to chromosome base number; (b) genome size to ploidy level, and (c) mean chromosome size to chromosome base number. In (a) and (b), black, red, and green points respectively represent diploids, tetraploids, and hexaploids; in (c), black and blue points represent points respectively included and excluded from regression line fitting. In each case, regression lines were fitted using both ordinary (solid line, ordinary font) and phylogenetic (dashed line, italic font) regression, the outlying *L. convallarioides* and *L. zebrina* being excluded from model fitting in (c).

5.3.2. Evolution of genome size, ploidy, and base number

All three genomic variables studied, i.e., genome size, ploidy, and chromosome base number, display significant phylogenetic signal (Table 4), with reconstructions identifying a diploid (Fig. 21a) genome of low to intermediate size (Fig. 22a), and a relatively high base number (Fig. 21b, c) as ancestral in *Lachenalia*. The inference of ancestral base number is sensitive to trait evolutionary model structure, a time-continuous evolutionary model identifying $x = 11$ and $x = 12$ as most probably ancestral (Fig. 21b) and a punctuational, speciation-linked evolutionary model identifying $x = 13$ and $x = 15$ as more probably ancestral (Fig. 21c). Regardless of the condition identified as ancestral, the genus displays a sequential reduction in chromosome base number, culminating in $x = 7$ in the *L. violacea*–*L. fistulosa* [35 taxa] and *L. elegans*–*L. rosea* [6 taxa] clades. This dysploid reduction series yields a sequence of comparatively diverse offshoot clades which differ in the dominant chromosome base number (e.g., $x = 11$ in the *L. namibiensis*–*L. juncifolia* clade [9 taxa]; $x = 9$ in the *L. undulata*–*L. attenuata* clade [11 taxa]; and $x = 8$ in the *L. framesii*–*L. liliiflora* clade [11 taxa]).

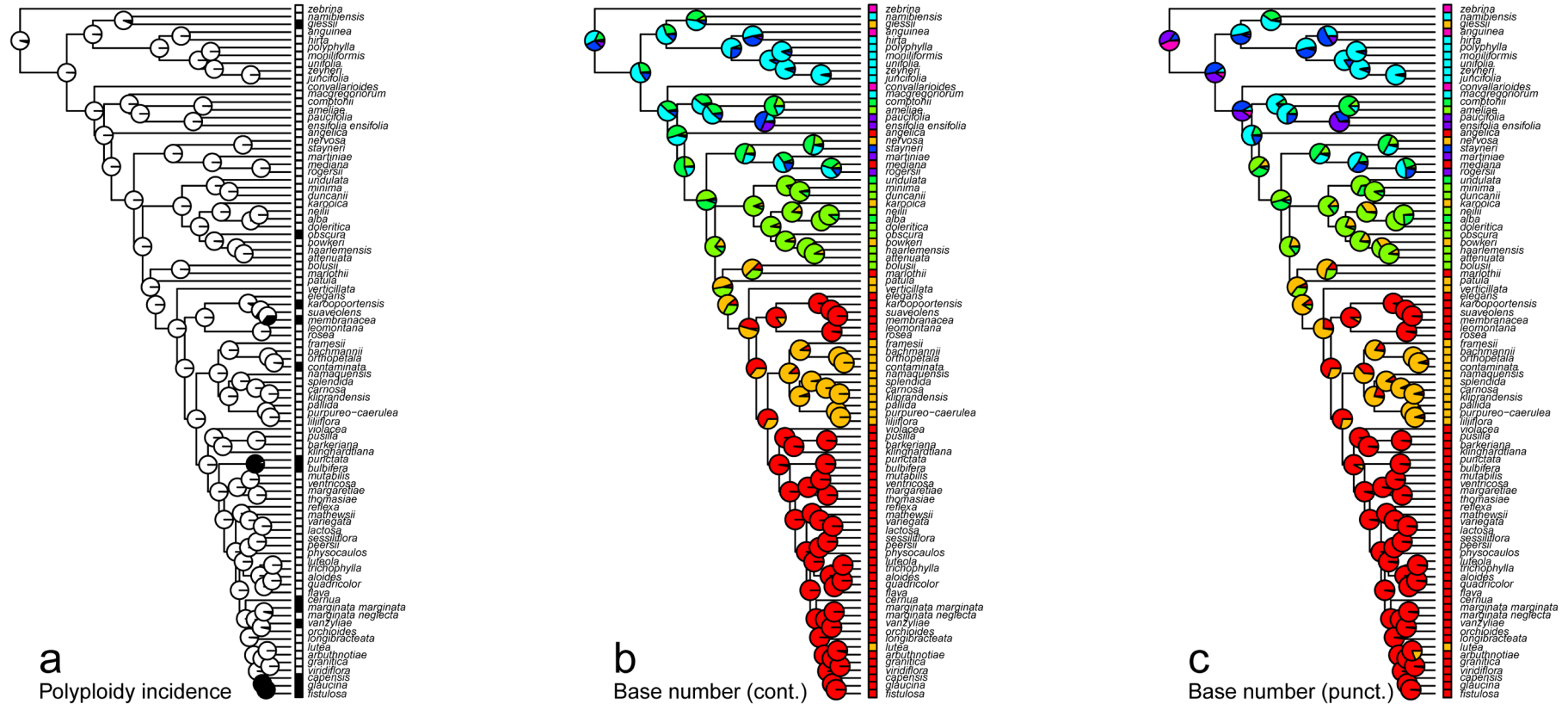


Figure 21. Maximum-likelihood character state reconstructions of (a) polyploidy incidence; and (b, c) chromosome base number. Where the reconstruction in (b) was done in the context of a tree with untransformed branch lengths (i.e., time-continuous trait evolution), that in (c) was done in the context of tree with branch lengths transformed ($\kappa = 0$) to be equal (i.e., punctuational, speciation-linked trait evolution). Colour coding is as follows: (a) white, no polyploids; black, polyploids recorded in the species; (b, c) pink, $x = 15$; purple, $x = 13$; dark blue, $x = 12$; turquoise, $x = 11$; fluorescent green, $x = 10$; ordinary green, $x = 9$; orange, $x = 8$; red, $x = 7$.

In contrast to dysploid transitions (Fig. 21b), which take place along the backbone of the phylogenetic tree and yield relatively diverse clades (six to 11 taxa per clade), transitions to polyploidy, while numerous ($n = 19$), tend to occur at the tips of the tree, mostly arising within terminal species (Fig. 21a). The only exceptions to this pattern are the *L. bulbifera*–*L. punctata* and *L. cernua*–*L. fistulosa* clades which comprise two and 16 taxa, respectively. Within the latter, nine species have polyploid members. Consistent with the strong, positive relationship between ploidy and genome size, transitions to larger genomes coincide broadly with transitions to polyploidy (Fig. 21a).

Like the three genomic variables, both fynbos occurrence and MGS duration display significant phylogenetic signal (Table 4), with reconstructions identifying an association with non-fynbos environments experiencing a short growing season as ancestral in *Lachenalia* (Fig. 22b, c). From this ancestral condition, there are multiple transitions to wetter fynbos environments. Both ordinary and phylogenetic regressions identify fynbos association but not MGS duration as influencing polyploidy and genome size (Fig. 19a), with the optimal models retaining only fynbos occurrence as a main effect (Table 5). Interestingly, the coefficients associated with this variable are consistently positive, indicating that the appearance of polyploids and large genomes is more likely in fynbos vegetation.

Table 4. Assessment of phylogenetic signal in (a) continuous and (b) discrete variables/traits. For each variable/trait, phylogenetic signal assessed by comparing either the observed variance of standardized contrasts (VSC_{obs}: continuous variables) or the observed consistency index (CI_{obs}: discrete traits) to a null distribution generated by randomly shuffling tip values 999 times and each time determining the relevant statistic.

Variable/trait	VSC _{obs} / CI _{obs}	VSC _{rand} / CI _{rand}	<i>P</i>
a) Continuous variables			
Log (Genome size)	0.012	0.017	0.002
Fynbos occurrence	0.027	0.034	<0.001
Moisture growing season duration	354.5	1008.7	<0.001
b) Discrete variables/traits			
Chromosome base number	0.333	0.164	<0.001
Polyploidy incidence	0.100	0.081	0.015

Table 5. Regression models of (a) log (genome size) and (b) polyploidy incidence, identified as optimal by AIC-based, backward stepwise regression. In each case, the optimal ordinary (left) and phylogenetic (right) regression model is shown.

