Systematics, diversification and ecology of the *Conophytum*-clade (Ruschieae; Aizoaceae)

by

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"All of nature begins to whisper its secrets to us through its sounds.
Sounds that were previously incomprehensible to our soul
now become the meaningful language of nature"

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—RUDOLF STEINER

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SUMMARY

The Ruschieae is the most diverse and speciose tribe within the large subfamily Ruschioideae (Aizoaceae), with approximately 71 genera and a distribution centred in the arid parts of the Greater Cape Floristic Region (GCFR) of South Africa. Recent phylogenetic analyses provided the first insights into generic relationships within the tribe, with a number of novel generic relationships discovered. The tribal phylogeny recovered 12 large clades, of which the *Conophytum*-clade was one the most morphologically diverse based on leaf and capsule characters. The *Conophytum*-clade is an early-diverging lineage of the Ruschieae and includes the following 10 genera: *Cheiridopsis* N.E.Br., *Conophytum* N.E.Br., *Enarganthe* N.E.Br., *Ihlenfeldtia* H.E.K.Hartmann, *Jensenobotrya* A.G.J.Herre, *Namaquanthus* L.Bolus, *Octopoma* N.E.Br., *Odontophorus* N.E.Br., *Ruschianthus* L.Bolus and *Schlechteranthus* Schwantes. The present study presents an expanded phylogenetic analysis of the *Conophytum*-clade, with the sampling of the majority of species in the genera and a representative sampling (56% of species) of the speciose genus *Conophytum.* Phylogenetic data for up to nine plastid gene regions (*atpB–rbcL, matK, psbJ–petA, rpl16, rps16, trnD–trnT, trnL–F, trnQ*^{UUG}*–rps16, trnS–trnG*) were produced for each of the sampled species. The produced plastid data was analyses using maximum parsimony, maximum likelihood and Bayesian inference. The combined plastid phylogenetic analyses were used in combination with morphological, anatomical and palynological data to assess generic and subgeneric circumscriptions within the clade.

Upon assessment of generic circumscriptions in the *Conophytum*-clade, the number of recognised genera in the clade decreased from ten to seven. *Arenifera* A.G.J.Herre, which had not been sampled in any phylogeny of the Ruschieae, and *Octopoma* were recovered as polyphyletic, with species placed in the *Conophytum*-clade, while the type species was placed in the xeromorphic clade of the tribal phylogeny. The species of *Arenifera* and *Octopoma*

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placed in the *Conophytum*-clade were subsequently included in *Schlechteranthus* upon assessment of generic circumscriptions between the taxa. Two morphological groupings were recognised within *Schlechteranthus*, one including the species of *Schlechteranthus* and the other including species previously recognised as *Arenifera* and *Octopoma.* These two morphological groupings were treated as subgenera, with the erection of the new subgenus *Microphyllus* R.F.Powell. A detailed taxonomic revision of subgenus *Microphyllus* is presented with a key to species, descriptions of the species (including a new species: *S. parvus* R.F.Powell & Klak), known geographical distributions and illustrations of the species.

In addition to the changes mentioned above, the expanded sampling and phylogenetic analyses of the *Conophytum*-clade recovered *Ihlenfeldtia* and *Odontophorus* embedded in *Cheiridopsis.* The species of *Ihlenfeldtia* were recovered with species of *Cheiridopsis* subgenus *Aequifoliae* H.E.K.Hartmann, while the species of *Odontophorus* were recovered as polyphyletic within the *Cheiridopsis* subgenus *Odontophoroides* H.E.K.Hartmann clade. *Cheiridopsis* was subsequently expanded to include the species of *Ihlenfeldtia* and *Odontophorus,* with these species accommodated in the subgenera of *Cheiridopsis.* The phylogenetic placement and relationship of these species was supported by the shared capsule morphology.

The expanded sampling of the clade did not resolve the phylogenetic relationship of the monotypic genera *Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus,* with these genera unresolved in the *Conophytum*-clade. These genera however, exhibit a unique combination of morphological characters, such as a glabrous leaf epidermis and variation in pollen exine and colpi structure, in contrast to the other genera of the clade. The assessment of the generic circumscription of these genera, based on the molecular, morphological, anatomical and palynological data suggested that the generic statuses of these monotypic genera should be maintained.

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The expanded phylogenetic sampling of the morphologically diverse and speciose genus *Conophytum* recovered the genus as monophyletic. This monophyly was supported by the unique floral type in *Conophytum,* with the fused petaloid staminodes forming a tube. None of the sectional classifications were recovered as monophyletic but the phylogenetic analyses did recover a few clades which more or less corresponded to the current sectional classification of the genus. A number of clades were also recovered which included species from a range of different sections. Diverse leaf and floral traits were shown to have evolved numerous times across the genus. This was particularly interesting with regards to the selected floral traits, as the phylogeny indicated a number of switches in floral morphologies across the genus. The floral diversity was assessed in complex species communities of *Conophytum* across the GCFR, where up to 11 species of *Conophytum* are found occurring sympatrically, and floral traits were shown to be different across the species within the communities. Pollination competition and adaptation were suggested as possible drivers of floral diversity in the genus, with differences in phenology, anthesis and floral morphology within the species complex communities. The unique floral type of *Conophytum* has enabled the species to develop a diverse range of specialised flowers, with a variety of structures, scents and colours, resulting in the diverse floral morphologies found across the genus.

The complex *Conophytum* species communities included both closely as well as distantly related species, suggesting the soft papery capsules of *Conophytum* are wind dispersed. This adaptation to long distance seed dispersal resulted in a significantly higher phylogenetic diversity in *Conophytum* when compared to its sister genus, *Cheiridopsis.* A population genetics study of *Conophytum* also suggested that the capsules may be wind dispersed, with an indication of genetic connectivity between the geographically isolated populations of *C. marginatum* Lavis across the Bushmanland Inselberg Region. Although the capsules are dispersed by wind, the seeds are released from the hygrochastic capsules by

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runoff during rainfall events. The relationship between seed dispersal and runoff is evident from the genetic structure of populations of *C. maughanii* N.E.Br. and *C. ratum* S.A.Hammer that occur on the tops and the surrounding bases of the inselbergs, as the drainage pattern was found to directly influence population structure in these species. In addition, the AFLP analyses provided insight into the conservation of the flagship species *C. ratum.* The summit populations of this species were shown to sustain the populations at the base of the Gamsberg. This finding is especially important, as the distribution of the species is restricted to the Gamsberg inselberg, where mining has already commenced as of this year.

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Chapter 1

General Introduction and Objectives

1.1 GENERAL INTRODUCTION

Aizoaceae is the largest succulent plant family in the world (Chesselet *et al.*, 2002), with ca. 125 genera and 1,845 species (Hartmann, 2001; Manning & Goldblatt, 2012; Snijman, 2013). The family is characterised by the presence of epidermal bladder cells, the formation of a perianth-stamen tube and hygrochastic capsules (Bittrich & Hartmann, 1988), and constitutes the largest angiosperm family in the arid parts of the Greater Cape Floristic Region (GCFR) of South Africa (Snijman, 2013). Despite the strong representation of Aizoaceae in the GCFR and the large number of taxa endemic to this region (Manning & Goldblatt, 2012; Snijman, 2013), it was identified as the top priority for taxonomic research in South Africa (Victor & Smith, 2011; Von Staden *et al.*, 2013). The Aizoaceae included the highest percentage of data deficient species (28%) of all plant families in South Africa (Victor & Smith, 2011), with 52% of taxa not treated in any taxonomic revision (Von Staden *et al.*, 2013).

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Although different subfamilial classifications for Aizoaceae have been proposed (Herre & Volk, 1948; Herre, 1971; Bittrich & Hartmann, 1988; Hartmann, 1991, 1993, 2001), evidence from phylogenetic data (Klak, 2003a; Thiede, 2004; Thiede *et al.*, 2007) supports the most recent classification of the family (Hartmann, 2001), recognising five subfamilies, viz. Aizooideae, Mesembryanthemoideae, Ruschioideae, Sesuvioideae and Tetragonioideae. Together the Aizooideae, Sesuvioideae and Tetragonioideae contain a small proportion of taxa in the family, i.e. only 12 genera distributed world-wide in tropical and temperate regions. In contrast, the Mesembryanthemoideae and Ruschioideae (previously treated as a distinct family, the Mesembryanthemaceae (Herre & Volk, 1948; Herre, 1971) comprise of ca. 113 genera with their diversity centred in the arid parts of the GCFR

(Manning & Goldblatt, 2012; Snijman, 2013). The subfamilies are distinguished by differences in nectary type and the position of the placenta in the ovary (Chesselet *et al.*, 2002). In Mesembryanthemoideae, the nectary is koilomorphic and the ovary half inferior with an axile placentation, while in Ruschioideae the nectary is a flat or lophomorphic meronectary or holonectary and the ovary is inferior with a basal or parietal placentation (Chesselet *et al.*, 2002). In addition, the tepals form a tube in Mesembryanthemoideae, with cortical bundles and expanding keels from the centre of the capsule to the tip of the valve. In contrast, the tepal bases are free, cortical bundles absent and the expanding keels are more or less confined to valves in Ruschioideae (Chesselet *et al.*, 2002).

The Ruschioideae is by far the largest and most diverse subfamily in the Aizoaceae, with approximately 111 genera and 1,600 species (Hartmann, 2001). The first tribal classification for the Ruschioideae was established by Schwantes (1947, 1957) and later replaced by the 12 morphological groups recognised by Hartmann (1988, 1991, 1998a). These groupings, however, did not provide a formal classification for the subfamily. As such, a formal classification was proposed by Chesselet *et al.* (2002), with four tribes (viz. Apateasieae, Dorotheantheae, Drosanthemeae and Ruschieae) recognised based on differences in floral nectaries, the degree of leaf succulence and the presence of covering membranes in the capsules (Hartmann, 1991; Chesselet *et al.*, 2002, 2004). This classification is also supported by phylogenetic data (Klak *et al.*, 2003a, 2003b, 2013). The tribe Ruschieae is the most diverse, with 71 genera, and is defined by the lophomorphic holonectary and basally fused leaves (Chesselet *et al.*, 2002, 2004). Early molecular phylogenetic analyses of this tribe suggested that it represents the most rapid and recent diversification in the family (Klak *et al.*, 2003a, 2003b), as well as the most rapid radiation of any plant group at the time (Klak *et al.*, 2004).

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Although early studies attempted to resolve phylogenetic relationships within the Ruschieae (Klak *et al.,* 2003a, 2003b), the resolution of these phylogenies was poor and only sampled a subset (44–53 genera) of the tribe. However, identification of additional variable plastid markers (Shaw *et al.,* 2005, 2007) provided improved resolution, allowing for an almost complete sampling of the tribe, with 69 genera sampled, excluding only *Arenifera* A.G.J.Herre and *Circandra* N.E.Br. (Klak *et al.,* 2013). Although only one representative was included for the majority of genera, up to 11 species of the larger genera were sampled. The tribal level phylogeny recovered 12 large clades (Klak *et al.,* 2013) and provided insights into generic relationships within the tribe for the first time. The tribal phylogeny recovered a number of polyphyletic genera as well as novel relationships between genera that had previously been considered unrelated (Klak *et al.*, 2013).

The groupings of Hartmann (1991) were based mainly on capsule morphology, in combination with leaf and floral characters. Generic circumscriptions in the subfamily have predominantly relied on capsule morphology (Brown, 1920; Schwantes, 1926; Ihlenfeldt, 1960; Ihlenfeldt & Bittrich, 1985; Ihlenfeldt & Struck, 1987; Hartmann, 1988, 1991), as the capsules often include complex internal structures (expanding keels, covering membranes and closing bodies) providing a range of characters to distinguish taxa. The fruits of the Aizoaceae are loculicidal hygrochastic capsules that open when in contact with water to release the seeds and the internal structures facilitate seed dispersal during rainfall events through a number of different dispersal mechanisms (Hartmann, 1991). Klak *et al.* (2013) reconstructed the different capsule types (i.e. weakly persistent simple capsules or strongly persistent complex capsules) and leaf epidermal type (mesomorphic or xeromorphic) which had been used to delimit groups in the tribe previously, onto their tribal phylogeny. The basal lineages were shown to have mostly mesomorphic, often ephemeral leaves with weakly persistent capsules, with trend towards xeromorphic, persistent leaves and strongly persisting

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capsules (Klak *et al.,* 2013). However, both capsules types, strongly and weakly persistent, had evolved multiple times across the tribe (Klak *et al.,* 2013) and as a consequence, the tribal phylogeny found that none of the morphological groups of Hartmann (1991) were monophyletic, with a number of clades including a mixture of taxa from these morphological groups.

One such morphological variable clade is the early-diverging *Conophytum*-clade, which included all seven capsule types and five of the morphological groups recognised by Hartmann (1988, 1991), as well as both weakly and strongly persistent capsules (Klak *et al.,* 2013). Despite the placement of the *Conophytum*-clade within the group of mesomorphic-leaved clades (clades A–J), the clade itself largely included genera with xeromorphic leaves (Klak *et al.,* 2013), although the diverse genus *Conophytum* N.E.Br. includes both leaf types (Hammer, 1993; Opel, 2005a). In addition, dwarf stemless plants, which were shown to have arisen multiple times in the Ruschieae, were also found in the *Conophytum*-clade (i.e. *Conophytum*), together with caespitose and shrubby genera (Klak *et al.*, 2013).

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The *Conophytum*-clade currently includes 10 genera: *Cheiridopsis* N.E.Br, *Conophytum, Enarganthe* N.E.Br., *Ihlenfeldtia* H.E.K.Hartmann, *Jensenobotrya* A.G.J.Herre, *Namaquanthus* L.Bolus, *Octopoma* N.E.Br., *Odontophorus* N.E.Br, *Ruschianthus* L.Bolus, and *Schlechteranthus* Schwantes (Klak *et al.,* 2013; Klak & Bruyns, 2016). One of these genera, *Octopoma,* was recovered as polyphyletic in the Ruschieae phylogeny (Klak *et al.,* 2013) with *Octopoma subglobosum* (L.Bolus) L.Bolus placed sister to species of *Polymita* N.E.Br. and *Schlechteranthus* Schwantes in the *Conophytum*-clade, while the type species, *O. octojuge* N.E.Br., was placed in the *Stomatium-Bergeranthus*-clade (Klak *et al.,* 2013). *Octopoma* was also identified as one of the priority genera for taxonomic revision (Victor & Smith, 2011; Von Staden *et al.,* 2013). *Arenifera,* which shares similar leaf and capsule characters with *Octopoma,* was not sampled in the tribal phylogeny (Klak *et al.,* 2013) and

therefore its relationship to other genera in the tribe is unknown. This genus was also listed as a high priority genus for taxonomic revision (Victor & Smith, 2011; Von Staden *et al.*, 2013).

Conophytum, with 106 species, is the most speciose and morphologically diverse genus in the *Conophytum*-clade and also one of the most diverse genera in the Ruschieae (Hartmann, 2001). The genus includes a range of leaf morphological and anatomical characters, such as leaf markings (spots and lines), epidermal windows, papillae, calcium oxalate crystals and bladder cells (Hammer, 2002; Opel, 2005a). *Conophytum* also exhibits a range of floral morphologies including differences in phenology, anthesis, floral structure, scent and colour (Liede & Hammer, 1991; Hammer, 2002; Jürgens & Manning, 2004). As a consequence of the leaf and floral diversity, the species of *Conophytum* are often quite charismatic and highly popular among succulent collectors. This popularity among collectors is so evident, that the majority of recent taxonomic work in the genus has been conducted by succulent collectors and growers (Hammer, 1993, 2002). However, despite its popularity, the species of *Conophytum* are poorly represented in herbarium collections, with almost 25% unaccounted for in national herbaria of South Africa. In addition, the species represented in the herbaria collections do not adequately represent the distribution range of the species. As a result, several of the species are currently listed as data deficient (Victor & Smith, 2011; Von Staden *et al.*, 2013). Not only is *Conophytum* unusual in the great number of species and diversity of morphologies included in the genus, but the species also form complex communities across the GCFR (Liede *et al.*, 1991), with up to 11 species occurring sympatrically (Young *et al.*, 2016).