Ordinary regression			Phylogenetic regression		
Variable	Coefficient	<i>P</i>	Variable	Coefficient	<i>P</i>
a) Response = Log (Genome size)					
Intercept	1.280	<0.001	Intercept	1.617	<0.001
Fynbos occurrence	0.147	0.020	Fynbos occurrence	0.141	0.017
Adj. R ² = 0.035			Adj. R ² = 0.037		
b) Response = Polyploidy incidence					
Intercept	−2.947	<0.001	Intercept	0.008	0.980
Fynbos occurrence	1.737	0.016	Fynbos occurrence	0.232	0.020
Adj. R ² = NA			Adj. R ² = 0.050		

5.3.3. Dysploidy and diversification

While dysploid transitions yield relatively diverse lineages, our data yield little evidence of an association between chromosome base number and diversification, at least when phylogenetic covariance is accounted for. Although ordinary regression reveals a negative relationship between these variables ($\beta = -0.023$, $P < 0.001$, $R^2 = 0.338$), this disappears when phylogenetic trait covariance is accounted for ($\beta = -0.001$, $P = 0.720$, $R^2 = 0$).

Moreover, we find no support for the idea that differences in base number facilitate species

coexistence. To the contrary, a conventional chi-square test suggests greater range overlap amongst species having identical base numbers ($\chi^2 = 5.894$, $df = 1$, $P = 0.015$), although this association loses significance when assessed in a manner that accounts for the non-independence of values in distance matrices ($P = 0.230$).

5.4. Discussion

5.4.1. Polyploidy and genome size evolution in *Lachenalia*

A strong positive relationship between ploidy and genome size in *Lachenalia*, indicates that, whole genome duplication is the primary driver of genome size variation. By contrast, except for the high base number (i.e., $x = 15$) species, whose genomes are large despite being diploid, dysploidy base number changes do not generally influence genome size, probably because they arise via chromosome fusion involving little or no loss or gain of genomic material and an increase in average chromosome size (Escudero et al. 2014). This pattern, which matches that observed in the daisy tribe Carduineae (Bureš et al. 2023), suggests that dysploidy has not played a role in genome downsizing in *Lachenalia*, with genome size apparently being relatively stable in the genus.

While polyploidy presents challenges for species tree inference and reliability (e.g., Oxelman et al. 2017; Ning et al. 2024), these are greater under allopolyploidy than autopolyploidy. Within *Lachenalia*, polyploidy arises almost exclusively within terminal species, seldom, if ever, giving rise to clades of multiple (polyploid) species. While the *L. bulbifera*–*L. punctata* and *L. cernua*–*L. fistulosa* clades appear exceptional in this regard, being identified by our analyses as ancestrally containing polyploids, the presence of several species having both diploid and polyploid individuals (e.g., *L. bulbifera*, *L. punctata*, *L. fistulosa*: Crosby 1986; Spies et al. 2008, 2009) suggests that the ancestors of these clades were diploid, with polyploidy arising repeatedly within them. Such a pattern of repeated polyploid evolution within terminal species, and a lack of long-term polyploid persistence, supports the hypothesis that, despite the enhanced genomic flexibility and adaptability conferred by polyploidy (Otto and Whitton 2000; Baniaga et al. 2020; Scarrow et al. 2021), most instances of polyploidization are evolutionary dead-ends (e.g., Wood et al. 2009; Mayrose et al. 2011; Escudero et al. 2014). Our data suggest that polyploidization in *Lachenalia* has generally arisen within species (i.e., autopolyploidy), consistent with a general absence of wild hybrids in the genus (G. Duncan, personal observation). Interestingly, where recent work suggests that

the benefits and establishment success of polyploidy are maximized under conditions of environment change and instability (Stebbins 1950; Brochmann et al. 2004; Fawcett and van de Peer 2010; van de Peer et al. 2017, 2021), the appearance of polyploidy and large genome size in *Lachenalia* is associated with fynbos, a vegetation type which has assembled and diversified under conditions of relative climatic and geological stability (Dynesius and Jansson 2000; Linder 2008; Verboom et al. 2014, 2020). In addition, since the soils occupied by fynbos are critically infertile, having low availabilities of several minerals including P (Cramer et al. 2014), a higher incidence of polyploids in fynbos-associated *Lachenalia* is surprising from a nutritional perspective (e.g., Guignard et al. 2016; Oberlander et al. 2016; Wang et al. 2021). While we had considered that the greater incidence of polyploids in fynbos may alternatively be linked to the reduced seasonality and longer moisture growing season of fynbos environments, we found no support for this idea. Perhaps the most important factor facilitating the emergence of polyploids in fynbos *Lachenalia*, then, is the tendency of many fynbos species to propagate clonally, often forming mats of interconnected ramets (e.g., *L. bulbifera*, *L. cernua*, *L. montana*). While this trait facilitates individual persistence over short-time scales in the face of sexual failure (Herben et al. 2017; van Drunen and Husband 2019), it does not offer a solution leading to population and species persistence in the face of changes in selective regimes taking place over evolutionary timescales. Thus, it is consistent with the failure of polyploid lineages to persist over longer timescales to give rise to multi-species clades.

5.4.2. Dysploidy and its diversification consequences

Our data provide broad support for the hypothesis that *Lachenalia* describes a dysploid reduction series (Goldblatt et al. 2012). Interestingly, where Goldblatt et al. (2012) proposed $x = 10$ as the starting point of this dysploid reduction series, apparently based on most species of *Massonia* having $2n = 18$ or $2n = 22$, our analyses identify higher chromosome base numbers, either $x = 11$ or $x = 12$ (time-continuous evolution), or $x = 13$ or $x = 15$ (punctuational, speciation-linked evolution), as ancestral in *Lachenalia*. The inference of a higher ancestral base number than that suggested by Goldblatt et al. (2012) is more consistent with the observation of $2n = 40$ in *Veltheimia* (Goldblatt et al. 2012), in *Pseudolachenalia isopetala* (Spies et al. 2008), and in *Massonia bifolia* (Wetschnig and Pfosser 2003; Goldblatt et al. 2012), which is sister to the rest of *Massonia*, these being the closest known relatives of *Lachenalia* (Pfosser et al. 2003; Buerki et al. 2012; Duncan et al. 2022).

In stark contrast to polyploids, lineages of *Lachenalia* experiencing dysploid change typically diversify to yield multi-species clades, examples including the $x = 11$ *L. namibiensis*–*L. juncifolia* clade (9 taxa), the $x = 9$ *L. undulata*–*L. attenuata* clade (11 taxa), the $x = 8$ *L. framesii*–*L. liliiflora* clade (11 taxa), the $x = 7$ *L. violacea*–*L. fistulosa* clade (35 taxa), and the $x = 7$ *L. elegans*–*L. rosea* (6 taxa) clade. Although this does not demonstrate that dysploidy stimulates diversification, it does suggest that dysploidy, unlike polyploidy, does not compromise diversification potential. One important way in which dysploid reduction might facilitate diversification is by establishing interspecific reproductive isolation, and so enabling species coexistence (Sader et al 2019; Valdés-Flórido et al. 2023). However, we find no evidence to show that coexistence in *Lachenalia* is facilitated by base number difference, at least at the broad spatial scale considered in our analyses. Instead, we find a tendency towards elevated coexistence amongst species having the same chromosome base number, this pattern probably being a consequence of clade diversification in *Lachenalia* being regionally localized. For example, most species belonging to the $x = 7$ *L. violacea*–*L. fistulosa* clade inhabit fynbos habitats in the southern GCFR, presumably because diversification of the clade was centred there.

5.5. Conclusions

In *Lachenalia* polyploids arise frequently but are ephemeral. This is consistent with a generally low incidence of polyploidy in the Cape flora, a pattern attributed to the prevalence of conditions (environmental stability, soil infertility) which do not favour the establishment and maintenance of large genomes (Oberlander et al. 2016). Relative to polyploidization events, which have probably been a ubiquitous feature of *Lachenalia* throughout its history (even though they have not persisted), dysploid changes have been relatively infrequent, but where they have arisen, they have commonly persisted to yield series of clades differing in chromosome base number. Our data suggest that in *Lachenalia* dysploidy does not significantly alter the diversification potential of lineages in which it arises, possibly because it has little effect on genome size.

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5.7. Author contributions

G.D. Duncan, G.A. Verboom, A.G. Ellis, and F.Forest conceived the study, designed the experiments and analysed the data. GDD, Z. Chumova, and P. Trávníček undertook the experimental work, and GDD performed data collection. GDD and GAV wrote the manuscript, with input from AGE, FF, ZC and PT.

CHAPTER 6. CONCLUSIONS

The phylogeny presented in this thesis illustrates the utility of next-generation sequence (NGS) data, with sequences from 378 target-capture nuclear loci yielding a well resolved and supported phylogenetic hypothesis of *Lachenalia*. This tree stands in stark contrast to earlier *Lachenalia* phylogenies, which were based on Sanger sequencing and lacked support and resolution on account of low levels of interspecific sequence divergence. The need for NGS data in estimating phylogenetic relationships in *Lachenalia* is likely a consequence of the recentness of its radiation, the genus associating predominantly with arid and semi-arid renosterveld and succulent karoo environments of Late Miocene–Pliocene age (Verboom et al. 2014; Hoffmann et al. 2015).

Analysis of NGS data provides strong support for the monophyly of *Lachenalia* in relation to the four outgroup taxa analysed (three species of *Massonia* and *Veltheimia capensis*), and identifies *L. isopetala* as distinct from the remainder of *Lachenalia*. This taxon possesses a number of unique morphological traits and a highly unusual diploid chromosome number, these features justifying its segregation as a new monotypic genus, *Pseudolachenalia*. Relationships within *Lachenalia* are also resolved with generally strong support, providing a solid foundation for a revised sectional classification. Although the phylogeny of *Lachenalia* is highly imbalanced, this study supports the recognition of ten sections, all monophyletic and generally characterized by morphological synapomorphies (Duncan et al. 2022).

Within *Lachenalia*, four species are shown to be polyphyletic, raising the possibility that each represents multiple species. This suggestion is supported by morphology, with the phylogenetically-isolated elements in each also being phenotypically divergent. Accordingly, these entities are here described as distinct species. Furthermore, the isolated placement of three subspecific taxa within section *Lachenalia* supports their elevation to species level (Duncan 2023; Duncan et al. 2022). Taken together, these patterns highlight the utility of well-resolved and well-supported species-level phylogenetic analysis, particularly one that reflects gene tree consilience across multiple loci, in the discovery of new species.

A well resolved and well supported phylogenetic hypothesis for *Lachenalia* also provides a valuable framework for downstream evolutionary studies, and two such studies are presented here, one addressing the evolution of reproductive safeguarding, and the other the evolution of ploidy, chromosome base number, and genome size. The first of these studies supports the interpretation of self-compatibility, autonomous selfing, and bird pollination, as adaptations

for reproductive safeguarding under conditions of low insect pollinator abundance. Where character reconstructions reveal all of these traits to have evolved repeatedly within *Lachenalia*, ordinary and phylogenetic regressions reveal an evolutionary association of these traits with fynbos heathland vegetation, and with flowering outside the austral spring insect abundance peak. There is no evidence, however, to support interpretation of clonal propagation as a reproductive safeguarding strategy, nor of any trade-off between reproductive safeguarding strategies (Duncan et al. 2024).

A second study, focusing on the evolution of dysploidy and polyploidy, and their consequences for genome size, reveal these to have sharply contrasting behaviours and effects. Where polyploidy is an important driver of genome size variation, dysploidy has little impact on genome size. Also, where polyploid lineages are invariably short-lived and recent in *Lachenalia*, consistent with suggestions that polyploidy represents an evolutionary dead-end, dysploid shifts invariably yield lineages which diversify to yield multi-species clades. Nevertheless, and although *Lachenalia* displays a sequential reduction in chromosome base number, this study finds no evidence to indicate that dysploid reduction influences diversification by facilitating coexistence. Surprisingly, polyploidy and large genome size are more frequent in infertile fynbos habitats, possibly because the environmental stability of the fynbos facilitates the persistence there of evolutionarily transient polyploids.

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APPENDIX 1

Taxa, voucher specimens and origin of plant material used in phylogenetic analysis of *Lachenalia*. All vouchers are housed in NBG. All specimens collected in South Africa, except *L. giessii*, *L. namibiensis* and *L. pearsonii* collected in Namibia. Abbreviations for geographical regions follow Brummitt (2001). Raw reads were submitted to the Europe Nucleotide Archive under accession PRJEB48649. All alignments, and Newick tree files can be found at Dryad (<https://doi.org/10.5061/dryad.9kd51c5jf>).

Lachenalia adamii G.D.Duncan, CPP-NC-Nieuwoudtville, *Harrower 4977*; ***Lachenalia alba*** W.F.Barker ex G.D.Duncan, CPP-NC-Nieuwoudtville, *Steiner 1366*; ***Lachenalia aloides*** (L.f.) Engl., CPP-WC-Malmesbury, *Duncan 282*; ***Lachenalia ameliae*** W.F.Barker, CPP-WC-Montagu, *Malan 90*; ***Lachenalia angelica*** W.F.Barker, CPP-NC-Hondeklipbaai, *Duncan 244*; ***Lachenalia anguinea*** Sweet, CPP-NC-Komaggas, *Williamson 3557*; ***Lachenalia arbuthnotiae*** W.F.Barker, CPP-WC-Matrosfontein, *Duncan 393*; ***Lachenalia arenicola*** G.D.Duncan & Helme, CPP-NC-Groen River, *Helme 7793*; ***Lachenalia argillicola*** G.D.Duncan, CPP-WC-Kotzesrus, *Helme 6082*; ***Lachenalia attenuata*** W.F.Barker ex G.D.Duncan, CPP-NC-Roggeveld, *Saunders s.n.*; ***Lachenalia aurioliae*** G.D.Duncan, CPP-WC-Beaufort West, *Duncan 394*; ***Lachenalia bachmannii*** Baker, CPP-WC-Piketberg, *Snijman 906*; ***Lachenalia barkeriana*** U.Müll.-Doblies & al., CPP-WC-Bitterfontein, *Duncan 440*; ***Lachenalia bolusii*** W.F.Barker, CPP-NC-Jenkinskop, *Snijman 167*; ***Lachenalia bowkeri*** Baker, CPP-EC-Port Elizabeth, *Steiner 1527*; ***Lachenalia buchbergensis*** Dinter, CPP-NC-Numees, *Lavranos s.n.*; ***Lachenalia bulbifera*** (Cirillo) Engl., CPP-WC-Saldanha, *Duncan 90*; ***Lachenalia calcicola*** (U.Müll.-Doblies & D.Müll.-Doblies) G.D.Duncan, CPP-WC-De Hoop, *Duncan 591*; ***Lachenalia callista*** G.D.Duncan & T.J.Edwards, CPP-WC-Piketberg, *Duncan 148*; ***Lachenalia campanulata*** Baker, CPP-EC-Katberg Pass, *Duncan 403*; ***Lachenalia canaliculata*** G.D.Duncan, CPP-NC-Victoria West, *Avenant s.n.*; ***Lachenalia capensis*** W.F.Barker, CPP-WC-Simonstown, *Duncan 139*; ***Lachenalia carnososa*** Baker, CPP-WC-Kamieskroon, *Duncan 185*; ***Lachenalia cernua*** G.D.Duncan, CPP-WC-Wolseley, *Duncan 470*; ***Lachenalia comptonii*** W.F.Barker, CPP-WC-Karoopoort, *Duncan 107*; ***“Lachenalia contaminata 1”*** [= *Lachenalia* sp. nov.], CPP-WC-Riviersonderend, *Malan 117*; ***Lachenalia contaminata*** Ait. **“2”**, CPP-WC-Rawsonville, *Townsend 378*; ***Lachenalia contaminata*** Ait. **“3”**, CPP-WC-Darling, *Duncan 321*; ***Lachenalia***

convallarioides Baker, CPP-EC-Makhanda, *Dold 1018*; ***Lachenalia corymbosa*** (L.) J.C.Manning & Goldblatt “1”, CPP-WC-Somerset West, *De Villiers s.n.*; ***Lachenalia corymbosa*** (L.) J.C.Manning & Goldblatt “2”, CPP-WC-Kraaifontein, *Duncan 623*; ***Lachenalia dasybotrya*** Diels, CPP-NC-Calvinia, *Thomas 8*; ***Lachenalia dehoopensis*** W.F.Barker, CPP-WC-De Hoop, *Scott s.n.*; ***Lachenalia doleritica*** G.D.Duncan, CPP-NC-Calvinia, *O’Farrell s.n.*; ***Lachenalia duncanii*** W.F.Barker, CPP-WC-Kliprand, *Duncan 189*; ***Lachenalia elegans*** W.F.Barker, CPP-NC-Nieuwoudtville, *Duncan s.n.*; ***Lachenalia ensifolia*** (Thunb.) J.C.Manning & Goldblatt subsp. *ensifolia* “1”, CPP-NC-Nieuwoudtville, *Duncan 372*; ***Lachenalia ensifolia*** (Thunb.) J.C.Manning & Goldblatt subsp. *ensifolia* “2” CPP-NC-Nieuwoudtville, *Duncan s.n.*; ***Lachenalia ensifolia*** (Thunb.) J.C.Manning & Goldblatt subsp. *maughanii* (W.F.Barker) G.D.Duncan, CPP-NC-Nieuwoudtville, *De Villiers s.n.*; ***Lachenalia fistulosa*** Baker, CPP-WC-Signal Hill, *NBG*; ***Lachenalia flava*** Andrews, CPP-WC-Tulbagh, *Duncan 523*; ***Lachenalia framesii*** W.F.Barker, CPP-NC-Knersvlakte, *Duncan 99*; ***Lachenalia giessii*** W.F.Barker, NAM-Zebrafontein, *Lavranos 19917*; ***Lachenalia glauca*** (W.F.Barker) G.D.Duncan, CPP-NC-Kamiesberg, *Duncan 416*; ***Lachenalia glaucophylla*** W.F.Barker, CPP-NC-Middelpos, *Duncan 540*; ***Lachenalia haarlemensis*** Fourc., CPP-WC-Avontuur, *Perry 3258*; ***Lachenalia hirta*** (Thunb.) Thunb., CPP-WC-Malmesbury, *Duncan 77*; ***Lachenalia isopetala*** Jacq., CPP-NC-Sutherland, *Snijman 986*; ***Lachenalia judithiae*** G.D.Duncan, CPP-WC-Mossel Bay, *Harrower 3330*; ***Lachenalia juncifolia*** Baker “1”, CPP-WC-Lemoentuin, *Van Jaarsveld 8897*; “***Lachenalia juncifolia*** 2” [= ***Lachenalia*** sp. nov.], CPP-NC-Komsberg, *Duncan 459*; ***Lachenalia karooica*** W.F.Barker ex G.D.Duncan, CPP-FS-Fauresmith, *Duncan 367*; ***Lachenalia karooopoortensis*** G.D.Duncan, CPP-WC-Karooport, *McMaster s.n.*; ***Lachenalia klinghardtiana*** Dinter, CPP-NC-Alexander Bay, *Van Jaarsveld 16663*; ***Lachenalia kliprandensis*** W.F.Barker, CPP-NC-Gamoep, *Duncan 188*; ***Lachenalia lactosa*** G.D.Duncan, CPP-WC-Kleinmond, *Duncan 417*; ***Lachenalia leipoldtii*** G.D.Duncan, CPP-WC-Matrosberg, *Harrower 192*; ***Lachenalia leomontana*** W.F.Barker, CPP-WC-Swellendam, *Botha s.n.*; ***Lachenalia liliiflora*** Jacq., CPP-WC-Durbanville, *Duncan 583*; ***Lachenalia longibracteata*** E.Phillips “1”, CPP-WC-Philadelphia, *Duncan 549*; ***Lachenalia longibracteata*** E.Phillips “2” CPP-WC-Piketberg, *Duncan 146*; “***Lachenalia longibracteata*** 3” [= ***Lachenalia*** sp. nov.], CPP-WC-Saldanha, *Duncan 119*; ***Lachenalia lutea*** G.D.Duncan, CPP-WC-Elgin, *Duncan 419*; ***Lachenalia luteola*** Jacq., CPP-WC-Ocean View, *Duncan 89*; ***Lachenalia macgregoriorum*** W.F.Barker, CPP-NC-Nieuwoudtville, *Barker 9766*; ***Lachenalia margaretae*** W.F.Barker, CPP-WC-Cederberg, *Paterson s.n.*; ***Lachenalia***