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The species of *Conophytum* have been comprehensively assessed by Hammer (1993, 2002), with 387 taxa reduced to synonymy and 16 sections recognised (Hammer, 2002). This subgeneric classification was tested by Opel (2005b) through the production of a morphological phylogeny based on leaf anatomical characters, in combination with floral

morphology. The morphological phylogeny recovered a number of clades which supported the sectional classification, as well as a few clades which included species from a range of sections (Opel, 2005b). Although *Conophytum* was recovered as monophyletic in Klak *et al.* (2013), only three species (of ca. 106) were sampled and therefore the infrageneric relationships within the genus remains unclear.

An expanded phylogenetic sampling of the *Conophytum*-clade is required to refine generic and infrageneric circumscriptions and relationships, resolve the suggested polyphyly of *Octopoma* and better understand the evolution of leaf, floral and capsule characters in the clade. In addition, a first molecular phylogenetic hypothesis for the speciose genus *Conophytum* could be used to assess phylogenetic relationships in the genus and within the species complex communities, providing insight into possible drivers of diversification.

1.2 OBJECTIVES

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In this study I use a multidisciplinary approach to evaluate relationships and circumscriptions across several taxonomic levels (genera, subgenera, sections, species, species communities and populations), within the *Conophytum*-clade of the Ruschieae. For the first time since the production of the tribal phylogeny, a recovered clade of the Ruschieae is expanded, therefore representing the first step to ultimately resolving relationships in the tribe as a whole. The specific objectives of this thesis were to:

1. Assess the phylogenetic placement, monophyly, generic circumscription and taxonomy of the multilocular genus *Arenifera*.
2. Resolve the reported polyphyly of *Octopoma* and refine the generic circumscription, relationships and taxonomy of the genus.

3. Expand phylogenetic sampling in the *Conophytum*-clade to suitably assess generic and infrageneric circumscriptions and relationships.

4. Explore the evolutionary history of selected morphological, anatomical and palynological characters in the *Conophytum*-clade.

5. Revise the taxonomy of genera within the *Conophytum*-clade where necessary.

6. Produce a digital key to the species of *Conophytum,* with comprehensive distribution data and images of the species.

As *Conophytum* is a diverse genus, with species exhibiting a range of floral traits as well as forming complex species communities, the genus lends itself as an ideal model group to:

7. Explore differences in phenology, anthesis and floral traits of species within complex *Conophytum* species communities to determine the contribution of these traits in maintaining species integrity.

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8. Assess the phylogenetic diversity of the species included in the complex species communities to provide insight into drivers of diversity in the genus.

9. Investigate the population dynamics of three *Conophytum* species endemic to the Bushmanland Inselberg Region to assess the genetic connectivity of species, including narrow endemic species and relatively widespread species, across the inselbergs and explore different seed dispersal mechanisms in the species.

Chapter 2

Materials and Methods

This chapter provides the detailed methods followed in each of the chapters of this thesis. The taxonomic sampling of the studied genera is included in the relevant chapters. Authorities for scientific plant names (according to Brummit & Powell, 1992) are provided at first mention in each of the chapters and are also available in Appendices A, B & C. Similarly, abbreviations used in this thesis are provided at first mention in each of the chapters.

2.1 MORPHOLOGICAL DATA

2.1.1 COLLECTION OF MATERIAL

Extensive field work was carried out over the last three years, with four field visits conducted to collect material of species in the *Conophytum*-clade. A total of 95 samples (57 species) were collected. This material was processed to produce herbarium specimens housed at NBG, and living collections housed at the Karoo Botanical Gardens (KBG). Fresh leaf material was also stored in desiccant silica gel for subsequent DNA extraction.

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While the majority of material was collected in the field, several rare species were obtained from greenhouse collections at KBG (Worcester, RSA), the South African National Biodiversity Institute (Kirstenbosch, RSA), as well as from the Sphaeroid Institute (California, USA). The voucher specimens for these samples (Appendix A–C) are housed at the Compton Herbarium (NBG).

2.1.2 Examination of Herbarium Specimens

The complete collections of the genera and species of the *Conophytum*-clade housed at BOL, NBG (including SAM) and PRE were examined. In addition, 54 of the 158 species were studied *in situ* during field visits. Specimens from the herbaria, as well as those collected during the present study, were used for examination of leaf, floral and capsule characters (Chapters 3–6).

2.1.3 Production of Digital DELTA Key for *Conophytum*

A digital key to the species of *Conophytum* N.E.Br. was produced using the DELTA Software package (Dallwitz *et al.*, 1993). Data were coded for each *Conophytum* species from Hammer (2002) and from the literature for subsequently described species (Hammer, 2009; Young *et al.*, 2011, 2015a, b; Rodgerson, 2012; Rodgerson &Young, 2013) as well as from field observations in DELTA Editor (Dallwitz *et al.*, 1999). The coded information was then used to produce a digital key using Intkey (Dallwitz *et al.*, 1995), and images (from field collections and C. Rodgerson) of each of the species, as well as selected diagnostic characters, were added to the digital key. The digital key is available at http://rpowell2.wixsite.com/conophytum-delta-key.

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This key provides a user-friendly tool for identification of *Conophytum.* The genus includes a large number of species (106 species) and due to the diverse range of leaf and floral morphologies of the species, the distinction between species is often unclear, making identification of species challenging. The key provides images which greatly aid in the identification of species, as well as images illustrating diagnostic characters. A digital key is also advantageous, as the user can select the order in which to use characters for identification, rather than following a predetermined order, as for instance in a dichotomous key. In addition, the key provides extensive distribution maps of the species, which are

essential in identification of the species, as many taxa are distinguished based on their distribution.

2.2 DISTRIBUTION AND SPECIES RICHNESS MAPS

2.2.1 DISTRIBUTION MAPS

Distribution data of the species were collected from the examined herbarium specimens as well as field collection notes. The distribution for each species was recorded using the Quarter Degree Reference System for South Africa (Edwards & Leistner 1971; also outlined in Leistner & Morris 1976). The basic unit in this system is the one-degree square of latitude and longitude, which is designated by a degree reference number (i.e. degrees of latitude and longitude of the north-west corner) and the district name of that grid. This grid reference system was used to produce maps for each of the species in *Schlechteranthus* subgenus *Microphyllus* R.F.Powell (Chapter 4).

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2.2.2 SPECIES RICHNESS QUARTER DEGREE SQUARE MAPS

The distribution data of the species of *Cheiridopsis* N.E.Br. and *Schlechteranthus* Schwantes was mapped in ArcMap (ESRI, 2011) for the genera and subgenera within each of the genera, respectively (Chapters 3, 5). A Quarter Degree Square (QDS) (Edwards & Leistner, 1971; Leistner & Morris, 1976) grid was overlaid onto the distribution data and the Spatial Join tool (ESRI, 2011) was used to join the two datasets. The number of species per QDS was calculated from the count data in the Spatial Join (ESRI, 2011). The Spatial Join analysis and count data were calculated separately for the genera and each of the subgenera, respectively, with separate maps for the genera and subgenera within them produced (Chapters 3, 5).

2.3 CHARACTER RECONSTRUCTION

Selected morphological characters were coded manually with a polarised outgroup (i.e. outgroup was coded as a '0' state) and reconstructed onto the BI consensus tree (Chapters 5 & 6), using the parsimony trace character history function in Mesquite version 3.04 (Maddison & Maddison, 2015). Intermediate taxa (taxa with more than one character state) were coded as polymorphic (i.e. 0/1) following the Mesquite manual (Maddison & Maddison, 2015).

2.4 PHYLOGENETIC DATA

2.4.1 DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total DNA was extracted from silica-dried leaf material (0.2 mg) using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. Depending on the study (Chapters 3, 5, 6), either nine or six chloroplast gene regions were amplified and sequenced. Nuclear regions were found to be problematic in the Ruschioideae by Klak *et al.* (2013), as they indicated gene duplication or multiple copies. The ETS nuclear region was tested in the present study on a subset of samples, but multiple paralogues were detected in the electropherograms of the resulting sequences indicating that there were possibly multiple copies and therefore nuclear regions were excluded. A portion of the *trnQ*^{UUG}*–rps16* intergenic spacer was amplified using the primers *trnQ*^{UUG} and *rps16x1* (Shaw *et al*., 2007). The *trnS–trnG* intergenic region was amplified using the primers *trnS* and *trnG* (Hamilton, 1999). The *trnL–F* region (consisting of the adjacent *trnL* intron and *trnL–F* intergenic spacer) was amplified using the extron primers c and f (Taberlet *et al*., 1991). The *rps16* region was amplified using the primers *rps16F* and *rps16R2* (Oxelman *et al*., 1997). The *rpl16* intron was amplified using primers *rpl16* 71F (Jordan *et al*., 1996) and *rpl16* 1516R (Kelchner & Clark, 1997). The intergenic spacer between the *atpB* and *rbcL*

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genes was amplified using primers 2 and 5 (Manen *et al*., 1994). The intergenic spacer *psbJ–petA* was amplified using primers *psbJ* and *petA* (Shaw *et al*., 2007) and part of the intergenic spacer *trnD–trnT* was amplified using primers *trnE* (Shaw *et al*., 2005) and *trnD* (Demesure *et al*., 1995). Selected species (Appendix A3) were sent to the Canadian Centre for DNA Barcoding (Guelph, Canada) for *matK* barcode sequencing, to contribute towards the International Barcode of Life project (iBOL, 2015) (Sequences available at http://www.boldsystems.org/ (Ratnasingham & Herberg, 2007), while a portion of the *matK* gene was amplified for the remaining species using DNA barcoding primers 3F-Kim and 1R-Kim (Cuenoud *et al*., 2002).

Polymerase chain reactions (PCRs) were performed in 25 µl reactions containing 22.5 µl Thermo Scientific 1.1X ReddyMix PCR Master Mix (Thermo Fischer Scientific, Inc.), 0.8 µl Bovine Serum Albumin, 0.6 µl sterile distilled water, 0.3 µl of each primer and 0.5 µl of DNA template. The PCR reactions were carried out using the following thermal conditions: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 8 min. For samples that did not amplify successfully, the protocol was adjusted to include a temperature ramp following Shaw *et al*. (2005). Successfully amplified samples were cleaned using the ExoSAP protocol of Werle *et al*. (1994) using 5 units of Exonuclease I and 0.5 units of Shrimp Alkaline Phosphatase. Automated sequencing was carried out by Macrogen (Seoul, Korea). Electropherograms obtained from the sequences were manually checked and inconsistencies in the sequences were edited where necessary using MEGA version 6 (Higgins *et al*., 1994).

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2.4.2 PHYLOGENETIC ANALYSES

The 585 new plastid sequences produced were analysed as specified in each of the respective chapters (Chapters 3, 5, 6). In general, the sequences were automatically aligned using the Clustal W function in MEGA version 6 (Higgins *et al*., 1994; Tamura *et al*., 2013). This alignment was checked manually and adjusted accordingly where required, with gaps positioned so as to minimize nucleotide mismatches. Hypervariable regions for *trnQ*^{UUG}*–rps16* (79 base pairs) and *trnS–trnG* (48 base pairs) were excluded from the analysis, and gaps were coded using simple indel coding (Simmons & Ochoterena, 2000).

The maximum parsimony (MP) algorithm was implemented in PAUP* version 4.0b4 (Swofford, 2000). Character transformations were unordered and equally weighted (Fitch, 1971). A heuristic search with 1,000 random sequence additions, tree bisection reconnection (TBR) branch-swapping, and the MULPARS option selected, was performed. All character transformations were treated with equal likelihood and a maximum of 10 trees were saved in each replicate to minimise swapping on local minima. Trees of the shortest length were saved and used as starting trees for a second round of TBR swapping with no limit on the number of trees saved, to ensure the shortest trees were recovered in the analysis. Node support was evaluated using the jackknife function in PAUP, with a full search, 1,000 replicates and a limit of 1,000 trees per replicate (Farris *et al*., 1996). Only jackknife support (JK) values greater than 50% were retained, and the following scale was used to evaluate support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Maximum likelihood (ML) analyses were performed using RAxML version 8.1.11 (Stamatakis, 2006) on the combined chloroplast data (excluding the coded indels). The analyses were run on the CIPRES Portal, version 3.3 (Miller *et al*., 2010), using the default settings. A maximum likelihood tree with bootstrap node support (BS) is presented in each of

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the relevant chapters using the following scale to evaluate support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Bayesian inference (BI) was performed on the combined chloroplast dataset, (including the coded indels), using MrBayes 3.2.3 (Ronquist & Huelsenbeck, 2003). The analyses were run on the CIPRES Portal, version 3.3 (Miller *et al*., 2010). Data were partitioned accordingly in each dataset and all parameters were unlinked (statfreq, revmat, shape, pinvar) between partitions. The standard deviation between the split frequencies stabilised below 0.01, providing evidence that a sufficient number of generations had been completed. Using Tracer v.1.5 (Rambaut & Drummond, 2009), suboptimal trees were discarded as the "burn-in" phase. Only support values greater than 0.5 were retained, and the following scale was used to evaluate support values: 0.50–0.94, weak; and 0.95–1.0, strong.

2.4.3 CONSTRAINT ANALYSIS

A constraint topological analysis was conducted in PAUP (Swofford, 2000), using the constrained monophyly option. *Cheiridopsis* (including *Ihlenfeldtia* H.E.K.Hartmann and *Odontophorus* N.E.Br.) was constrained to monophyly, to test whether trees including the two outlying *Cheiridopsis* species (*C. pearsonii* N.E.Br. and *C. peculiaris* N.E.Br.), placed outside of the main clade of *Cheiridopsis* species (Chapter 5), were significantly different from those that did not include them in the genus. A Templeton-Wilcoxon test (Swofford, 2000) was run to test for a significant difference between the two trees produced under topological constrained and unconstrained parameters (Chapter 5).

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2.5 ANATOMICAL DATA

Fresh leaf material was fixed in formalin-aceto-alcohol (FAA). The FAA was prepared using 90 ml of 70% ethanol, 5 ml of 40% formalin and 5 ml of glacial acetic acid (De Neergaard *et al*., 2001). Leaf material obtained from herbarium specimens was re-hydrated and then also fixed in FAA. The material was embedded in paraffin wax (Rudall, 1995), and 12–15 µm transverse sections from the central third of the leaf were cut using a Reichert-Jung autocut microtome (Model 2040). The sections were then double-stained with alcian blue and safranin. Permanent slides were made using Entellan and viewed with a Zeiss compound microscope and photographed using an Olympus SC30 camera (Chapters 3 & 5).

2.6 POLLEN EXINE AND COLPI STRUCTURE

Variation of pollen exine structure was surveyed for selected taxa of the *Conophytum*-clade (Chapter 5). The pollen samples were prepared using glycerin jelly for viewing under a light microscope (Erdtman, 1960). Glycerin jelly was prepared following Kearns *et al.* (1993). A small amount of the jelly was placed on a cleaned slide and the pollen was dusted onto it. A cover slip was then placed over the jelly and the slide was placed on a heated plate. The pollen was then viewed using a Zeiss compound microscope.

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2.7 MULTIVARIATE ANALYSES

2.7.1 PRINCIPAL COMPONENT ANALYSES (PCA)

A principal components analysis (PCA) was performed for each of the communities (Chapter 7) using the ‘R’ statistical package *prcomp* (Venables & Ripley, 2002). Trait data were obtained from the coded matrix used to produce the digital DELTA Key (Appendix E4) and these data were used to produce a binary data matrix with floral traits for species of *Conophytum*, and where appropriate for subspecies, coded within a community (Chapter 7).

The only instance where more than one subspecies of a species was found in a community was at Umdaus (Chapter 7), where both *C. flavum* N.E.Br. subsp. *flavum* N.E.Br. and subsp. *novicium* (N.E.Br.) S.A.Hammer occurs; however, these subspecies do not differ in floral morphology and therefore did not pose a problem in the multivariate analyses. Traits that showed no variation within the community (i.e. either absent or present in all the species within the community) were excluded from the analyses

Species in all the communities form temporal guilds, separated by different flowering times (anthesis) and flowering seasons (phenology). As a result species across the different temporal guilds would not compete for pollinators and would be reproductively isolated from one another (Nosil *et al.*, 2008). To illustrate the separation of species within each of these temporal guilds, anthesis and phenology were coded for the species in each of the different communities, with a PCA for each of the communities produced (Chapter 7).

Additional PCAs were produced for each of the temporal guilds (Chapter 7) within each community (e.g. diurnal autumnal flowering species) using the following floral traits: flower colour, flower structure, presence of filamentous staminodes and scented flowers (Table 7.1 of Chapter 7). A PCA could not be produced for temporal guilds with only one or two species; however, in the case of two species within a guild, the floral differences were reported.

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2.7.2 PRINCIPAL COORDINATE ANALYSES (PCOA)

The maximum likelihood (ML) tree for the expanded sampling of *Conophytum* (Chapter 6), was used to calculate pairwise genetic values for each species sampled in the tree using the *cophenetic.phylo* function in the 'R' statistical package 'ape' (Felsenstein, 2001; Paradis *et al.*, 2016). The pairwise distances for each of the species in the community (Chapter 7) were manually compiled into separate matrices in Microsoft Excel (2010) and a Principal

Coordinate Analyses (PCoA) was calculated on the data using the 'R' statistical tool *pcoa*, available from the package 'ape' (Paradis *et al.*, 2016) for each of the communities (Chapter 7). The PCoAs were plotted using the *biplot* function (Paradis *et al.*, 2016) and the axes were set to a standard scale across the communities. Pairwise genetic distance PCoAs were produced for each community as well as for the different temporal guilds of each community (Chapter 7). Genetic distance PCoAs could not be produced for temporal guilds with only one or two species, but in the case of two species, the genetic pairwise distance value was reported. Species for which phylogenetic data were absent, i.e. species not sampled in the phylogeny (Appendix A2), were excluded from these analyses.

2.8 PHYLOGENETIC DIVERSITY

Distribution data was obtained for *Conophytum* from Young & Desmet (2016) and from Powell *et al.* (submitted b; Chapter 5) for *Cheiridopsis* and QDS with three or more species were manually identified in each of the genera (Chapter 7). The maximum likelihood (ML) tree produced in Chapter 6 was used to calculate phylogenetic diversity (PD) in the identified QDS. Faith's PD was calculated in R using the pd function available from the picante package (Faith, 1992; Kembel *et al.*, 2010). The PD values for each QDS in *Conophytum* and *Cheiridopsis* were manually recorded and mapped in ArcMap (ESRI, 2011).

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Differences in PD values of *Conophytum* and *Cheiridopsis* was compared in shared QDS and tested using a pairwise t-test. As phylogenetic diversity can be linked to species richness (Rodrigues & Gaston, 2002), a proportional PD value was also calculated by dividing the total PD score by the number of species in a QDS and a significant difference between the genera was also tested using a pairwise t-test.

2.9 AFLP METHODS

2.9.1 EXTRACTION, AMPLIFICATION AND SEQUENCING

Total DNA was extracted from silica-dried leaf material (0.2 mg) for the samples of *C. marginatum* Lavis, *C. maughanii* N.E.Br. and *C. ratum* S.A.Hammer (Chapter 8) using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions for all the samples collected. The AFLP reactions were carried out following the AFLP Plant Mapping Protocol (PN 4303146F) – Applied Biosystems using a modified protocol, with all reactions carried out using half the protocol quantities. Genomic DNA (150ng) was digested using the restriction enzymes *Mse*I and *Eco*RI before ligating to double-stranded *Mse*I and *Eco*RI adapters in a restriction-ligation reaction. A preselective amplification was carried out on the restriction/ligation products using DreamTaq Master Mix (2X; Thermo Scientific) without dilution and 0.5 µl preselective primer pairs. The selective amplifications were also carried out using DreamTaq Master Mix.

The genome size of *Conophytum* (*C. marginatum*) was estimated as 10.69 pg (2C) using flow cytometry (Doležel et al., 2007) which is within the 'regular' genome size range according to the protocol. A primer trial was carried out on the preselective amplifications of a few samples using the four small genome selective primers pairs used by Ellis et al. (2007) for their study on *Argyroderma* N.E.Br. Also nine regular genome size primer combinations, corresponding to the base pairs of the primers selected by Ellis et al. (2007), were selected for trial, with three regular genome size primer pairs chosen (E.ACT/M.CAT, E.ACG/M.CTT, E.ACC/M.CAA) for the complete study. The preselective amplification products from all samples were amplified using these three selective primer pairs and the fragments produced were run on an Applied Biosystems 3730 DNA Analyzer with the ROX GS500 size standard.

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2.9.2 AFLP ANALYSES

The resulting electropherograms were analysed in GeneMapper Version 4.0 (PE Applied Biosystems, Foster City, CA, USA). Fragments were automatically identified within the size range of 50-500 base pairs and a bin width of 1 base pair. The selected peaks were scored manually and exported as a binary presence/absence matrix.

The separate matrices for the three selected primers were combined in Microsoft Excel 2010 to produce a combined matrix for each of the respective species (*C. marginatum, C. maughanii* and *C. ratum*). Molecular analysis of variance (AMOVA) was used to test the hierarchical structure of genetic variance across the populations using GenAlEx for binary diploid data (Peakall & Smouse, 2012). The results of the AMOVA were used to produce a PCoA in GenAlEx to investigate the molecular variance of individuals across the populations.

Genetic diversity in each of the species was investigated using AFLPsurv version 1.0 (Vekemans et al., 2010), estimating Nei's genetic diversity and the number of polymorphic loci. Pairwise Fst values were calculated using 10,000 permutations for the dataset.

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Genetic structure was inferred using STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) for K=1–10. Using an assumed admixture and independent allele frequency model (Pritchard et al., 2000), an analysis of 10 reps for the number of K (population clusters) with 1,000,000 MCMC (Markov chain Monte Carlo) iterations and a burn-in period of 100,000 steps were run. The simulation results were uploaded to STRUCTURE Harvester (Evanno et al., 2005) and ΔK was calculated to estimate the number of genetic clusters for the populations in each of the species, as well as between populations of *C. maughanii* and *C. ratum.* The results for the genetic clusters of the selected K values were mapped for the different populations of each species in ArcMap version 10 (ESRI, 2011).

As drainage has been previously shown to be indicative of population structure (Ellis *et al.,* 2007), drainage lines were identified manually from images in Google Earth 2015 and mapped onto the figures. Elevation transects, also indicating drainage patterns, were drawn across the *Conophytum ratum* populations to produce an elevation transect in Google Earth 2015. The proportion of genetic clusters for individuals in each population were mapped onto these transects.

Chapter 3

Phylogenetic placement and generic re-circumscriptions of the multilocular genera *Arenifera, Octopoma* and *Schlechteranthus* (Aizoaceae: Ruschieae): Evidence from anatomical, morphological and plastid DNA data

3.1 INTRODUCTION

Octopoma N.E.Br. is a genus of woody shrubs with multilocular capsules, comprised of nine species distributed across the Greater Cape Floristic Region (GCFR) (Hartmann, 2001). The genus was last revised by Hartmann (1996), where it was suggested that the circumscription of the genus may be unnatural. The possible polyphyly of the genus was highlighted by Klak *et al*. (2013), however, only two of the nine species were sampled. In tribal phylogeny the type of the genus, *Octopoma octojuge* N.E.Br., was placed sister to *Zeuktophyllum* N.E.Br. and *Smicrostigma* N.E.Br., while *Octopoma subglobosum* (L.Bolus) L.Bolus was recovered as sister to the multilocular genera *Schlechteranthus* Schwantes and *Polymita* N.E.Br. within the *Conophytum*-clade.

Arenifera A.G.J.Herre is another shrubby genus with multilocular capsules which is distributed in the GCFR and the genus includes four species (Hartmann, 1996, 2001). The phylogenetic position and relationship of *Arenifera* remains uncertain, as the genus has not been sampled in any phylogenetic studies. An affinity with *Psammophora* Dinter & Schwantes has been postulated (Bolus, 1927a; Herre 1948; Hartmann, 1996), based on the distinctly sticky leaves. However, *Psammophora*

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does not share multilocular capsules with *Arenifera* (Hartmann, 2001), and therefore the placement of *Arenifera* in the subfamily is unclear.

In this study, the polyphyly of *Octopoma* is assessed by expanding the current phylogenetic analyses to include eight of the nine species in the genus. The phylogenetic placement and relationships of *Arenifera* are also determined, with all of the five species (including a new species) sampled. Generic circumscriptions of the sampled taxa and their relationships to sister genera were assessed. In addition, leaf anatomical and morphological characters of the relevant taxa were investigated to provide additional evidence to further inform generic circumscriptions and relationships in *Arenifera*, *Octopoma* and *Schlechteranthus.*

3.2 MATERIALS AND METHODS

3.2.1 TAXON SAMPLING

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Field visits were conducted to collect and study the species of *Arenifera*, *Octopoma* and *Schlechteranthus in situ*. Twelve (*Arenifera pungens* H.E.K.Hartmann, *Arenifera* sp. nov., *A. spinescens* L.Bolus, *A. stylosa* (L.Bolus) H.E.K.Hartmann, *Octopoma abruptum* N.E.Br., *O. connatum* (L.Bolus) L.Bolus, *O. inclusum* N.E.Br., *O. nanum* (L.Bolus) Klak, *O. tetrasepalum* (L.Bolus) H.E.K.Hartmann, *Schlechteranthus albiflorus* (L.Bolus) Klak, *S. hallii* L.Bolus, *S. maximiliani* Schwantes) of the seventeen species in these three genera were located in the field. The collected material was processed for phylogenetic, anatomical and morphological study following the methods outlined in Section 2.1.1 of Chapter 2.

A total of 125 herbarium specimens were examined, which included all *Arenifera, Octopoma* and *Schlechteranthus* specimens held at BOL, NBG and SAM.

Further investigation of *Octopoma abruptum*, known only from the type locality, showed that it was not distinct from the sympatric *O. rupigenum* (L.Bolus) L.Bolus and is therefore considered conspecific with this species (Powell *et al*., submitted a; Chapter 4).

All five species of *Arenifera* (including an undescribed species) and seven of the eight species of *Octopoma* (*O. quadrisepalum* could not be relocated) were sampled in the phylogeny. In addition, two anomalous species of the morphologically similar genus *Leipoldtia* L.Bolus were included: the large flowered *L. gigantea* Klak, and a potential new species (*L.* sp.) with 8 to 10 rather than the 10 to 16 locules usually found in the genus. In order to assess the phylogenetic relationships of *Octopoma* and *Arenifera*, the Ruschieae dataset of Klak *et al*. (2013) was expanded with 106 new sequences, for nine chloroplast gene regions. This data set (Klak *et al*., 2013) included all genera in the subfamily, excluding *Arenifera*, *Circandra* N.E.Br. and the insufficiently known *Calamophyllum* Schwantes. As in Klak *et al*. (2013), the trees were rooted with *Cleretum papulosum* (L.f.) N.E.Br. and *Conicosia pugioniformis* N.E.Br. These taxa were selected as outgroups as, with Drosanthemeae, they represent the closest related tribes to Ruschieae, Dorotheantheae and Apateasieae (Hartmann, 1996; Klak *et al*., 2003b; Klak *et al*., 2013). Nuclear regions were excluded as they have been shown to be problematic (Klak *et al*., 2013), due to gene duplication or multiple copies. Voucher information and GenBank accession numbers for sequences produced in this study are provided in Appendix A. Voucher information and accession numbers for the remainder of the taxa can be found in Klak *et al*. (2013).

Fresh leaf material of five species of *Arenifera* (*A. pillansii* (L.Bolus) A.G.J.Herre, *A. pungens*, *Arenifera* sp. nov., *A. spinescens*, *A. stylosa*), eight species of

Octopoma (*O. abruptum, O. connatum, O. inclusum, O. nanum, O. octojuge, O. quadrisepalum, O. subglobosum, O. tetrasepalum*) and three species of *Schlechteranthus* (*S. albiflorus, S. hallii, S. maximiliani*) was used (Appendix B). Fresh leaf material was not available for two species of *Octopoma* (*O. octojuge* and *O. quadrisepalum*), and for these samples, leaf material was collected from herbarium (NBG) specimens. These taxa were selected as they are representative of the taxa added to the phylogenies and *Schlechteranthus* was added as it was shown to be sister to *Octopoma* (Klak *et al*., 2013).

3.2.2 PHYLOGENETIC DATA

Total DNA was extracted and amplified following the methods outlined in Section 2.4.1 of Chapter 2 for the nine plastid gene regions *atpB-rbcL, matK, psbJ-petA, rpl16, rps16, trnD-trnT, trnL–F, trnQ-rps16* and *trnS-trnG*.

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The 106 new sequences were automatically aligned into the existing Ruschieae matrices of Klak *et al* (2013) following the methods outlined in Section 2.4.2 of Chapter 2. The combined chloroplast dataset included a total of 8259 characters (including coded indels) and were analysed using Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) following the methods outlined in Section 2.4.2 of Chapter 2.

In the BI analysis, the combined plastid data were partitioned into 10 partitions (nine gene regions and one coded indel partition). Following Klak *et al*. (2013), the most complex model (GTR + G + I) was implemented for the gene regions partitions (Huelsenbeck & Rannala, 2004). The standard coding model in MrBayes was used for the coded indel partition (Ronquist *et al*., 2011). Two simultaneous runs were

completed for 10^7 generations with a sampling frequency of 100. The standard deviation between the split frequencies stabilised below 0.01 and suboptimal trees were discarded as the "burn-in" phase The remaining 75,001 trees were used to construct a 50% majority rule consensus tree with posterior probabilities (PP).

3.2.3 ANATOMICAL DATA

Transverse leaf anatomical sections for the sampled species of *Arenifera*, *Octopoma* and *Schlechteranthus* (Appendix B) were prepared following the methods outlined in Section 2.5 of Chapter 2.

3.3 RESULTS

3.3.1 PHYLOGENETIC ANALYSES

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Following Klak *et al*. (2013), the chloroplast matrices for the nine gene regions were not analysed separately due to low sequence divergence, but rather only in combination. The combined chloroplast matrix for the nine gene regions consisted of 8,236 unambiguously aligned positions and 23 binary scored indels, resulting in 1,247 variable characters and 570 parsimony informative characters. In the MP analysis, 129 trees were retained with a tree length of 2,652 steps (consistency index (CI)=0.78; retention index (RI)=0.68). The topologies recovered in the MP, ML and BI analyses were all consistent with those presented in Klak *et al*. (2013) as well as with one another, albeit with expected differences in resolution and support values. The BI phylogeny was the most resolved with well-supported clades (Fig. 3.1). The phylogeny produced in the ML analysis identified the majority of the clades, however, support for these clades was low, but increased within subclades (Fig. 3.2), while the MP

phylogeny showed moderate support for the main clades, but was otherwise poorly resolved (Fig. 3.3).

Octopoma was recovered as polyphyletic in all three analyses (Figs. 3.1–3.3). The type of the genus (*O. octojuge),* together with the closely related *O. nanum*, were recovered together (Little Karoo *Octopoma*, Fig. 3.1) in a clade with *Zeuktophyllum* and *Smicrostigma* (PP=0.88, Fig. 3.1). In contrast, the remaining species of *Octopoma* (*O. abruptum, O. connatum, O. tetrasepalum, O. inclusum, O. subglobosum*) were placed in the *Conophytum*-clade (PP=0.99, BS=69).