marginata W.F.Barker subsp. *marginata*, CPP-NC-Nieuwoudtville, *Duncan* 194; *Lachenalia marginata* W.F.Barker subsp. *neglecta* Schltr. ex G.D.Duncan, CPP-WC-Clanwilliam, *Duncan* 238; *Lachenalia marlothii* W.F.Barker ex G.D.Duncan, CPP-NC-Bulletrap, *Symmonds s.n.*; *Lachenalia martiniae* W.F.Barker, CPP-WC-Clanwilliam, *Duncan* 141; *Lachenalia martleyi* G.D.Duncan, CPP-WC-Theewaterkloof, *Duncan* 651; *Lachenalia mathewsii* W.F.Barker, CPP-WC-Vredenburg, *Duncan* 200; *Lachenalia mediana* Jacq. subsp. *mediana*, CPP-WC-Bot River, *Duncan* 576; *Lachenalia mediana* Jacq. subsp. *rogersii* (Baker) G.D.Duncan, CPP-WC-Saaron, *Duncan* 484; *Lachenalia membranacea* (W.F.Barker) G.D.Duncan, CPP-WC-Vanrhyns Pass, *Duncan* 101; *Lachenalia minima* W.F.Barker, CPP-WC-Bitterfontein, *Duncan* 172; *Lachenalia moniliformis* W.F.Barker, CPP-WC-Worcester, *Perry* 795; *Lachenalia montana* Schltr. ex W.F.Barker, CPP-WC-Hermanus, *Duncan* 268; *Lachenalia multifolia* W.F.Barker, CPP-WC-Gemsbokkloof, *Snijman* 1215; *Lachenalia mutabilis* G.Lodd. ex Schult. & Schult.f. [= "*L.mutabilis* 1"], CPP-WC-Saldanha, *Duncan* 87; "*Lachenalia mutabilis* 2" [= *Lachenalia* sp. nov.], CPP-NC-Middelpos, *Duncan* 666; *Lachenalia namaquensis* Schltr. ex W.F.Barker, CPP-NC-Kosies, *Van Berkel* 273; *Lachenalia namibiensis* W.F.Barker, NAM-Namuskluft, *Lavranos* 19955; *Lachenalia nardousbergensis* G.D.Duncan, CPP-WC-Piketberg, *Harrower* 247; *Lachenalia neilii* W.F.Barker ex G.D.Duncan, CPP-NC-Nieuwoudtville, *Duncan* 196; *Lachenalia nervosa* Ker Gawl., CPP-WC-Swellendam, *Botha s.n.*; *Lachenalia obscura* Schltr. ex G.D.Duncan, CPP-NC-Calvinia, *Duncan* 108; *Lachenalia orchioides* (L.) Ait. subsp. *glaucina* (Jacq.) G.D.Duncan, CPP-WC-Kirstenbosch, *Duncan* 131; *Lachenalia orchioides* (L.) Ait. subsp. *orchioides*, CPP-WC-Stellenbosch, *Duncan* 130; *Lachenalia orchioides* (L.) Ait. subsp. *parviflora* (W.F.Barker) G.D.Duncan, CPP-WC-Woodstock, *Clausen s.n.*; *Lachenalia orthopetala* Jacq., CPP-WC-Durbanville, *Duncan* 582; *Lachenalia pallida* Ait. [= "*L. pallida* 1"], CPP-WC-Vanrhynsdorp, *Duncan* 289; *Lachenalia pallida* Ait. [= "*L. pallida* 2"], CPP-WC-Citrusdal, *Duncan* 66; *Lachenalia patentissima* G.D.Duncan, CPP-WC-Cape Point, *Duncan* 471; *Lachenalia patula* Jacq., CPP-WC-Klawer, *Duncan* 647; *Lachenalia paucifolia* (W.F.Barker) J.C.Manning & Goldblatt, CPP-WC-Langebaan, *Duncan* 170; *Lachenalia pearsonii* (R.Glover) W.F.Barker; NAM-Karasburg, *Bruyns* 3554; *Lachenalia peersii* Marloth ex W.F.Barker, CPP-WC-Hermanus, *Van Zijl* 126; *Lachenalia perryae* G.D.Duncan, CPP-WC-Whitehill, *Gie s.n.*; *Lachenalia physocaulos* W.F.Barker, CPP-WC-Robertson, *Duncan* 288; *Lachenalia polyphylla* Baker, CPP-WC-Gouda, *Duncan* 348; *Lachenalia polypodantha* Schltr. ex W.F.Barker subsp. *polypodantha*, CPP-NC-Pellaberg,

Van Jaarsveld 13207; ***Lachenalia punctata*** Jacq., CPP-WC-Saldanha, *Duncan 91*;
Lachenalia purpureo-caerulea Jacq., CPP-WC-Darling, *Hiemstra 192*; ***Lachenalia pusilla***
 Jacq., CPP-WC-Aurora; *Duncan 210*; ***Lachenalia pygmaea*** (Jacq.) G.D.Duncan, CPP-NC-
 Kamiesberg, *Tsang s.n.*; ***Lachenalia quadricolor*** Jacq., CPP-WC-Langebaan, *Duncan 152*;
Lachenalia reflexa Thunb., CPP-WC-Potsdam, *Hansford s.n.*; ***Lachenalia rosea*** Andrews,
 CPP-WC-Cape Agulhas, *Crous 240*; ***Lachenalia salteri*** W.F.Barker, CPP-WC-Elim, *Nicklin*
136; ***Lachenalia sargeantii*** W.F.Barker, CPP-WC-Napier, *Duncan 535*; ***Lachenalia schelpei***
 W.F.Barker, CPP-NC-Calvinia, *Duncan 401*; ***Lachenalia sessiliflora*** Andrews, CPP-WC-
 Bredasdorp, *Hilton-Taylor s.n.*; ***Lachenalia splendida*** Diels, CPP-WC-Elandsbaai, *Wisura*
2718; ***Lachenalia stayneri*** W.F.Barker, CPP-WC-Worcester, *Stayner s.n.*; ***Lachenalia***
suaveolens (W.F.Barker) G.D.Duncan, CPP-NC-Nieuwoudtville, *Hiemstra 650*; ***Lachenalia***
summerfieldii G.D.Duncan, CPP-NC-Middelpos, *Duncan 562*; ***Lachenalia thomasiae***
 W.F.Barker ex G.D.Duncan, CPP-WC-Algeria, *Thomas 271*; ***Lachenalia thunbergii***
 G.D.Duncan & T.J.Edwards, CPP-WC-Riebeek Kasteel, *Rourke 1846*; ***Lachenalia***
trichophylla Baker, CPP-NC-Kamieskroon, *Van Zijl s.n.*; ***Lachenalia undulata*** Masson ex
 Baker, CPP-WC-Knersvlakte, *Duncan 100*; ***Lachenalia unifolia*** Jacq., CPP-WC-Potsdam,
Allen s.n.; ***Lachenalia vanzyliae*** (W.F.Barker) G.D.Duncan, CPP-WC-Piketberg, *Thomas*
s.n.; ***Lachenalia variegata*** W.F.Barker, CPP-WC-Matrosfontein, *Duncan 392*; ***Lachenalia***
ventricosa Schltr. ex W.F.Barker, CPP-WC-Pakhuis Pass, *Duncan 191*; ***Lachenalia***
verticillata W.F.Barker, CPP-NC-Steinkopf, *Müller-Doblies 88125*; ***Lachenalia violacea***
 Jacq., CPP-WC-Clanwilliam, *Duncan 240*; ***Lachenalia viridiflora*** W.F.Barker, CPP-WC-St
 Helena Bay, *Duncan 410*; ***Lachenalia whitehillensis*** W.F.Barker, CPP-WC-Whitehill,
Duncan 242; ***Lachenalia wrightii*** Baker, CPP-WC-Hopefield, *Clausen s.n.*; ***Lachenalia***
zebrina W.F.Barker, CPP-NC-Calvinia, *Du Plessis s.n.*; ***Lachenalia zeyheri*** Baker, CPP-
 WC-Ceres, *Van Zijl s.n.*; ***Massonia bifolia*** (Jacq.) J.C.Manning & Goldblatt, CPP-NC-
 Springbok, *Steiner 1753*; ***Massonia depressa*** Houtt., CPP-NC-Gamoep, *Duncan 382*;
Massonia pustulata Jacq. CPP-WC-Gansbaai, *Duncan 518*; ***Veltheimia capensis*** (L.) DC.,
 CPP-WC-Ladismith, *Duncan 132*.