Arenifera was also recovered as polyphyletic (Figs. 3.1–3.3), with the type species, *A. pillansii* (Fig. 3.1, *Arenifera* s.s.), placed in the xeromorphic clade (PP=0.74, *sensu* Klak *et al*., 2013), while the remaining four non-sticky *Arenifera* species (*A. pungens, Arenifera* sp. nov., *A. spinescens, A. stylosa*) were recovered among the Namaqualand species of *Octopoma* in the *Conophytum*-clade (PP=0.99, BS=69).

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Within the *Conophytum*-clade, the non-sticky *Arenifera* and Namaqualand *Octopoma* species formed a subclade *O. subglobosum-A. spinescens* (PP=1, JK=61, BS=92), sister (PP=0.97, BS=57) to the monophyletic *Schlechteranthus* (PP=1, JK=92, BS=99). Together these three groups (non-sticky *Arenifera,* Namaqualand *Octopoma* and *Schlechteranthus*), formed a subclade strongly supported in the Bayesian analysis (PP=0.97, BS=57, Fig. 3.1).

Both of the new accessions of *Leipoldtia* were recovered together with the previously included species of *Leipoldtia* (PP=1.0, BS=67, Fig. 3.1).

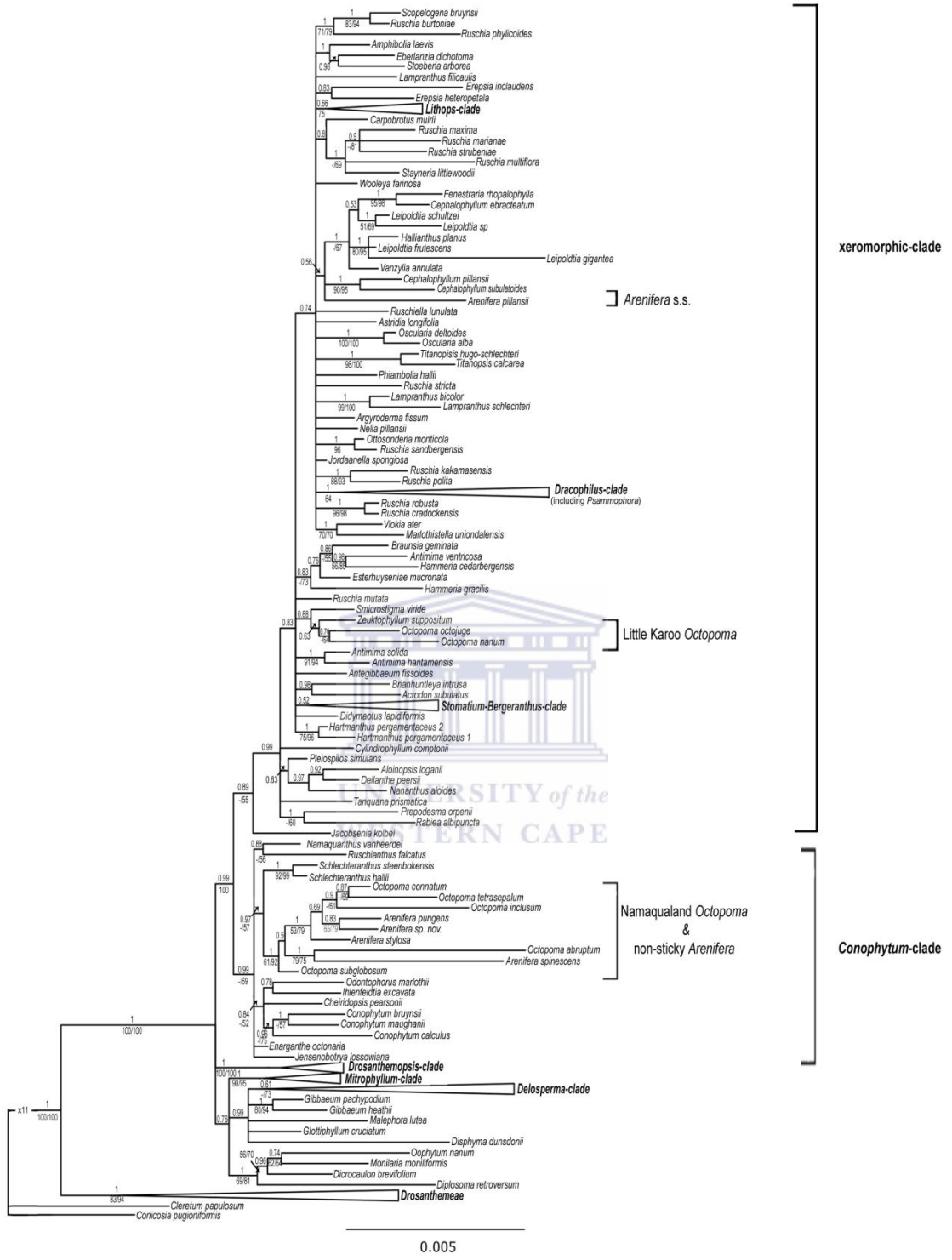

Figure 3.1. Majority Rule Consensus tree from Bayesian analysis of nine chloroplast markers showing phylogenetic relationships in Ruschieae, specifically the placement of new accessions of *Arenifera* and *Octopoma* in the tribe. Posterior probability (PP) values above 0.5 are indicated above the branches. Jackknife support values (JS) and Bootstrap supports (BS) above 50 and above from the maximum parsimony and maximum likelihood analyses are indicated below the branches. Brackets indicate the placement of taxa and clades discussed.

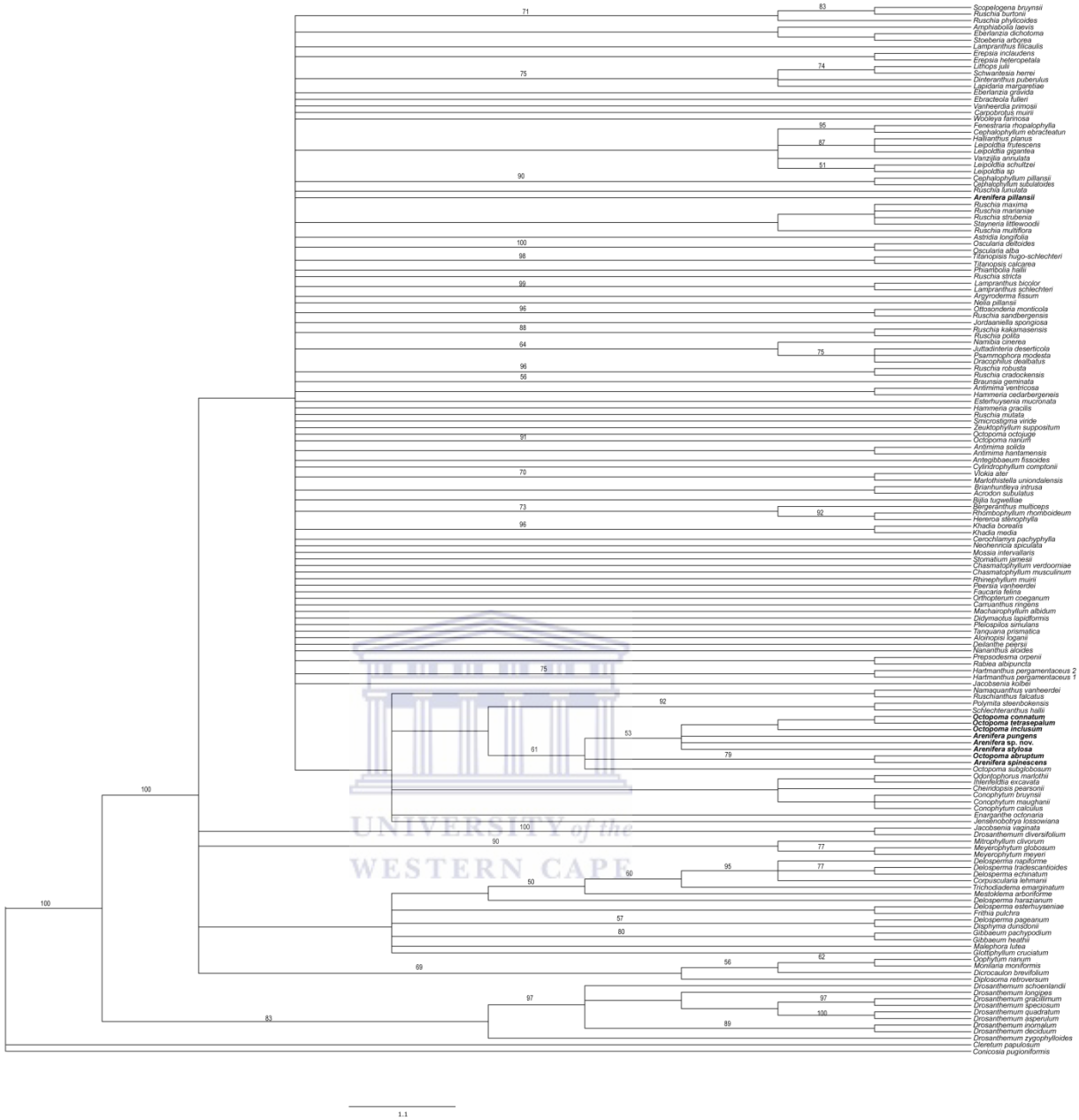

Figure 3.2. Maximum parsimony estimate showing the phylogenetic relationships in Ruschieae, specifically the placement of new accessions of *Arenifera* and *Octopoma* (bold) in the tribe. Jackknife support of 50 and above are indicated above the branches.

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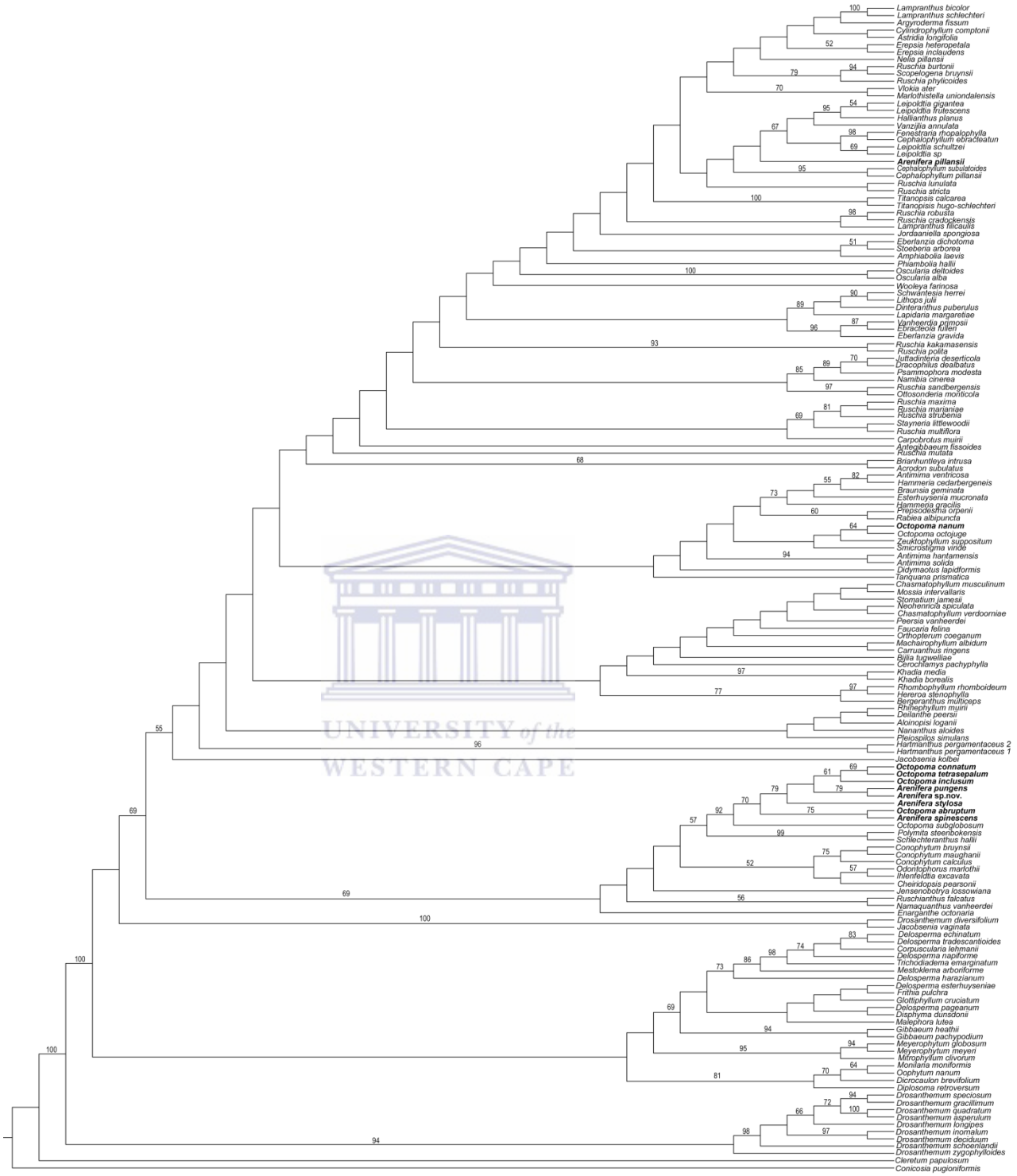

Figure 3.3. Maximum-likelihood estimate showing the phylogenetic relationships in Ruschieae, specifically the placement of new accessions of *Arenifera* and *Octopoma* (bold) in the tribe. Bootstrap support of 50 and above is indicated above the branches.

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3.3.2 LEAF ANATOMY

Leaf anatomical characters are summarised in Table 3.1. The epidermis was conspicuously smooth in *Arenifera pillansii* and three species of *Octopoma* (*O. octojuge, O. nanum, O. quadrisepalum*; Fig. 3.4A, D). The outer wall of the epidermal cells formed blunt papillae concentrated around the stomata in the remaining species studied (*Arenifera pungens, Arenifera* sp. nov.*, A. spinescens, A. stylosa, Octopoma abruptum, O. connatum, O. inclusum, O. subglobosum, O. tetrasepalum, Schlechteranthus albiflorus, S. hallii, S. maximiliani*; Fig. 3.4B, E). In *Arenifera pungens, Arenifera* sp. nov.*, A. spinescens, A. stylosa, Octopoma abruptum, O. connatum, O. inclusum, O. subglobosum* and *O. tetrasepalum* the papillae extended away from the stomata, decreasing in density (Table 3.1). The epidermal cells of *Schlechteranthus* (*S. albiflorus, S. hallii*, *S. maximiliani*) were anticlinally elongated (Fig. 3.4B). The epidermal cells in *Octopoma octojuge, O. nanum, O. quadrisepalum* were slightly paraclinally elongated (Fig 3.4A), while the epidermal cells of the remaining species were isodiametric (Fig. 3.4D, E, F). Prominent bladder cells, that almost obscure the epidermis, were only found in *Arenifera pillansii* (Fig. 3.4D), whereas bladder cells in the other species were inconspicuous. Calcium oxalate crystals were deposited on the outer paraclinal walls in all species, but the crystals were poorly developed in *Arenifera pillansii* (Fig 3.4D). In *Schlechteranthus* (*S. albiflorus, S. hallii, S. maximiliani*), the crystals were also deposited on the anticlinal walls of the epidermal cells (Fig. 3.4B). A hypodermis was only found in three species (*Octopoma octojuge, O. nanum* and *O. quadrisepalum*; Fig. 3.4A).

.

Table 3.1. Table summarising important morphological and anatomical characters for species of *Arenifera*, *Octopoma* and *Schlechteranthus*.

Species	Leaf mucro present	Leaf surface sticky	Number of locules	Closing body closing >½ of locule	Calcium oxalate crystals deposited on outer paraclinal wall of epidermal cell	Hypodermis present	Bladder cells prominent	Outer wall of epidermal cells forming blunt papillae concentrated around the stomata	Papillae decreasing away from the stomata	Epidermal cells anticlinally elongated	Stomata hidden by parastomal cells
Arenifera pillansii	-	+	7-8		Poorly developed	-	+	-	N.A.	-	-
Arenifera pungens	+	-	7-9	-	+	-	-	+	+	-	-
Arenifera sp.nov.	+	-	7-9	-	+	-	-	+	+	-	+
Arenifera spinescens	+	-	7-9	-	+	-	-	+	+	-	-
Arenifera stylosa	+	-	7-9	-	+	-	-	+	+	-	+
Octopoma abruptum	+	-	7-9	-	+	-	-	+	+	-	-
Octopoma connatum	+	-	7-9	-	+	-	-	+	+	-	-
Octopoma inclusum	+	-	7-9	-	+	-	-	+	+	-	+
Octopoma nanum	-	-	8	+	+	+	-	-	N.A.	-	-
Octopoma octojuge	-	-	8	+	+	+	-	-	N.A.	-	-
Octopoma quadrisepalum	-	-	8	+	+	+	-	-	N.A.	-	-
Octopoma subglobosum	+	-	7-9	-	+	-	-	+	+	-	-
Octopoma tetrasepalum	+	-	7-9	-	+	-	-	+	+	-	+
Schlechteranthus albiflorus	+	-	10-12	+	+ on anticlinal wall	-	-	+	-	+	+
Schlechteranthus hallii	+	-	10-12	+	+ on anticlinal wall	-	-	+	-	+	+
Schlechteranthus maximiliani	+	-	10-12	+	+ on anticlinal wall	-	-	+	-	+	+

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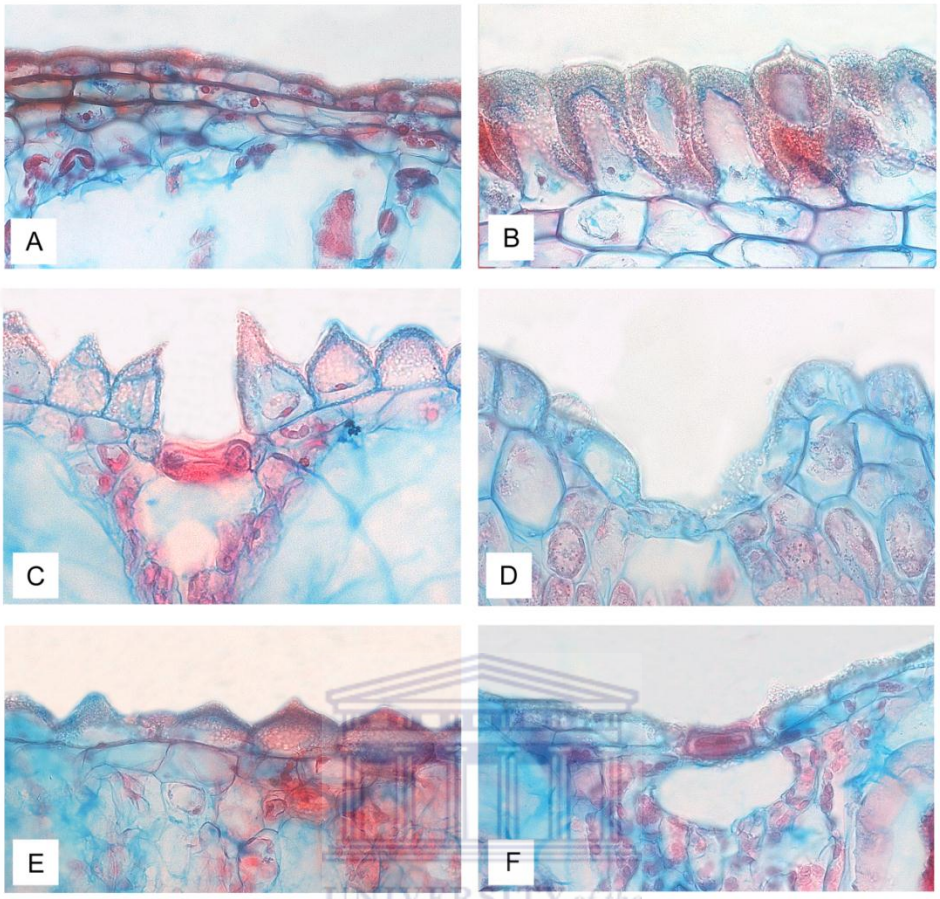

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Figure 3.4. Transverse sections through the leaves of *Arenifera*, *Octopoma* and *Schlechteranthus* species showing characters of taxonomic importance. (A) Hypodermis found in Little Karoo *Octopoma* species, *O. nanum*; (B) prominently thickened and anticlinally elongated oblong epidermal cells in subgenus *Schlechteranthus, S. hallii*; (C) stomata in depression, sunken and hidden by parastomal cell, *O. inclusum*; (D) bladder cells in *A. pillansii*; (E) papillate epidermis in *Schlechteranthus* subgenus *Microphyllus, Arenifera pungens* (=*Schlechteranthus pungens*); (F) stomata in depression, not sunken or hidden, *O. connatum*. Vouchers: (A) *Klak 2426* (BOL); (B) *Powell 71* (NBG); (C) *Powell 35* (NBG); (D) *Bruyns 9136* (BOL); (E) *Powell 28* (NBG); (F) *Powell 10* (NBG). Scale = 50µm

Tanniferous idioblasts were present in a ring below the epidermal cells in all the species, although the density of idioblasts varied. Similarly, raphide bundles were present at different densities across the species.

Two types (Form I and Form II) of stomatal protection, described by Ihlenfeldt & Hartmann (1982), were identified. In both these forms, the leaf surface is sculptured

by the tanniferous idioblasts below the epidermis, with elevations above the tanniferous idioblasts and depressions between the tanniferous idioblasts, as found in all species (Fig. 3.4D, F). In the first form the stomata are distributed in the depressions, but are not protected further by any additional structures. This form was observed in *Arenifera pillansii, A. pungens, A. spinescens, Octopoma abruptum, O. connatum, O. nanum, O. octojuge, O. quadrisepalum, O. subglobosum, O. tetrasepalum* (Fig. 3.4D, F). In the second form the stomata are sunken in the depressions and protected by parastomal cells which overarch the guard cells. This form was observed in *Arenifera* sp. nov., *A. stylosa, Octopoma inclusum, O. tetrasepalum, Schlechteranthus albiflorus, S. hallii, S. maximiliani* (Fig. 3.4C).

3.4 DISCUSSION

3.4.1 POLYPHYLY OF *OCTOPOMA*

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When described by Brown (1930), the genus *Octopoma* was contrasted only to the similar multilocular genus *Leipoldtia* and distinguished by the connate leaves, contiguous expanding keels and absence of valve wings. Subsequently, additional species have been described, based mainly on the 8-locular capsules and connate leaves, however, the absence of valve wings and keel characters are no longer diagnostic for the genus. The artificial nature of the genus was noted by Hartmann (1996) and Klak (2010), who speculated that *Octopoma* may not be monophyletic. Two groups were identified by Hartmann (1996) based on capsule morphology, with the first group comprised of *O. octojuge, O. quadrisepalum* and *O. subglobosum* with valve wings present and large closing bodies. The second group, *O. abruptum, O. connatum, O. inclusum,* and *O. tetrasepalum,* possess small closing bodies and lack

valve wings. Klak (2010), however, hypothesised a slightly different division based on geographical groupings, the first for those in the Little Karoo (*O. octojuge, O. nanum, O. quadrisepalum*) and the second for those in Namaqualand (*O. abruptum, O. connatum, O. inclusum, O. subglobosum, O. tetrasepalum*). Only two species (*O. octojuge* and *O. subglobosum*), both from Hartmann's (1996) first group but representing the two geographical groupings of Klak (2010), were included in the phylogeny of Klak *et al*. (2013). The two species were not recovered together but rather allied with very different clades. In all of our phylogenetic analyses (Figs. 3.1–3.3), *Octopoma* is confirmed as polyphyletic, with species allocated to one of two clades, corresponding to the two main geographical disjunctions as suggested by Klak (2010). The Namaqualand species (*O. connatum, O. inclusum, O. quadrisepalum, O. subglobosum, O. tetrasepalum*) were placed in the *Conophytum*-clade (PP=0.99, BS=69), while the Little Karoo species (*O. octojuge*, the generitype, and *O. nanum*), were placed sister to *Zeuktophyllum* and *Smicrostigma* (PP=0.88) in a subclade in the xeromorphic clade (Fig. 3.1, Klak *et al*., 2013).

The Little Karoo species of *Octopoma* (together with a third species not included in the phylogenies, *O. quadrisepalum*) differ from their congeners in leaf characters, namely the absence of a prominent mucro, smooth epidermal cells and the presence of a hypodermis in the lamina (Fig. 3.4A). The capsules are also distinguished by the larger closing bodies (blocking $^{5}/_{6}$ of the locule). In contrast, the Namaqualand species have leaves with a prominent mucro, epidermal cells that form blunt papillae and a lamina without a hypodermis (Fig. 3.4C, F). The capsules also have smaller closing bodies blocking $^{1}/_{3}$ of the locule.

The Little Karoo *Octopoma* (hereafter referred to as *Octopoma* s.s.) is recovered with *Zeuktophyllum* and *Smicrostigma*, both also Little Karoo endemics, forming a small, albeit weakly supported clade (PP=0.88). These three genera (*Octopoma* s.s., *Smicrostigma, Zeuktophyllum*) share solitary flowers without a hypanthium, multilocular capsules with covering membranes (Hartmann, 2001) and sunken stomata (Ihlenfeldt & Hartmann, 1982), although only slightly sunken in *Zeuktophyllum*. In fact, *Zeuktophyllum calycinum* (L.Bolus) H.E.K.Hartmann was originally described in *Octopoma* (Hartmann, 2001). However, *Zeuktophyllum* differs from *Octopoma* s.s. in capsule morphology, with 10 locules (8 or 9 in *Octopoma* s.s.) and the presence of funicular hairs (absent in *Octopoma* s.s.). The presence of closing bodies distinguishes *Octopoma* s.s. further, as they are absent in both *Zeuktophyllum* and *Smicrostigma* (Hartmann, 2001).

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3.4.2 POLYPHYLY OF *ARENIFERA*

Arenifera was recovered as polyphyletic in all three of the phylogenetic analyses (Figs 3.1–3.3), with the type species, *A. pillansii* (*Arenifera* s.s.; Fig. 3.1) recovered in the xeromorphic clade (PP=0.74) and the remaining, non-sticky *Arenifera* species (*A. pungens, A. spinescens, Arenifera* sp. nov. and *A. stylosa*) placed in the *Conophytum*-clade (PP=0.99, BS=69). *Arenifera pillansii* is readily distinguished from it congeners by the sticky, non-papillate leaf surface, prominent bladder cells (Fig. 3.4D), solitary flowers and 4-lobed calyces. The leaf surfaces of the other species are not sticky, with epidermal cells that form blunt papillae (similar to those found in Namaqualand *Octopoma* and *Schlechteranthus*), inconspicuous bladder cells and flowers in 3-flowered dichasia with 5- or 6- lobed calyces.

The prominently sticky leaves of *Arenifera pillansii* suggest an affinity with the similarly sticky-leaved genus *Psammophora* (Bolus, 1927a; Herre 1948; Hartmann, 1996). However, this relationship was not recovered in any of the phylogenetic trees. As in Klak *et al*. (2013) *Psammophora* is placed within the *Dracophilus*-clade (collapsed in Fig. 3.1), while *Arenifera pillansii* (*Arenifera* s.s.) is recovered within the xeromorphic clade (PP=0.74, Fig 3.1). *Arenifera* s.s. is retained here as a monotypic genus pending further investigation of its allies.

3.4.3 EXPANSION OF *SCHLECHTERANTHUS*

The non-sticky species of *Arenifera* and Namaqualand species of *Octopoma* were recovered as a subclade (hereafter referred to as the *O. subglobosum–A. spinescens* subclade, PP=1.0, JK=61, BS=92) within the *Conophytum*-clade (PP=97, BS=69, Fig. 3.1), with species of both the non-sticky *Arenifera* and Namaqualand *Octopoma* interspersed within one another (Fig. 3.1). Excluding the spinescent inflorescences of *Arenifera* (Fig. 3.5E), these species are indistinguishable and share a number of morphological and anatomical characters. They are generally compact subshrubs (100–400 mm in height; Fig. 3.5A, H), with small leaves, epidermal cells forming blunt papillae, capsules with 6 to 9 locules with closing bodies that block $^{1}/_{3}$ of the locule (Fig. 3.5D). Together species of the *O. subglobosum–A. spinescens* subclade (PP=1, JK=61, BS=92) are placed sister to *Schlechteranthus* (PP=0.97, BS=57).

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Figure 3.5. Morphological characters of *Schlechteranthus* s.l. (A) Compact shrub, *Octopoma inclusum;* (B) flowers with stamens collected in a cone, *Octopoma subglobosum* (P. Burgoyne); (C) the larger capsule with large closing bodies, closing ¾ of locule in *Schlechteranthus* subgenus *Schlechteranthus*, *S. maximiliani*; (D) the smaller capsules of *Schlechteranthus* subgenus *Microphyllus* with smaller closing bodies which close $^{1}/_{3}$ of locule, *Octopoma connatum;* (E) the cymose inflorescence forming spines in *Arenifera pungens*; (F) the densely leafy, larger-leaved *Schlechteranthus hallii;* (G) the papillate leaves with prominent mucro, *Octopoma subglobosum;* (H) the spiny shrub *Arenifera pungens.*

Schlechteranthus, as currently circumscribed (Klak & Bruyns, 2016), is poorly distinguished from the species of the *O. subglobosum–A. spinescens* subclade. *Schlechteranthus* is distinguished by the larger leaves (5–30 x 4.5–9.0 mm long), larger capsules (6–11 x 4–9 mm) with more locules (10 to 12) and larger closing bodies (blocking ¾ of the locule; Fig. 3.5C), whereas species of the *O. subglobosum–A. spinescens* subclade have smaller leaves (3.5–20.0 x 1.5–4.5 mm long), smaller capsules (2–6 x 2–6 mm) with fewer locules (6 to 9) and smaller closing bodies (blocking $^{1}/_{3}$ of locule). The epidermal cells also differ, with those of *Schlechteranthus* anticlinally elongated with calcium oxalate crystals deposited on the anticlinal walls (Fig. 3.4B), while those of the *O. subglobosum–A. spinescens* subclade are isodiametric with few crystal deposits (Fig. 3.4C, E, F). The leaf surface of *Schlechteranthus* species appears to be only slightly papillate, with the outer wall of the epidermal cells forming blunt papillae that are only concentrated around the stomata. *Schlechteranthus holgatensis* Klak is an exception, with conspicuously raised papillae throughout the leaf surface (Klak & Bruyns, 2016). The leaf surface of species in the *O. subglobosum–A. spinescens* subclade appears more papillate, with papillae present extending away from the stomata, but decreasing in density. *Schlechteranthus* species are also generally larger shrubs (up to 450 mm in height; Fig. 3.5F), while species of the *O. subglobosum–A. spinescens* subclade are generally smaller shrubs (100–400 mm in height) (Fig. 3.5A, H).