APPENDIX 2

Morphological characters and character states used in phylogenetic analysis of *Lachenalia*.
 Character and state numbers correspond to the data matrix in Appendix 3.

1 Bulb shape: globose (0), ovoid (1); **2 Bulb tissue colour:** white (0), yellow (1); **3 Bulb tunic texture:** hard (0), spongy (1); **4 Bulb apex:** short (0), extended (1); **5 Bulb apex texture:** fasciculate (0), not fasciculate (1); **6 Bulbil production:** no (0), yes (1); **7 Offset production:** solitary (0), offsets (1); **8 Concrecence:** absent (0), concrecent (1); **9 Cataphyll apex:** obtuse (0), acute (1); **10 Cataphyll adhesion to leaf bases:** not adhering (0), adhering (1); **11 Leaf number:** one (0), two (1), $\geq$ three (2); **12 Leaf orientation:** prostrate (0), suberect (1), erect (2); **13 Leaf shape:** ovate (0), lanceolate (1), linear (2); **14 Leaf base markings:** absent (0), banded (1), spotted (2); **15 Leaf pustules:** pustulate (0), absent (1); **16 Leaf upper surface grooves:** shallowly depressed (0), strongly depressed (1); **17 Leaf upper surface trichomes:** absent (0), simple (1), stellate (2); **18 Leaf upper surface maculation:** absent (0), always (1); **19 Distal lamina profile:** flat (0), canaliculate (1); **20 Basal lamina profile:** flat (0), canaliculate (1); **21 Adaxial lamina sculpturing:** striate (0), laevigate (1), ridged (2), transverse (3); **22 Adaxial lamina transverse distribution:** exclusively transverse (0), transverse 1: median finely striate, sides transverse (1), transverse 2: median ridged, sides transverse (2); **23 Abaxial lamina sculpturing:** striate (0), laevigate (1), ridged (2), transverse (3); **24 Leaf margin anatomy:** simple (0), flabellate (1), multiple (2), sclerotic (3); **25 Leaf margin shape:** flat (0), crisped (1); **26 Leaf margin trichomes:** absent (0), ciliolate (1), ciliate (2), stellate (3); **27 Leaf apex shape:** naviculate (0), acute (1); **28 Seedling leaf orientation:** prostrate (0), erect (1); **29 Seedling leaf shape:** flat (0), terete (1); **30 Inflorescence type:** racemose (0), spicate (1); **31 Peduncle elevation at anthesis:** subterranean (0), aerial (1); **32 Peduncle inflation:** not inflated (0), inflated (1); **33 Rachis apical anthocyanins:** absent (0), present (1); **34 Rachis apical carotenoids:** absent (0), present (1); **35 Rachis apical chlorophylls:** absent (0), present (1); **36 Rachis apical structural white:** present (0), absent (1); **37 Lower bract shape:** ovate (0), cup (1); **38 Upper bract shape:** lanceolate (0), ovate (1); **39 Flower arrangement:** spiral (0), whorled (1); **40 Pedicel orientation at anthesis:** erect/suberect (0), patent (1), curved (2), absent (3); **41 Floral symmetry:** actinomorphic (0), zygomorphic (1); **42 Perianth shape:** tubular (0), oblong bell (1), narrow bell (2), wide bell (3), urn (4); **43 Perianth tube length:** long (0), short (1); **44 Perianth tube shape:** tubular (0), cup (1), disc (2); **45 Inner to outer perianth segment length:** subequal (0), inner longer (1); **46 Perianth colour:** maroon (0), white/cream (1), yellow (2), pink/mauve (3), grey (4), brown (5), blue (6), orange (7), red (8), green (9), purple (10); **47 Outer perianth segment shape:** oblong (0), ovate (1); **48 Outer perianth segment length:** short (0), long (1); **49 Outer perianth segment apex:** recurved (0), flat (1); **50 Inner perianth segment shape:** linear (0), obovate (1), oblong (2); **51 Inner**

perianth segment length: short (0), long (1); **52 Ventral perianth segment extension:** shorter (0), equal (1), longer (2); **53 Filament orientation:** straight (0), narrow (1) recurved (2), declinate (3); **54 Filament colour:** white (0), purple (1); **55 Filament length:** short (0), long (1); **56 Filament relative lengths:** equal (0), subequal (1); **57 Outer filament insertion:** upper (0), medial (1), basal (2); **58 Inner filament insertion:** upper (0), medial (1); **59 Ovary shape:** obovoid (0), ellipsoid (1); **60 Style orientation:** straight (0), declinate (1); **61 Pollen exine sculpturing:** rugose (0), reticulate (1); **62 Infructescence elongation:** elongated (0), not elongated (1); **63 Capsule shape:** obovoid (0), ellipsoid (1); **64 Capsule orientation:** same as perianth (0), bends up (1), bends down (2); **65 Capsule length:** short (0), long (1); **66 Seed shape:** globose (0), ovoid (1), oblong (2); **67 Seed length:** short (0), medium (1), long (2); **68 Seed width:** narrow (0), medium (1), wide (2); **69 Testa lustre:** glossy (0), matte (1); **70 Testa primary sculpturing:** smooth (0), rugose (1); **71 Testa secondary sculpturing:** absent (0), reticulate (1), colliculate (2); **72 Strophiole length:** rudimentary (0), medium (1), long (2); **73 Strophiole texture:** ridged (0), smooth (1); **74 Strophiole inflation:** not inflated (0), inflated (1); **75 Raphe inflation:** absent (0), inflated (1); **76 Chalazal collar length:** absent (0), short (1), long (2).

APPENDIX 3

Morphological data matrix used in phylogenetic analysis of *Lachenalia*.

APPENDIX 4

Taxa, morphological/phenological data and tests of trait/selective regime association in *Lachenalia*.