The species of *Schlechteranthus* and the *O. subglobosum–A. spinescens* subclade do, however, share epidermal cells that form blunt papillae, which are also shared with a number of other genera in the *Conophytum*-clade (*Cheiridopsis* N.E.Br., *Conophytum* N.E.Br., *Ihlenfeldtia* H.E.K.Hartmann and *Odontophorus* N.E.Br.). The

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species are distinguished from other genera in the *Conophytum*-clade by a combination of features. They are woody shrubs with prominently mucronate leaves (inconspicuous in *Schlechteranthus maximiliani* and *Arenifera* sp. nov.) and epidermal cells forming blunt to conspicuously raised papillae (Fig. 3.4G), white to magenta flowers with few to many filamentous staminodes surrounding the stamens in a loose cone, with stamens arranged in a cone (Fig. 3.5B), and multilocular capsules (7 to 12 locules). Upon assessment of generic circumscription in the *Schlechteranthus*-clade, it is apparent there are two species groups; one comprised of species of the *O. subglobosum–A. spinescens* subclade, and the other comprised of species of *Schlechteranthus* s.s. However, the distinguishing characters for these two groups are largely size-dependant (apart from locule number), i.e. leaf size, capsule size, closing body size and general shrub size. Therefore, based on the phylogenetic results, morphological and anatomical examination of the species, the circumscription of *Schlechteranthus* s.s. is here expanded to include the species of the *O. subglobosum–A. spinescens* subclade. As such, *Schlechteranthus* s.l. is defined as a genus of woody shrubs, with prominently (rarely inconspicuous) mucronate leaves and epidermal cells forming blunt to conspicuously raised papillae, white to magenta flowers with few to many filamentous staminodes surrounding the stamens in a loose cone, with stamens arranged in a cone and multilocular capsules (6–12 locules). The two main subgroups within the expanded genus are accommodated as subgenera. Subgenus *Schlechteranthus* includes the species with large capsules (6–11x 4–9 mm) with 10–12 locules, large closing bodies (blocking > ½ of locule), and epidermal cells anticlinally elongated with calcium oxalate crystals deposited on the anticlinal walls, and subgenus *Microphyllus* R.F.Powell includes the species with small capsules (2–6 x 2–6 mm)

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with 7–9 locules, small closing bodies (blocking < ½ of locule) and isodiametric epidermal cells, without crystals on the anticlinal walls.

Species of *Schlechteranthus* s.l. are further distinguished from species in the *Conophytum*-clade in their woody, shrubby habit. Although *Enarganthe* N.E.Br. and *Namaquanthus* L.Bolus share this shrubby habit, *Schlechteranthus* s.l. is distinguished from *Namaquanthus* by the presence of closing bodies (absent in *Namaquanthus*) and from *Enarganthe* by the trigonous leaves that are fused at the base (leaves cylindrical and not fused at the base in *Enarganthe*) (Hartmann, 2001). The large multilocular capsules and leaf mucro are also shared with some genera in the *Conophytum*-clade (*Cheiridopsis, Conophytum, Ihlenfeldtia* and *Odontophorus*), however, *Schlechteranthus* s.l. is distinguished by the non-sheathing leaves (persistent partly or fully sheathing in *Cheiridopsis, Conophytum, Ihlenfeldtia* and *Odontophorus*) and the woody non-caespitose shrubby habit with stems that are never reduced (caespitose shrublets with highly reduced stems in *Cheiridopsis, Conophytum, Ihlenfeldtia* and *Odontophorus*) (Hartmann, 2001).

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3.5 KEY TO THE MULTILOCULAR (≥ 6) TAXA OF RUSCHIEAE WITH AN ERECT SHRUBBY HABIT, XEROMORPHIC LEAF EPIDERMIS AND CAPSULES WITH CLOSING BODIES:

1. Capsules 6–8(–9)-locular.....2
1. Capsules (9–)10–18-locular.....9
2. Leaves large and chunky, 25-70 (-120) mm long, 5-30 mm broad and thick 3
2. Leaves small and slender, 3–25 mm long, 4–6 mm broad and thick 6
3. Flowers in annually enlarged, persistent inflorescences ..... ***Ottosonderia***
3. Inflorescence not persistent, but formed and ripening annually, new ones formed every year 4

4. Base of capsules shallow, bowl-shaped; closing bodies stalked and large, blocking the exit of the locules..... ***Antimima***
4. Base of capsules deep and funnel-shaped; closing bodies hook-shaped, small, not blocking exit of locules 5
5. Calyx 6-lobed; capsules mostly 6-locular, valves open and close repeatedly..... ***Astridia***
5. Calyx 4-lobed; capsules (5-) 6-8-locular (within a specimen), valves opening fully, but not closing completely again..... ***Stayneria***
6. Leaf surface sticky, with sand adhering to the surface with age; calyx 4-lobed..... ..... ***Arenifera* s.s.**
6. Leaf surface never sticky, free of sand; calyx 4–6-lobed.....7
7. Base of capsules shallow and bowl-shaped; capsules 6-locular..... ***Antimima***
7. Base of capsules deep and funnel-shaped; capsules 6–8(–9)-locular.....8
8. Leaves with epidermis smooth; hypodermis present; closing bodies large, blocking the exit of locules; old peduncles never forming spines ..... ***Octopoma* s.s.**
8. Leaves with epidermal cells forming blunt to conspicuously raised papillae; hypodermis absent; closing bodies small, not blocking the exit of locules; sometimes old peduncles forming spines ..... ***Schlechteranthus* subg. *Microphyllus***
9. Densely branched shrubs; leaves with a prominent mucro, rarely inconspicuous .... ***Schlechteranthus* subg. *Schlechteranthus***
9. Loosely branched shrubs; leaves without a mucro.....10
10. Leaves heterophyllous; branches rarely erect, more often spreading to climbing .... ***Vanzijlia***
10. Leaves homophyllous; branches erect or ascending 11
11. Flowers pink (rarely white or yellow); base of capsule deep and funnel-shaped..... ..... ***Leipoldtia***
11. Flowers yellow; base of capsule shallow and bowl-shaped ..... ***Cephalophyllum***

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3.6 TAXONOMIC TREATMENT

3.6.1 ***Arenifera*** A.G.J.Herre, Sukkulentenkunde 2: 35. 1948. *Type*: *A. pillansii* (L.Bolus) A.G.J.Herre. (=*Psammophora pillansii* L. Bolus).

Arenifera is here reduced to a monotypic genus restricted to the Richtersveld and characterised by its shrubby habit, sticky leaves without an apical mucro, prominent bladder cells, solitary flowers with 4-lobed calyces, with old peduncles forming blunt spines and multilocular capsules with covering membranes and conspicuous rodlet-shaped closing bodies.

Species: *Arenifera pillansii* (L.Bolus) A.G.J.Herre.

3.6.2 ***Octopoma*** N.E.Br., Gard. Chron. Ser. III. 87: 126, in clavi. 1930. *Type: O. octojuge* N.E.Br.

Octopoma is here reduced to include only three species centred in the Little Karoo. The genus is distinguished by the shrubby habit, leaves with a smooth epidermis, absence of a prominent mucro with a hypodermis, and capsules with 6–8 locules and large closing bodies (blocking $^{5}/_{6}$ of locule).

Species: *Octopoma octojuge* N.E.Br., *O. nanum* (L.Bolus) Klak, *O. quadrisepalum* (L.Bolus) H.E.K.Hartmann.

3.6.3 ***Schlechteranthus*** Schwantes, Monatsschr. Deutsch. Kakteen-Ges. 1: 16. 1929, **emend. nov.** R.F.Powell. *Type*: *S. maximiliani* Schwantes.

Polymita N.E.Br., Gard. Chron., Ser. III. 87: 72, in clavi. 1930. *Type*: *P. pearsonii* N.E.Br. (= *P. albiflora* (L.Bolus) L.Bolus).

The circumscription of *Schlechteranthus* is expanded here to include five species of *Octopoma* and four species of *Arenifera*. As such, it now comprises of 14 species, with a distribution centred in Namaqualand, extending from Clanwilliam and the Tanqua Karoo, northwards into the Richtersveld. The genus is distinguished by the shrubby habit, leaves with a prominent mucro (rarely inconspicuous) with epidermal cells forming blunt to conspicuously raised papillae, white to magenta flowers with few to many filamentous staminodes surrounding the stamens in a loose cone, with anthers arranged in a cone and 7- to 12-locular capsules. Two subgenera are recognised to accommodate the differences in epidermal cell shape, cuticle thickness, locule number and variation in shrub, leaf and capsule size.

3.6.3.1 ***Schlechteranthus*** subg. ***Schlechteranthus***. *Type: S. maximiliani*.

The subgenus is distinguished by the large leaves (5–30 x 3.5–9.0 mm), large capsules (6–11 x 4–9 mm) with 10 to 12 locules, and larger closing bodies that block ¾ of the locule. It is further distinguished by the anticlinally elongated epidermal cells with calcium oxalate crystal deposits on the anticlinal walls. The outer walls of the epidermal cells form blunt to conspicuously raised papillae which are only concentrated around the stomata. This results in the leaf appearing only slightly papillate (with the exception of *S. holgatensis*). Six species are accommodated in the subgenus (Klak & Bruyns, 2016) distributed from the Kamiesberg north into the Richtersveld.

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Species: *Schlechteranthus albiflorus* (L.Bolus) Klak, *S. diutinus* (L.Bolus) Klak, *S. maximiliani* Schwantes, *S. hallii* L.Bolus, *S. holgatensis* and *S. steenbokensis* (H.E.K.Hartmann) Klak

3.6.3.2 ***Schlechteranthus*** subg. ***Microphyllus*** R.F.Powell, **subg. nov.** *Type*: *S. pungens* (H.E.K.Hartmann) R.F.Powell (=*Arenifera pungens* H.E.K.Hartmann)

The subgenus can be distinguished by the smaller leaves (3.5–5.0 x 4–6 mm), smaller capsules (2–6 x 2–6 mm) with 7 to 9 locules and small closing bodies that block $^{1}/_{3}$ of the locule. The epidermal cells also differ in that they are isodiametric with no crystals deposited on the anticlinal wall. The outer walls of the epidermal cells form blunt papillae which are concentrated around the stomata but extend away from the stomata, decreasing in density. This results in the leaf appearing more papillate than in subg. *Schlechteranthus.* Eight species are accommodated within the subgenus occurring from Calvinia northwards into the Richtersveld.

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Schlechteranthus abruptus (A.Berger) R.F.Powell**, comb. nov.** *Mesembryanthemum abruptum* A.Berger in Bot. Jahrb. Syst. 57: 638. 1922. *Octopoma abruptum* (A.Berger) N.E.Br., Gard. Chron. 3: 126. 1930. *Type:* South Africa, Western Cape Province: Brandewynrivier, between Clanwilliam and Calvinia, *Schlechter 10828* (isotype: BOL!).

Octopoma rupigenum (L.Bolus) L.Bolus in J. S. African Bot. 33: 306. 1967, **syn. nov**. *Type*: South Africa, Western Cape Province: near Pakhuis, Clanwilliam Div., common on rocks between Pakhuis and Buishoekfontein, *L.Bolus sub NBG 1504/33* (holotype: BOL!).

Schlechteranthus connatus (L.Bolus) R.F.Powell, **comb. nov.** *Ruschia connata* L.Bolus, Notes Mesembryanthemum 1: 139. 1928. *Octopoma connatum* (L.Bolus) L.Bolus in J. S. African Bot. 29: 49. 1963. *Type*: South Africa, Northern Cape Province: between Doornpoort and Brakfontein, *Pillans 5794* (holotype: BOL!).

Schlechteranthus inclusus (L.Bolus) R.F.Powell, **comb. nov.** *Mesembryanthemum inclusum* L.Bolus in Ann. Bolus Herb. 4: 40. 1926. *Octopoma inclusum* (L.Bolus) N.E.Br., Gard. Chron. 3: 126. 1930. *Type*: South Africa, Northern Cape Province: Slopes overlooking the sea, south of Hondeklip Bay, Namaqualand, *Pillans 17758* (holotype: BOL!).

Schlechteranthus pungens (H.E.K.Hartmann) R.F.Powell, **comb. nov.** *Arenifera pungens* H.E.K.Hartmann in Bradleya 14:37. 1996. *Type*: South Africa, Northern Cape Province: Namaqualand, *Hartmann, Dehn, Gölling, Rust & Stüber 25739* (holotype: HBG–image!).

Schlechteranthus spinescens (L.Bolus) R.F.Powell, **comb. nov.** *Ruschia spinescens* L.Bolus, Notes Mesembryanthemum 2: 175. 1930. *Arenifera spinescens* (L.Bolus) H.E.K.Hartmann in Bradleya 14:38. 1996. *Type*: South Africa, Western Cape Province: Whitehill near Matjiesfontein, Laingsburg, *Compton 19081* (holotype: BOL!).

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Schlechteranthus stylosus (L.Bolus) R.F.Powell, **comb. nov.** *Ruschia stylosa* L.Bolus, Notes Mesembryanthemum 1: 144. 1928. *Arenifera stylosa* (L.Bolus) H.E.K.Hartmann in Bradleya 14: 38. 1996. *Type*: South Africa, Northern Cape Province: hills N.E. of Arris Drift, *Pillans 5742* (holotype: BOL!).

Schlechteranthus subglobosus (L.Bolus) R.F.Powell, **comb. nov.** *Ruschia subglobosa* L.Bolus, Notes Mesembryanthemum 1: 140. 1928. *Octopoma subglobosa* (L.Bolus) L.Bolus in J. S. African Bot. 29: 49. 1963. *Type*: South Africa, Northern Cape Province: hills on north side of O'kiep, Little Namaqualand, *Pillans 5844* (holotype: BOL!).

Schlechteranthus tetrasepalus (L.Bolus) R.F.Powell, **comb. nov.** *Ruschia tetrasepala* L.Bolus, Notes Mesembryanthemum 2: 373. 1932. *Octopoma tetrasepalum* (L.Bolus) H.E.K.Hartmann, Bradleya 16: 74. 1998. *Type*: South Africa, Western Cape Province: between the town and [the] Sout River, *Luckhoff sub BOL 20203* (holotype: BOL!).

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Chapter 4

A Taxonomic Revision of *Schlechteranthus* subgenus *Microphyllus* (Ruschieae; Aizoaceae)

4.1 INTRODUCTION

Schlechteranthus Schwantes is a genus of succulent shrubs included in the diverse and speciose tribe Ruschieae, with species endemic to the arid parts of the Greater Cape Floristic Region (Jürgens, 1991; Hartmann, 1996, 1998, 2001; Manning & Goldblatt, 2012; Snijman, 2013; Klak & Bruyns, 2016). The genus is placed in the *Conophytum*-clade (Klak *et al.*, 2013; Powell *et al.*, 2016; Chapter 3) and is distinguished from other genera in the clade by its shrubby habit, leaves with a papillate epidermis and a prominent mucro, white to magenta cone-type flowers, with stamens arranged in a cone and 7- to 12-locular capsules with pointed expanding keels. Generic circumscription of the genus was recently assessed resulting in the expansion of the genus and erection of subgenus *Microphyllus* R.F.Powell (Klak & Bruyns. 2016; Powell *et al.*, 2016; Chapter 3).

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The phylogenetic analysis of Klak *et al.* (2013) placed *Schlechteranthus* sister to the previously recognised genus *Polymita* N.E.Br. An assessment of generic circumscription of these genera, led to the expansion of *Schlechteranthus* to include *Polymita*, with a taxonomic revision for these species presented in Klak & Bruyns (2016). *Schlechteranthus* and *Polymita* were placed sister to *Octopoma subglobosum* (L.Bolus) L.Bolus in the tribal phylogeny produced by Klak *et al.* (2013), but as only two species of *Octopoma* N.E.Br. were sampled, generic relationships between these species were unclear.

Powell *et al.* (2016; Chapter 3) expanded the phylogenetic sampling of *Octopoma* to include seven of the eight species, as well as the genus *Arenifera* A.G.J.Herre, which had not been sampled in any previous phylogenetic analyses. The results indicated that both

Octopoma and *Arenifera* were polyphyletic, with five *Octopoma* and four *Arenifera* species placed sister to *Schlechteranthus* in the *Conophytum*-clade, while the type species of the genera were placed in the xeromorphic clade (Klak *et al.*, 2013; Powell *et al.*, 2016; Chapter 3). The assessment of generic circumscriptions based on phylogenetic, morphological and anatomical data, of these species, specifically in relation to their sister genus *Schlechteranthus*, revealed two groups, which were recognised as subgenera (Powell *et al.*, 2016; Chapter 3). Subgenus *Schlechteranthus* includes species of *Schlechteranthus* and the previously recognised genus *Polymita*, while subgenus *Microphyllus* includes the species previously included in *Arenifera* and *Octopoma*. The subgenera are distinguished from one another by differences in epidermal cell shape, cuticle thickness, locule number and variation in shrub, leaf and capsule size (Powell *et al*., 2016; Chapter 3).

The species of subgenus *Microphyllus* were previously included in *Eberlanzia* Schwantes, *Mesembryanthemum* L. and *Ruschia* Schwantes (Berger, 1922; Bolus, 1926, 1928, 1930, 1932; Brown, 1930), but were subsequently moved into *Arenifera* based on the shared inflorescence structure, capsule shape and presence of spines and into *Octopoma* based on the shared 8-locular capsules (Hartmann, 1996; Hartmann, 1998). The need for a taxonomic revision of these species was recognised even prior to the erection of the subgenus, with *Arenifera* and *Octopoma* identified as priority genera for taxonomic revision by Von Staden *et al.* (2013).

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This study presents the first taxonomic revision for *Schlechteranthus* subgenus *Microphyllus* and includes the descriptions of nine species, including one new species, as well as a key to the species, notes on their ecology and comprehensive distribution maps.

4.2 MATERIALS AND METHODS

4.2.1 EXAMINATION OF MORPHOLOGICAL CHARACTERS

The complete collections of *Schlechteranthus* (160 specimens) housed at BOL, NBG (including SAM) and PRE were examined. In addition, all of the species of subgenus *Microphyllus* were studied *in situ.* During field visits, material was collected and processed following Section 2.1.1 of Chapter 2. This material was used for the examination of leaf, floral and capsule characters to compare and identify diagnostic morphological characters across the subgenus.

4.2.2 PHYLOGENETIC ANALYSES

A molecular and morphological cladistic analysis for subgenus *Microphyllus* was conducted following the methods outlined in Section 2.4.2 of Chapter 2, using the molecular phylogenetic data produced in Powell *et al.* (2016; Chapter 3) and eight coded morphological characters for the morphological analyses. Both the molecular and morphological data was analysed for the species in the subgenus, as well as for two selected outgroup taxa from subgenus *Schlechteranthus*. The two outgroup taxa (*S. hallii* L.Bolus and *S. steenbokensis* (H.E.K.Hartmann) Klak)), were selected as these species of subgenus *Schlechteranthus* were included in the molecular phylogenetic analysis of Powell *et al.* (2016; Chapter 3) and were recovered sister to subgenus *Microphyllus*.

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The molecular and morphological dataset was analysed separately using Bayesian inference in MrBayes version 3.2.3 (Ronquist & Huelsenbeck, 2003). The molecular data was analysed following Powell *et al.* (2016) and the methods outlined in Section 2.4.2 of Chapter 2, with the combined plastid dataset partitioned into 10 partitions. The partitions were all unlinked and the GTR+G+I model was implemented (Huelsenbeck & Rannala, 2004), with two simultaneous runs completed for 10^7 generations. For the morphological dataset, the

standard model (Ronquist *et al.*, 2011) was implemented and two simultaneous runs were completed for 15^5 generations. In both analyses, the split frequencies stabilised below 0.01 and suboptimal trees were discarded as the "burn-in" phase. A 50% majority-rule consensus tree was constructed from the remaining trees in the separate analyses and the posterior probability (PP) values are reported on the trees, respectively.

The maximum parsimony algorithm was implemented for both the molecular and morphological data separately as outlined in Section 2.4.2 of Chapter 2 and the strict consensus tree with bootstrap support values are presented for the molecular and morphological data.

The coded morphological characters were reconstructed onto the Bayesian inference consensus trees of both the molecular and morphological analysis following the methods outlined in Section 2.3 of Chapter 2.

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4.2.3 DISTRIBUTION AND SPECIES RICHNESS MAPS

Data on the distribution of the species are presented as maps produced following the methods outlined in Section 2.2.1 of Chapter 2. Species richness maps, indicating the number of species per Quarter Degree Square (QDS) were also produced for the genus and subgenera following the methods of Section 2.2.2 in Chapter 2.

4.3 RESULTS AND DISCUSSION

4.3.1 HABIT

Schlechteranthus subgenus *Microphyllus* is comprised of woody shrubs or shrublets, some of which are markedly compact, including *S. connatus* (L.Bolus) R.F.Powell, *S. inclusus* (L.Bolus) R.F.Powell (Fig. 4.1H), *S. parvus* and *S. tetrasepalus* (L.Bolus) R.F.Powell, only reaching 150 mm in height, while the larger shrubs, *S. pungens* (H.E.K.Hartmann)

R.F.Powell and *S. stylosus* (L.Bolus) R.F.Powell, grow up to 400 mm tall. The species share an upright to spreading habit, although *S. connatus* is often decumbent.

4.3.2 BRANCHES AND LEAF ARRANGEMENT

Branches are up to 9(–13) mm in diameter, with the widest branches found in the very compact *S. tetrasepalus*. The upper branches are usually brown, but in *S. pungens* they are distinctively white, while in *S. spinescens* (L.Bolus) R.F.Powell they are often whitish-grey.

The leaves are usually crowded towards the tips of the branches in subgenus *Microphyllus*, however, in *S. subglobosus* (L.Bolus) R.F.Powell the opposite leaf pairs are evenly spaced along the branches with visible internodes (Fig. 4.1E). In *S. inclusus* the leaves are decussate and tightly packed along the branch and due to this leaf arrangement, the internodes are not visible (Fig. 4.1G). In addition, the old blackened leaves remain on the branch in *S. inclusus* (Fig. 4.1G), in contrast to all the other species in the subgenus, where old leaves fall off the branch.

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4.3.3 LEAVES

The leaves in species of subgenus *Microphyllus* are triquetrous to fatly trigonous and mucronate (Fig. 4.2), although in *S. parvus* the mucro is not prominent. Leaves display various shapes in outline, including globose, ovoid, oblong to lanceolate (Fig. 4.2). Leaf size ranges include 1.5–20.0 x 1.5–5.0 mm and the leaf pair bases are fused from $^{1}/_{5}$ to ¾ of the leaf length (Fig. 4.2). Leaf colour is usually green, ranging from bright to deep green and sometimes yellow-green. The leaves are slightly papillate, due to the epidermal cells forming blunt papillae, defined as low protuberances in the centre of the epidermal cell (Opel, 2005a). These blunt papillae result in a slightly velvety appearance of the leaf, but never appear hairy to the naked eye. In *S. abruptus* (A.Berger) R.F.Powell, the protuberances are slightly

Figure 4.1. (A) Internal structure of capsules in *Schlechteranthus* subgenus *Microphyllus*, with arrows indicating closing bodies (CB) and covering membranes (CM); (B) spinescent inflorescence of *Schlechteranthus pungens* held above terminal leaf pair; (C) capsules of *S. connatus* on long central axis indicated by the arrow; (D) magenta flower of *S. stylosus* with the stamens arranged in a cone in the centre of the flower, as is diagnostic of the genus *Schlechteranthus*; (E) evenly spaced leaves along the stem in *S. subglobosus*; (F) cup-shaped bracteole in *S. inclusus*, placed at the base of the capsule; (G) old leaves that remain on stem in *S. inclusus*; (H) compact habit of *S. inlusus* found growing on a gneiss rocky outcrop along a river course. Photographs: R.F.Powell.

extended, creating a trichome-like structure, which results in a prominently velvety leaf. The leaf surface of *S. subglobosus* is also slightly different from the rest of the species due to the stout, dome-shaped epidermal cells, which result in a rough leaf surface.

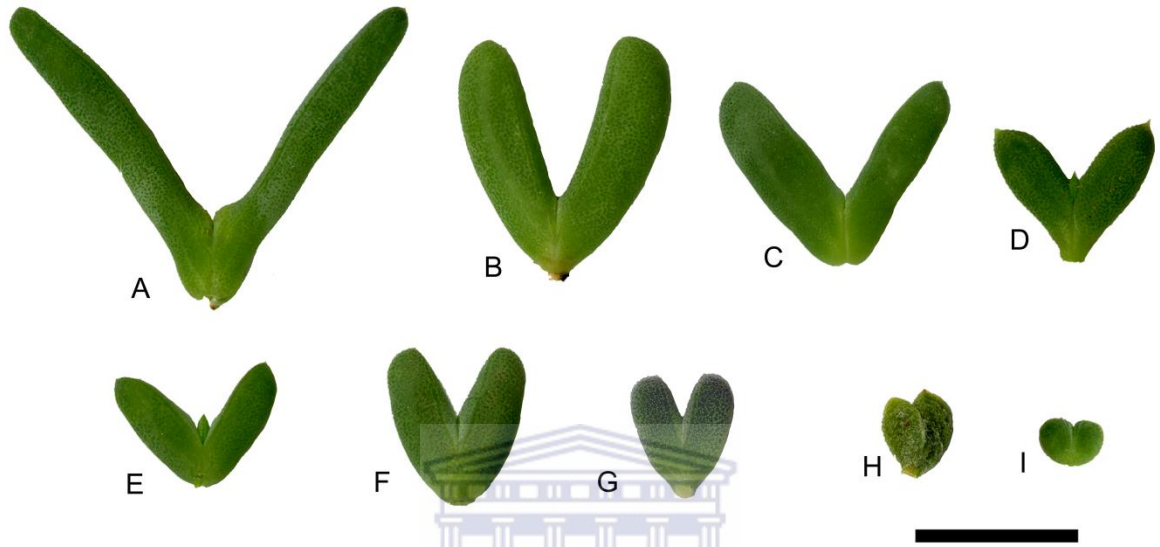

Figure 4.2. Leaves of *Schlechteranthus* subgenus *Microphyllus*, illustrating the variation in leaf size and degree of fusion of the leaf pairs. (A) *Schlechteranthus connatus*; (B) *S. spinescens*; (C) *S. abruptus*; (D) *S. subglobosus*; (E) *S. pungens*; (F) *S. inclusus*; (G) *S. tetrasepalus*; (H) *S. stylosus*; (I) *S. parvus*. Vouchers: (A) *Powell 10* (NBG); (B) *Klak 2424* (BOL); (C) *Powell 5* (NBG); (D) *Powell 33* (NBG); (E) *Powell 28* (NBG); (F) *Powell 35* (NBG); (G) *Klak 2411* (BOL); (H) *Powell 75* (NBG); (I) *Powell 41*(NBG). Scale = 1 cm.

4.3.4 INFLORESCENCE STRUCTURE

Flowers in species of subgenus *Microphyllus* are either solitary or arranged in cymose inflorescences. The non-spiny species, i.e. *S. abruptus*, *S. connatus*, *S. inclusus*, *S. subglobosus* and *S. tetrasepalus*, all share solitary flowers (Fig. 4.1C, F), with the flowers on a central primary axis, as found in species of subgenus *Schlechteranthus* (Table 4.1; Fig. 4.3B; Appendix D1).

The central primary axis is sometimes prominently extended, ranging from 1 ½ times to 10 times longer than the capsule length (as found in *S. connatus* (Fig. 4.1C), *S. subglobosus* and *S. tetrasepalus*). This character was recovered as a synapomorphy in the

morphological phylogeny for these species (Table 4.1; Fig. 4.3B; Appendix D1). In the remaining species, as well as in species of subgenus *Schlechteranthus,* the primary axis is proportionally equal to the length of the capsule (Table 4.1; Fig. 4.3B; Appendix D1).

The length of the primary axis also affects the position of the bracteole, with the bracteole positioned more than $^{1}/_{2}$ way down the primary axis in *S. connatus* (Fig. 4.1C) and *S. tetrasepalus,* while in *S. abruptus* and *S. inclusus* the bracteoles are positioned at the base of the capsule (less than ¼ of the way down the primary axis (Fig. 4.1G). The bracteoles of *S. subglobosus* are positioned the lowest, more than $^{2}/_{3}$ down the primary axis. Furthermore, in *S. inclusus,* the bracteoles are prominently cup-shaped (Fig. 4.1F). The bracteole is absent in *S. parvus, S. pungens, S. spinescens* and *S. stylosus,* grouping these species in the morphological phylogeny (Table 4.1; Fig. 4.3B; Appendix D1).

Schlechteranthus parvus, S. pungens, S. spinescens and *S. stylosus* share a cymose inflorescence with spines. Both these characters, together with the loss of the bracteole, were recovered as synapomorphies in the morphological phylogeny for the species (Table 4.1; Fig. 4.3B; Appendix D1). The arrangement of the spines is directly linked to the inflorescence structure, as the spines are derived from the old peduncles and in some species (*S. parvus, S. stylosus, S. pungens*), additional spines are derived from aborted axillary buds. In *S. spinescens,* the spines are arranged in a simple cyme on the primary axis, with blunt spines derived solely from old peduncles where the capsules have fallen off (Table 4.1; Fig. 4.4A; Appendix D1). Although all the species share this spine type, this is the only spine formation found in *S. spinescens.* In *S. parvus, S. pungens* and *S. stylosus* (Table 4.1; Figs. 4.3, 4.4B, C; Appendix D1), there are additional sharp spines derived from aborted axillary buds, which are arranged in compound cymes (Figs. 4.1B, 4.4B, C). In *S. parvus* and *S. stylosus,* the spines are arranged in a simple cyme on the secondary axes (Fig. 4.4B), while in *S. pungens,*

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the spines are arranged in a compound cyme on the secondary axes and in a simple cyme on the tertiary axes (Fig. 4.4C).

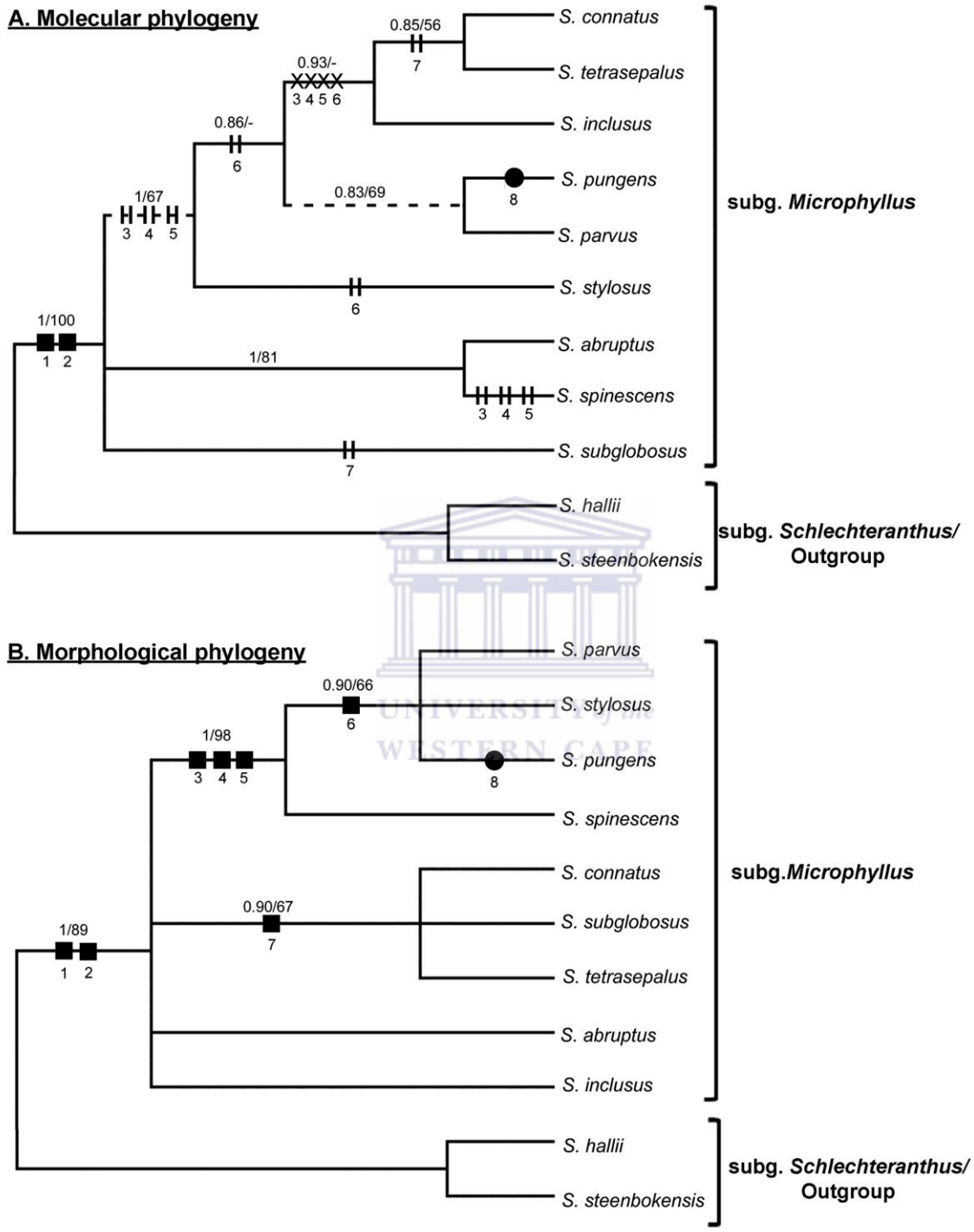

Figure 4.3. (A) Consensus tree from the Bayesian inference analysis from genetic plastid data (following Powell *et al.*, 2016), tree length=156, Consistency index (CI)=0.94, Retention index(RI)=0.76. (B) Consensus tree from the Bayesian inference analysis from coded morphological characters (Appendix D1) for species of *Schlechteranthus* subgenus *Microphyllus* and two species of *Schlechteranthus* subgenus *Schlechteranthus* (outgroup taxa); tree length = 8, Consistency index (CI)=1.00, Retention index (RI)=1.00. Posterior probabilities (PP) and bootstrap (BS) values are indicated above the branches. ■ = synapomorphy without reversal, ● = autapomorphy, || = convergence, X = loss. Character identification numbers are indicated below the symbols.