Taxa	In.tree	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Non.sprn	Offsets	Herb.spec	Herb.spec	Fyn.prop	Poll.code	N.auto	N.geit	N.outc	Ov.auto	Ov.geit	Ov.outc	Sd.auto	Sd.geit	Sd.outc	Al.ovary	Al.seed	SCI.ovary	SCI.seed	Al.bin	SCI.bin	RA
adamii	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2	1	0	0	0	5	5	5	0	0.2	1	0	0.2	4.6	0	0	0.2	0.043478	0	0	1
alba	1	0	0	0	0	0	2	22	4	0	0	0	0	0	1	28	0	0	0																
algoensis	0	0	0	0	0	0	20	24	0	0	0	0	0	0	0	1	43	12	0.27907	3	3	3	0	0.333333	1	0	0.333333	27.33333	0	0	0.333333	0.012195	0	0	0
aloides	1	0	0	0	0	4	0	0	0	0	0	0	0	1	2	4	0	0	1	4	4	4	0	0.25	1	0	0.5	6	0	0.25	0.833333	0	0	1	
ameliae	1	0	0	0	0	0	7	10	0	0	0	0	0	0	0	17	0	0	0	5	5	5	0	0	0.8	0	5	0	0	0	0	0	0	0	
angelica	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	10	10	10	0	0	0.8	0	10.5	0	0	0	0	0	0	0	
anguinea	1	0	0	0	0	1	16	26	3	0	0	0	0	0.021739	1	46	0	0	0	6	8	8	0	0	1	0	0	7.25	0	0	0	0	0	0	
arbutunotiae	1	0	0	0	0	2	1	9	2	1	0	0	0	0.2	1	20	20	1	0																
arenicola	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0	4	0	0	0	2	2	2	0	1	1	0	4	17.5	0	0	1	0.228571	0	1	0
argillicola	1	0	0	1	1	1	0	0	0	0	0	0	0	1	0	4	0	0	0																
attenuata	1	0	0	0	0	0	1	11	0	0	0	0	0	0	1	11	0	0	0																
aurioliae	1	0	0	0	3	9	23	5	0	0	0	0	0	0.3	2	43	0	0	0															1	
bachmannii	1	0	0	0	0	0	10	17	2	0	0	0	0	0	2	25	0	0	0	8	8	8	0	0.75	1	0	1.625	8.5	0	0	0.75	0.191176	0	0	1
barbarae	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	6	6	0	0	6	6	6	0	0	0	1	0	3.333333	0	0	0	0	0	0	
barkeriana	1	0	0	2	3	2	0	0	0	0	0	0	0	1	0	4	4	0	0	4	4	4	1	1	13.75	10	9.75	1	1.410256	1	1	0.025641	1	1	1
bolusii	1	0	0	0	0	0	2	9	5	0	0	0	0	0.125	0	16	0	0	0	3	3	3	0	0.333333	1	0	0.666667	5.666667	0	0	0.333333	0.117647	0	0	0
bowkeri	1	0	0	0	0	8	16	6	3	0	0	0	0	0.242424	1	37	0	0	0	3	3	3	0	0.666667	1	0	1.333333	7.333333	0	0	0.666667	0.181818	0	0	0
brunsii	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	2	1	0																
buchubergensis	1	0	0	1	1	6	0	0	0	0	0	0	0	1	0	11	0	0	0	2	2	2	0	0	1	0	0	6	0	0	0	0	0	0	
bulbifera	1	0	7	28	50	55	31	11	0	0	0	0	0	0.769231	2	194	192	0.989691	1	7	7	7	0	1	1	0	1.714286	20.28571	0	0	1	0.084507	0	0	1
calicicola	1	0	0	2	0	0	0	0	0	0	0	0	0	1	0	4	4	1	0	3	0	3	1	1	1	19.66667	17.66667	1	1.113208	1	1	1	1		
callista	1	0	0	0	0	0	1	8	0	0	0	0	0	0	1	13	13	1	1	6	6	6	0	0.5	1	0	1.5	5.333333	0	0.5	0.28125	0	1	1	
campanulata	1	0	0	0	0	0	0	1	1	10	11	0	0	0.913043	2	29	0	0	0															1	
canaliculata	1	0	0	0	0	1	4	11	6	0	0	0	0	0.045455	1	23	0	0	0																
capensis	1	0	0	0	0	0	2	8	13	2	0	0	0	0.08	1	24	24	1	0	6	6	6	0	0.666667	1	0	3	20.33333	0	0	0.666667	0.147541	0	0	0
carnosa	1	0	0	0	0	3	29	16	1	0	0	0	0	0.061224	1	41	0	0	0	6	7	7	0	0.857143	0.714286	0	1.857143	11.42857	0	1.2	0.1625	0	0	0	
cernua	1	0	0	0	0	1	0	4	3	1	0	0	0	0.222222	1	7	7	1	0	10	10	10	0	1	1	0	9.8	16.2	0	0	1	0.604938	0	1	0
comptonii	1	0	0	0	0	0	0	21	9	1	0	0	0	0.032258	0	34	0	0	0	9	9	9	0	0	0.888889	0	0	9.444444	0	0	0	0	0	0	0
concordiana	0	0	0	0	0	0	2	11	2	0	0	0	0	0	0	14	0	0	0																
congesta	0	0	0	0	2	3	3	2	0	0	0	0	0	0.5	0	12	0	0	0															1	
contaminata	1	0	0	0	0	0	3	34	46	0	0	0	0	0	2	125	0	0	0															0	
convallarioides	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	3	0	0	0	7	7	7	0	0.857143	0.857143	0	2.714286	5	0	0	1	0.542857	0	1	0
corymbosa	1	1	13	6	0	0	0	0	0	0	0	0	0	1	2	22	0	0	0																
dasybotrya	1	0	0	0	0	0	3	10	3	0	0	0	0	0	0	17	0	0	0																
dehoopensis	1	0	0	0	0	0	3	4	0	0	0	0	0	0	1	5	5	1	0	5	5	5	0	0.6	1	0	4.4	13.8	0	0	0.6	0.318841	0	1	0
doleritica	1	0	0	0	0	0	1	8	2	0	0	0	0	0	0	12	0	0	0	3	3	3	0	0.666667	1	0	2.666667	14.33333	0	0	0.666667	0.186047	0	0	0
duncanii	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	6	6	6	0	0	1	0	16.16667	0	0	0	0	0	0	0	
elegans	1	0	0	0	0	0	1	5	0	0	0	0	0	0	0	6	6	1	0	10	10	10	0	0.7	1	0	4.2	8.6	0	0	0.7	0.488372	0	1	0
ensifolia_ensifolia	1	0	27	65	12	0	0	0	0	0	0	0	0	1	1	140	0	0	0																
ensifolia_maughanii	1	0	0	5	2	0	0	0	0	0	0	0	0	1	1	6	0	0	0	10	10	10	0	0.7	0.9	0	3.8	8.3	0	0	0.777778	0.457831	0	1	1
filamentosa	1	0	0	0	0	0	5	12	2	0	0	0	0	0	2	15	9	0.6	0																
fistulosa	1	0	0	0	0	0	7	26	32	10	0	0	0	0.133333	1	78	0	0	0	7	7	7	0	0	0.857143	0	0	27.42857	0	0	0	0	0	0	
flava	1	0	0	0	0	0	1	2	4	2	0	0	0	0.222222	1	9	8	0.888889	0	10	10	10	0	1	1	0	11.4	43	0	0	1	0.265116	0	1	1
framesii	1	0	0	0	0	2	9	5	0	0	0	0	0	0.125	1	13	0	0	0																
giessii	1	0	0	0	0	3	18	7	0	0	0	0	0	0.107143	0	19	0	0	0	5	5	5	0	0.6	1	0	1.4	7.8	0	0	0.6	0.179487	0	0	0
glauca	1	0	0	0	0	0	4	7	0	0	0	0	0	0	0	9	0	0	0																
glauцина	1	0	0	0	0	0	1	4	12	0	0	0	0	0	1	10	10	1	0	10	10	10	0	0.8	0.9	0	3	11.7	0	0	0.888889	0.25641	0	1	0
glaucoephylla	1	0	0	0	0	0	0	2	9	0	0	0	0	0	0	10	0	0	0																
granitica	1	0	0	0	5	2	2	1	0	0	0	0	0	0.7	0	10	0	0	0	6	6	6	0	1	1	0	9.166667	24.5	0	0	1	0.37415	0	1	0
haarlemensis	1	0	0	0	0	0	1	8	1	1	0	0	0	0.090909	1	10	0	0	0	7	7	7	0	0.571429	0.714286	0	1.142857	3.428571	0	0.8	0.333333	0	1	0	
hirta	1	0	0	0	0	2	10	64	6	0	0	0	0	0.02439	0	60	0	0	0	7	7	7	0	0	0	1	0	20.14286	0	0	0	0	0	0	
inconspicua	0	0	0	0	0	1	7	0	0	0	0	0	0	0.125	0	8	0	0	0																
inflata	1	0	0	0	0	0	1	3	0	0	0	0	0	0.2	0	4	0	0	0	4	4	4	0	1	1	0	9.5	26	0	0	1	0.365385	0	1	0
judithiae	1	0	0	0	0	0	5	8	3	0	0	0	0	0	1	14	7	0.5	0																
juncifolia	1	0	0	0	0	0	13	55	32																										

Taxa = Taxon name

In.tree = Taxon included in phylogenetic tree: 0 = false, 1 = true

Mar-Feb = Number of records (flowering herbarium specimens) from a given month (March to February)

Non.spring.prop = Proportion of records from outside the peak (August-October) spring season

Offsets = Annual offset production: 0 = no offsets produced, 1 = one offset produced, 2 = two or more offsets produced

Herb.spec.total = Total number of records

Herb. spec. fynbos = Number of records from fynbos sites

Fyn.prop = Proportion of records from fynbos sites

Poll. code = Pollination mode: 0 = insect pollinated, 1 = bird pollinated

N.auto = Number of replicate autofertility trials

N.geit = Number of replicate geitonogamy trials

N.outc = Number of replicate outcross trials

Ov.auto = Proportion of autofertility trials resulting in ovary swelling

Ov.geit = Proportion of geitonogamy trials resulting in ovary swelling

Ov.outc = Proportion of outcross trials resulting in ovary swelling

Sd.auto = Mean number of seeds produced by autofertility trial plants

Sd.geit = Mean number of seeds produced by geitonogamy trial plants

Sd.outc = Mean number of seeds produced by outcross trial plants

Al.ovary = Number of autofertility trial plants showing ovary swelling, divided by the number of outcross trial plants showing ovary swelling

Al.seed = Mean number of seeds produced by autofertility trial plants, divided by the mean number of seeds produced by outcross trial plants

SCI.ovary = Number of geitonogamous trial plants showing ovary swelling, divided by the number of outcross trial plants showing ovary swelling

SCI.seed = Mean number of seeds produced by geitonogamous trial plants, divided by the mean number of seeds produced by outcross trial plants

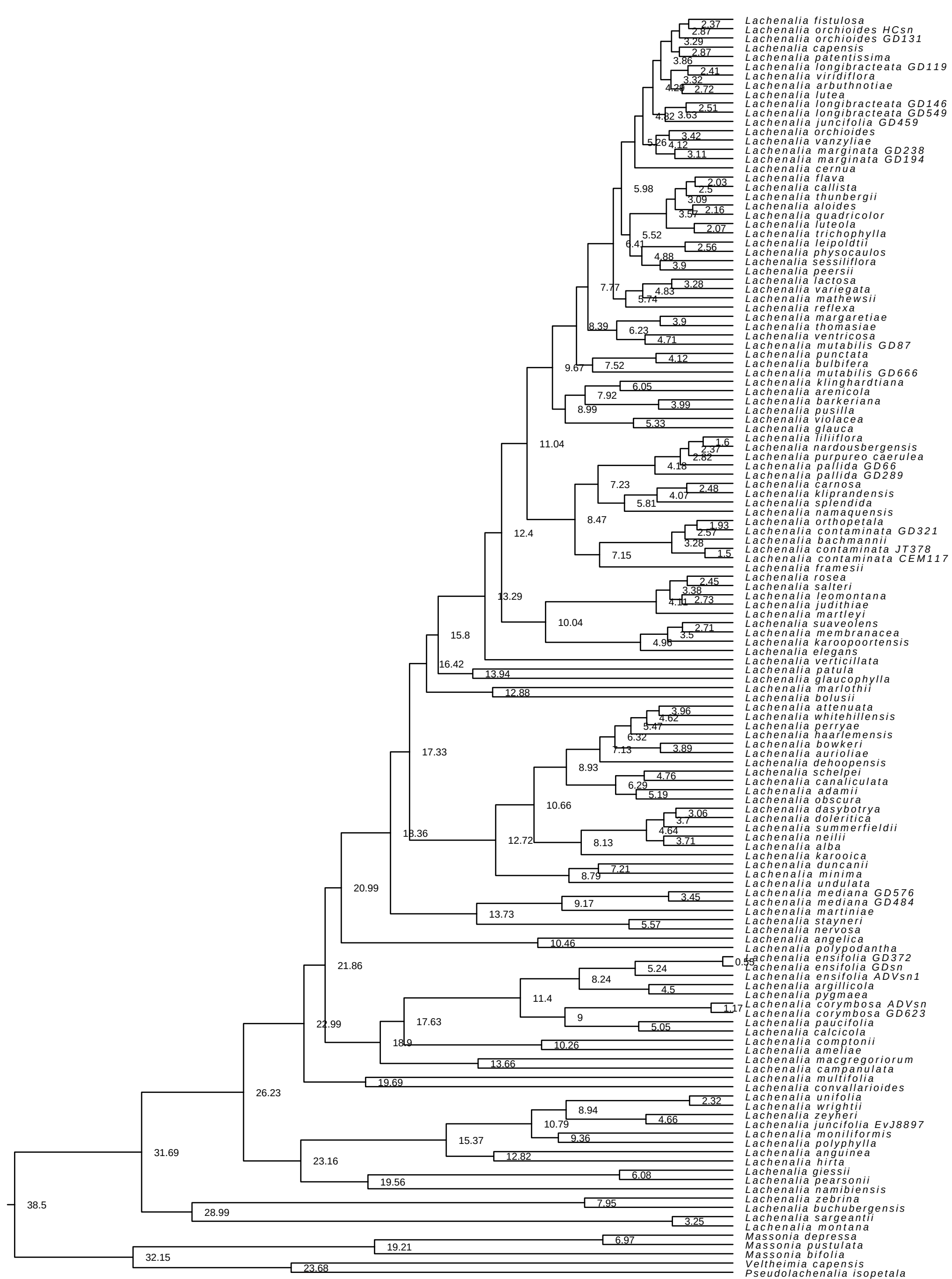
Al.bin = Binary autofertility index based on seed production: 0 = non-autofertile ($Al.seed < 0.2$), 1 = autofertile ($Al.seed > 0.2$)

SCI.bin = Binary self-compatibility index based on seed production: 0 = self-incompatible ($SCI.seed < 0.2$), 1 = self-compatible ($SCI.seed > 0.2$)

RA = Reproductive assurance: 0 = absent, 1 = present (i.e., species is bird pollinated and/or produces multiple offsets and/or is autofertile)

APPENDIX 5

Phylogenetic tree of genus *Lachenalia* based on the RAxML concatenated analysis and made ultrametric using treePL and a secondary calibration from Buerki et al. (2012). Ages are indicated at the nodes in million years ago (Ma).



APPENDIX 6

Genomic and environmental data matrix used in regression analyses of *Lachenalia*. Taxa marked with an * indicate that the same accession was used to determine chromosome count and genome size.

No	Phyl.seq	Species	Count	Base.no	X2n.or.higher	Low.no	High.no	D.B	Base.score	Gen.size	Ploidy	Polyploids	Chr.size	Veg.type	Fyn.score	Fyn.prop	MGS.min	MGS.med	MGS.max
30	45	L. adamii	0							2.79	2	0		Ren	0	0	0	0	0
21	40	L. alba	1	10	20	20	20	2	4	3.27	2	0	0.08175	Ren	0	0	0	0	0
90	107	L. aloides*	1	7	14	14	14	2	1	3	2	0	0.107142857	Thi	0	0	125	150	175
110	20	L. ameliae*	1	9	18	18	18	2	3	3.26	2	0	0.090555556	Suc/Ren	0	0	0	0	0
5	29	L. angelica*	1	7	14	14	14	2	1	3.47	2	0	0.123928571	Suc	0	0	0	0	0
123	9	L. anguinea	1	15	30 (1)+2B	30	30	2	8	5.81	2	0	0.096833333	Suc	0	0	0	38	100
79	120	L. arbuthnotiae	1	7	14	14	14	2	1	2.97	2	0	0.106071429	Fyn	1	1	150	175	200
95	85	L. arenicola	0							3.95	2	0		Suc	0	0	0	0	0
105	27	L. argillicola	0							3.21	2	0		Suc	0	0	0	0	0
24	55	L. attenuata	1	9	18	18	18	2	3	3.15	2	0	0.0875	Ren	0	0	0	38	100
27	50	L. aurioliae	0							2.78	2	0		Suc	0	0	0	25	50
55	72	L. bachmannii	1	8	16	16	16	2	2	3.23	2	0	0.1009375	Ren	0	0	125	150	175
97	87	L. barkeriana	1	7	14	14	14	2	1	3.23	2	0	0.115357143	Suc	0	0	0	0	0
13	57	L. bolusii	1	9	18	18	18	2	3	3.84	2	0	0.106666667	Des/Suc/Fyn	0	0	0	0	0
28	51	L. bowkeri	1	8	16	16	16	2	2	6.94	2	0	0.216875	Ren/Thi	0	0	0	113	225
2	2	L. buchubergensis	0							6.94	2	0		Des	0	0	0	0	0
58	90	L. bulbifera*	1	7	14, 28, 42, 49, 56	14	56	8	1	13.25	8	1	0.118303571	Thi/Fyn	1	0.99	0	100	200
108	23	L. calcicola	0							6.63	2	0		Fyn	1	1	150	175	200
87	109	L. callista	0							3.06	2	0		Fyn	1	1	150	175	200
113	19	L. campanulata	0							5.25	2	0		Ren/Thi	0	0	0	125	225
31	47	L. canaliculata	0							5.5	2	0		Suc/Ren	0	0	0	0	0
75	122	L. capensis*	1	7	28	28	28	4	1	5.62	4	1	0.100357143	Fyn	1	1	150	175	200
50	82	L. carnosa*	1	8	16	16	16	2	2	3.23	2	0	0.1009375	Suc	0	0	0	0	0
68	111	L. cernua	1	7	28	28	28	4	1	5.54	4	1	0.098928571	Fyn	1	1	100	150	200
111	21	L. comptonii	1	10	20	20	20	2	4	2.94	2	0	0.0735	Suc	0	0	0	0	0
53	73	L. contaminata	1	8	16, 32	16	32	4	2	3.67	4	1	0.05734375	Ren	0	0	100	163	225
115	17	L. convallarioides*	1	15	30	30	30	2	8	6.54	2	0	0.109	Ren/Thi	0	0	25	50	100
107	22	L. corymbosa	0							2.8	2	0		Ren	0	0	100	125	150
19	43	L. dasybotrya	0							2.8	2	0		Suc	0	0	0	0	0
29	49	L. dehoopensis	0							3.01	2	0		Fyn	1	1	150	163	175
18	42	L. doleritica	1	9	18	18	18	2	3	2.97	2	0	0.0825	Suc	0	0	0	0	0
14	37	L. duncanii*	1	9	18	18	18	2	3	2.47	2	0	0.068611111	Suc	0	0	0	0	0
38	61	L. elegans	1	7	14	14	14	2	1	2.73	2	0	0.0975	Fyn	1	1	0	0	0
104	25	L. ensifolia_ensifolia	1	13	26	26	26	2	7	3.99	2	0	0.076730769	Suc/Ren	0	0	0	138	364
103	26	L. ensifolia_maughanii	0							3.21	2	0		Ren	0	0	0	0	0
56	71	L. filamentosa	0							4.37	2	0		Ren/Fyn	1	0.6	125	150	175
82	125	L. fistulosa	1	7	14, 28	14	28	4	1	2.88	4	1	0.051428571	Ren	0	0	200	225	364
88	110	L. flava	1	7	14	14	14	2	1	3.24	2	0	0.115714286	Ren/Fyn	1	0.89	150	175	200
52	70	L. framesii	1	8	16	16	16	2	2	2.61	2	0	0.0815625	Suc	0	0	0	0	0
124	6	L. giessii	1	8	32	32	32	4	2	4.57	4	1	0.07140625	Des	0	0	0	0	0
100	84	L. glauca	0							3.61	2	0		Suc	0	0	0	38	100
81	124	L. glaucina*	1	7	28	28	28	4	1	5.79	4	1	0.103392857	Ren/Fyn	1	1	200	225	364
101	58	L. glaucophylla	0							4.4	2	0		Suc	0	0	0	0	0
78	119	L. granitica	1	7	14	14	14	2	1	3.13	2	0	0.111785714	Thi	0	0	100	125	150
26	52	L. haarlemensis*	1	9	18	18	18	2	3	3.43	2	0	0.095277778	Ren	0	0	0	25	50
122	8	L. hirta	1	11	22	22	22	2	5	2.91	2	0	0.066136364	Suc/Ren	0	0	0	100	175
57	89	L. inflata	0							3.2	2	0		Suc	0	0	0	0	0
43	69	L. judithiae	0							4.16	2	0		Ren/Fyn	1	0.5	200	225	364
116	12	L. juncifolia	1	11	22	22	22	2	5	3.35	2	0	0.076136364	Suc/Ren/Fyn	1	0.6	0	138	225
17	39	L. karooica	1	8	16	16	16	2	2	3.67	2	0	0.1146875	Suc	0	0	0	38	100
35	62	L. karooipoortensis	1	7	42	42	42	6	1	6.5	6	1	0.077380952	Suc	0	0	100	125	150
96	86	L. klinghardtiana	1	7	14	14	14	2	1	3.99	2	0	0.1425	Des	0	0	0	0	0
49	81	L. kliprandensis	1	8	16	16	16	2	2	3.35	2	0	0.1046875	Suc	0	0	0	0	0
74	117	L. komsbergensis	0							3.12	2	0		Suc	0	0	0	0	0
65	98	L. lactosa*	1	7	14	14	14	2	1	3.28	2	0	0.117142857	Fyn	1	1	175	200	225
91	100	L. leipoldtii	0							3.54	2	0		Ren/Fyn	0	0	125	150	175
42	68	L. leomontana*	1	7	14	14	14	2	1	4.23	2	0	0.151071429	Fyn	1	1	125	138	150
46	77	L. liliiflora	1	8	16	16	16	2	2	3.86	2	0	0.120625	Ren	0	0	150	175	200
73	116	L. longibracteata	1	7	14	14	14	2	1	5.94	2	0	0.212142857	Ren/Thi/Fyn	1	0.63	50	113	150
80	121	L. lutea	1	8	16	16	16	2	2	2.94	2	0	0.091875	Ren/Fyn	0	0	125	188	364
85	105	L. luteola	1	7	14	14	14	2	1	3.7	2	0	0.132142857	Fyn	1	1	150	175	200
112	18	L. macgregoriorum*	1	11	22	22	22	2	5	2.91	2	0	0.066136364	Ren	0	0	0	0	0
61	93	L. margaretiae*	1	7	14	14	14	2	1	3.27	2	0	0.116785714	Fyn	1	1	50	75	100
72	115	L. marginata_marginata	1	7	28	28	28	4	1	3	4	1	0.053571429	Fyn	1	1	100	125	150
71	114	L. marginata_neglecta*	1	7	14	14	14	2	1	3.19	2	0	0.113928571	Fyn	1	1	100	125	150
12	56	L. marlothii*	1	7	14	14	14	2	1	3.75	2	0	0.133928571	Suc	0	0	0	0	0
9	33	L. martiniae*	1	13	26	26	26	2	7	3.6	2	0	0.069230769	Suc/Ren/Fyn	1	1	50	100	125
39	65	L. martleyi	0							3.86	2	0		Ren	0	0	200	225	364
67	97	L. mathewsii*	1	7	14	14	14	2	1	3.85	2	0	0.1375	Ren/Thi	0	0	50	75	100
11	35	L. mediana	1	7	14	14	14	2	1	3.83	2	0	0.136785714	Ren	0	0	125	150	175
36	63	L. membranacea	1	7	14, 28	14	28	4	1	5.47	4	1	0.097678571	Fyn	1	1	100	125	150
15	38	L. minima	1	9	18	18	18	2	3	2.21	2	0	0.061388889	Suc	0	0	0	0	0
120	10	L. moniliformis*	1	11	22	22	22	2	5	3.92	2	0	0.089090909	Ren	0	0	50	100	125
4	4	L. montana	0							6.1	2	0		Fyn	1	1	175	200	225
114	16	L. multifolia	0							5.94	2	0		Suc	0	0	0	0	0
63	95	L. mutabilis*	1	7	14	14	14	2	1	2.48	2	0	0.088571429	Suc/Ren/Fyn	1	0.39	0	100	175
51	79	L. namaquensis*	1	8	16	16	16	2	2	3.02	2	0	0.094375	Suc	0	0	0	0	0
126	5	L. namibiensis	1	11	22	22	22	2	5	3.45	2	0	0.078409091	Des	0	0	0	0	0
47	78	L. nardousbergensis	0							3.46	2	0		Fyn	1	1	100	125	150
22	41	L. neilii*	1	9	18	18	18	2	3	2.85	2	0	0.079166667	Ren	0	0	0	0	0
8	32	L. nervosa*	1	8	16	16	16	2	2	3.32	2	0	0.10375	Ren	0	0	125	150	175
33	46	L. obscura*	1	9	18, 36	18	36	4	3	5.22	4	1	0.0725	Suc	0	0	0	0	0
69	112	L. orchiodoides	1	7	14	14	14	2	1	6.04	2	0	0.215714286	Ren/Fyn	1	0.1	100	175	364
54	74	L. orthopetala	1	8	16	16	16	2	2	3.45	2	0	0.1078125	Ren	0	0	125	150	175
44	75	L. pallida	1	8	16	16	16	2	2	3.24	2	0	0.10125	Ren/Thi	0	0	100	138	175
83	126	L. parviflora	0							3.1	2	0		Ren	0	0	200	225	364
76	123	L. patentissima	0							5.									

Column name	Description	Scoring (if relevant)
No	Species or taxon number	
Phyl. Seq	Phylogenetic sequence number	
Species	Alphabetical species or taxon name	
Count	Chromosome count published in the literature	
Base.no	Chromosome base number	
X2n.or.higher	Diploid chromosome number or higher ploidy levels	
Low.no	Lowest recorded diploid chromosome number	
High.no	Highest recorded diploid chromosome number	
D.B	Diploid base	2 = diploid, 4 = tetraploid, 6 = hexaploid, 8 = octoploid
Base.score	Chromosome base score	7 = 1, 8 = 2, 9 = 3, 10 = 4, 11 = 5, 12 = 6, 13 = 7, 15 = 8
Gen.size	Genome size in picograms (pg)	
Ploidy	Ploidy level	2 = diploid, 4 = tetraploid, 6 = hexaploid, 8 = octoploid
Polyploids	Polyploids absent or present	0 = absent, 1 = present
Chr.size	Chromosome size in micrometers (μm)	
Veg.type	Vegetation type	Des = Desert, Fyn = Fynbos, Ren = Renosterveld, Suc = Succulent Karoo, Thi = Thicket
Fyn.score	Fynbos score, presence in fynbos	0 = false, 1 = true
Fyn.prop	Proportion of records from fynbos sites	0 = never recorded in fynbos, 1 = only recorded in fynbos
MGS.min	Moisture Growing Season minimum duration in days	
MGS.med	Moisture Growing Season median duration in days	
MGS.max	Moisture Growing Season maximum duration in days	