Table 4.1. Character matrix used for the morphological cladistic analyses for each of the species in *Schlechteranthus*. Description of characters and character states are included in Appendix D1.

Species	1	2	3	4	5	6	7	8
S. abruptus	1	1	0	0	0	0	0	0
S. connatus	1	1	0	0	0	0	1	0
S. hallii	0	0	0	0	0	0	0	0
S. inclusus	1	1	0	0	0	0	0	0
S. parvus	1	1	1	1	1	1	0	0
S. pungens	1	1	1	1	1	1	0	1
S. spinescens	1	1	1	1	1	0	0	0
S. steenbokensis	0	0	0	0	0	0	0	0
S. stylosus	1	1	1	1	1	1	0	0
S. subglobosus	1	1	0	0	0	0	1	0
S. tetrasepalus	1	1	0	0	0	0	1	0

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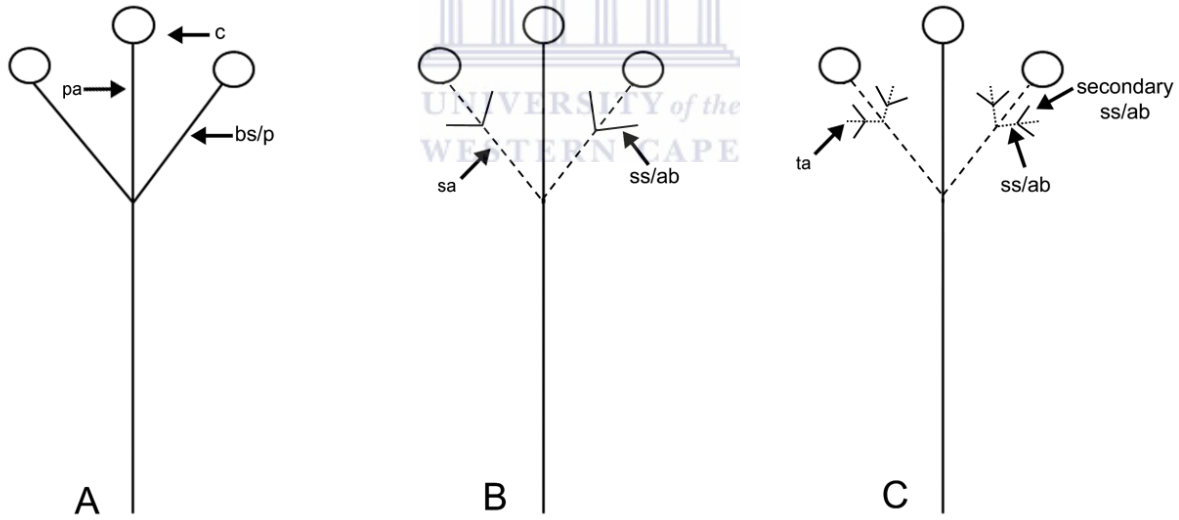

Figure 4.4. Inflorescence structure and spine arrangement in the spiny species, *Schlechteranthus parvus, S. pungens, S. spinescens* and *S. stylosus*. (A) Inflorescence arranged in a simple cyme on the primary axis (pa), with blunt spines (bs) derived from old peduncles (p) where capsules (c) have fallen off, as found in *S. spinescens*; (B) inflorescence arranged in compound cyme on primary axis and in simple cyme on secondary axes (sa) (dashed lines) with sharp spines (ss) derived from aborted buds (ab) on the two outer axes (as in *S. parvus* and *S. stylosus*), blunt spines also sometimes present from capsules falling off; (C) inflorescence arranged in compound cyme on primary axis, compound cyme on the secondary axes and in a simple cyme on the tertiary axes (ta) (dotted lines) as found in *S. pungens*, with sharp spines (ss) derived from aborted buds (ab) and blunt spines also sometimes present where capsules have fallen off.

4.3.5 FLORAL MORPHOLOGY

The calyx is always green, with 4–6 sepals and the petaloid staminodes are more or less equal, although inner row is sometimes slightly shorter. The flowers are white to magenta and sometimes a combination thereof, i.e. magenta tips with a white central eye. Filamentous staminodes are occasionally absent (*S. tetrasepalus,* sometimes absent in *S. stylosus*) and the number of filamentous staminodes varies from a few (up to only 3) to many, in which case they form a complete series around the stamens. *Schlechteranthus spinescens* and *S. parvus* have filamentous staminodes that form a complete series around the stamens, which is a useful character to distinguish *S. parvus* (Fig. 4.5A) from *S. stylosus.* Some species have few to many filamentous staminodes, such as *S. connatus* and *S. pungens,* forming a variable complete to incomplete series around the stamens. The remaining species, *S. abruptus, S. inclusus, S. stylosus* and *S. subglobosus,* always have only a few filamentous staminodes (sometimes absent in *S. stylosus*), forming an incomplete series around the stamens. The stamens are always collected into a cone in the centre of the flower (Fig. 4.1D), as is diagnostic of the genus. If filamentous staminodes are present (absent in some species), these surround the stamens, also forming a cone. The flowers include 6–9 stigmas, with the nectary in a ring.

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Figure 4.5. A new species of *Schlechteranthus*, *S. parvus*. (A) Flower of *S. parvus* with many filamentous staminodes (FS), forming a complete series around the stamens; (B) old flower of *S. parvus*, with spines developing from aborted apical buds; (C) spine arrangement in compound cymose in *S. parvus*; (D) branch of *S. parvus* cut at base to illustrate small size and spines; (E) *Schlechteranthus parvus* in habitat, illustrating the small size of the species and display of dense spines.

4.3.6 CAPSULE MORPHOLOGY

The capsules are 7–9 locular (Fig. 4.1A), as opposed to the 10–12 locules found in subgenus *Schlechteranthus* (Powell *et al.*, 2016; Chapter 3). Capsule shape may be useful to distinguish some species in subgenus *Microphyllus*. The top of the capsules is usually convex, but sometimes only slightly so, with a somewhat flattened apex, as found in *S. subglobosus* (Fig. 4.6A). The base of the capsule is funnel-shaped in all the species (Fig. 4.6A), with the exception of *S. connatus*, where the base is subglobose (Fig. 4.6B). The subglobose base, in combination with the convex top, results in an almost globose capsule in *S. connatus*. The capsules possess complete covering membranes with small closing bodies that block $^1/_3$ of the locule (Figs. 4.1A, 4.6C). The size of the closing bodies was recovered as a synapomorphy for this subgenus (Table 4.1; Fig. 4.3; Appendix D1). Closing rodlets are conspicuous in all species, with the rodlets protruding distally in *S. connatus* and *S. tetrasepalus.* Valve wings may be present or absent, and if present, they are usually narrow ($^1/_4$ to $^1/_3$ width of valve). Valve wings are absent in *S. abruptus*, *S. connatus*, *S. inclusus*, *S. parvus* and *S. stylosus*.

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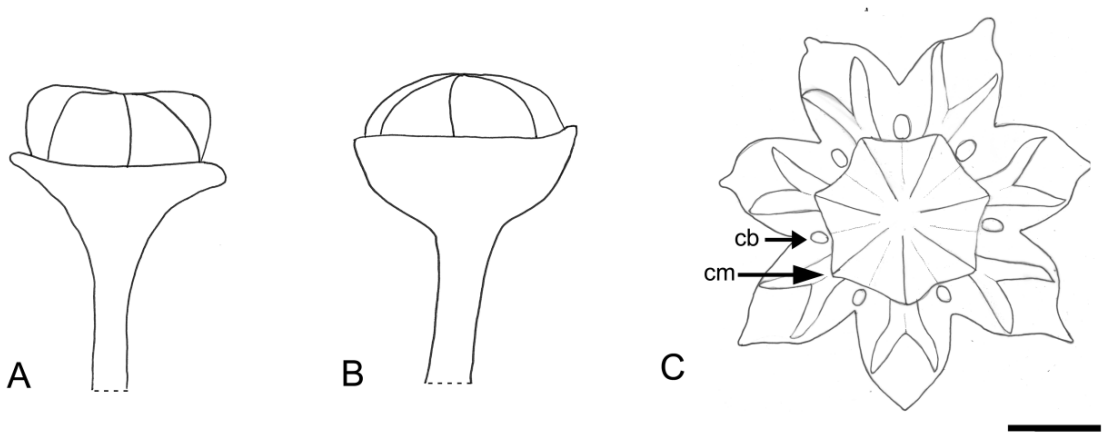

Figure 4.6. Capsule shape and structure in *Schlechteranthus* subgenus *Microphyllus*. (A) Capsule with flattened apex and funnel-shaped base, as found in *S. pungens*; (B) *Schlechteranthus connatus* with convex top and subglobose base; (C) internal capsule structure found in subgenus *Microphyllus* (*S. connatus*), arrows indicating the closing bodies (cb) and covering membranes (cm). Scale = 1 cm.

4.3.7 PHYLOGENETIC RELATIONSHIPS AND CHARACTER EVOLUTION

The trees recovered from the analyses of the molecular plastid data and morphological coded data are strongly incongruent with very different topologies and groupings of the species (Figs. 4.3, 4.7, 4.8). The subgenus *Microphyllus* is, however, recovered as monophyletic in both analyses (PP=1, BS=100 in the molecular tree; PP=1, BS=89 in the morphological tree), supported by the 7 to 9-locular capsules with small closing bodies.

In the molecular analysis (Figs. 4.3A, 4.7), the recovered clades are not supported by morphological characters; however, there is a slight distinction in geographical distributions of the species. The two species recovered in a well-supported clade, *S. abruptus* and *S. spinescens* (PP=1, BS=81), have a slightly more southern centre of distribution (Fig. 4.9B, F) while the distributions of the majority of species (*S. connatus, S. inclusus, parvus, S. pungens, S. spinescens, S. styosus, S. tetrasepalus*), recovered in a strongly supported clade (PP=1, BS=67), are centred in Namaqualand (Fig. 4.9A, C–E, G–I). The distribution of the remaining unresolved species, *S. subglobosus* (Fig. 4.3A), is also centred in Namaqualand with the distribution of this species is restricted to this region (Fig. 4.9D).

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In contrast, the weak geographical pattern was not recovered in the morphological analysis, as the trees are incongruent (Figs. 4.3, 4.7, 4.8). In the morphological analysis of subgenus *Microphyllus*, the clade including *S. parvus, S. pungens, S. spinescens* and *S. stylosus* (PP=1, BS=98; Figs. 4.3B, 4.8) is supported by three synapomorphic characters, i.e. the presence of spines, flowers arranged in a cymose inflorescence and the loss of the bracteole (Table 4.1; Appendix D1). These characters, when mapped onto the molecular tree, were shown to have converged twice, with a subsequent loss of these characters in *S. connatus, S. inclusus* and *S. tetrasepalus* (Fig. 4.3A). The convergence of spines is not unusual in the Aizoaceae, as spines are shown to have evolved numerous times in the Ruschioideae, for example in *Antimima* N.E.Br., *Drosanthemum* Schwantes and *Leipoldtia*

L.Bolus (Hartmann & Stüber, 1993). Spine type, specifically the type where spines are derived from aborted buds (Table 4.1; Appendix D1) supported the grouping of *S. parvus*, *S. pungens* and *S. stylosus* (PP-0.90, BS=66) in the morphological analysis (Fig. 4.3B), but was recovered as a convergence in the molecular analysis (Fig. 4.3A). The lengthened primary axis (Table 4.1; Appendix D1), grouped *S. connatus*, *S. subglobosus* and *S. tetrasepalus* (PP=0.90, BS=67) in the morphological analysis (Fig. 4.3B), while it was recovered as a convergence in the molecular analysis for the clade including *S. connatus* and *S. tetrasepalus* (PP=0.85, BS=56) and for the unresolved *S. subglobosus* (Fig. 4.3A).

In light of the incongruence between the two phylogenetic datasets, we have arranged the species in the taxonomic treatment according to the sequence suggested by the morphological analysis (Fig. 4.3B), so that morphologically similar or easily confused taxa are arranged together, for the sake of practicality and ease of identification.

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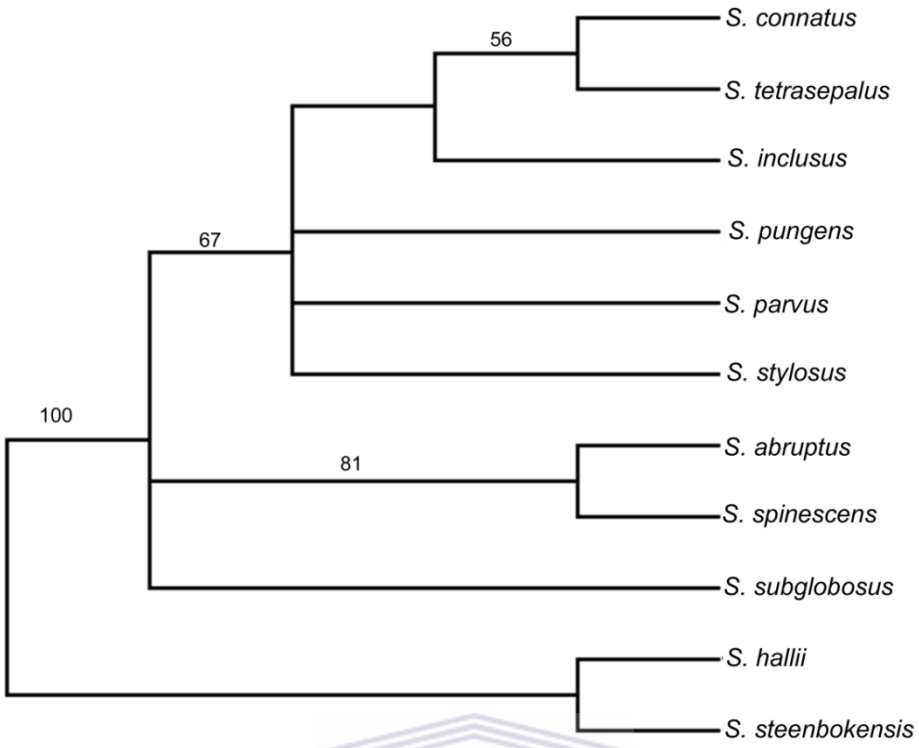

Figure 4.7. Maximum parsimony estimate based on nine plastid gene regions showing the phylogenetic relationships of *Schlechteranthus* subgenus *Microphyllus*. Bootstrap support values greater than 50 are indicated above the branches.

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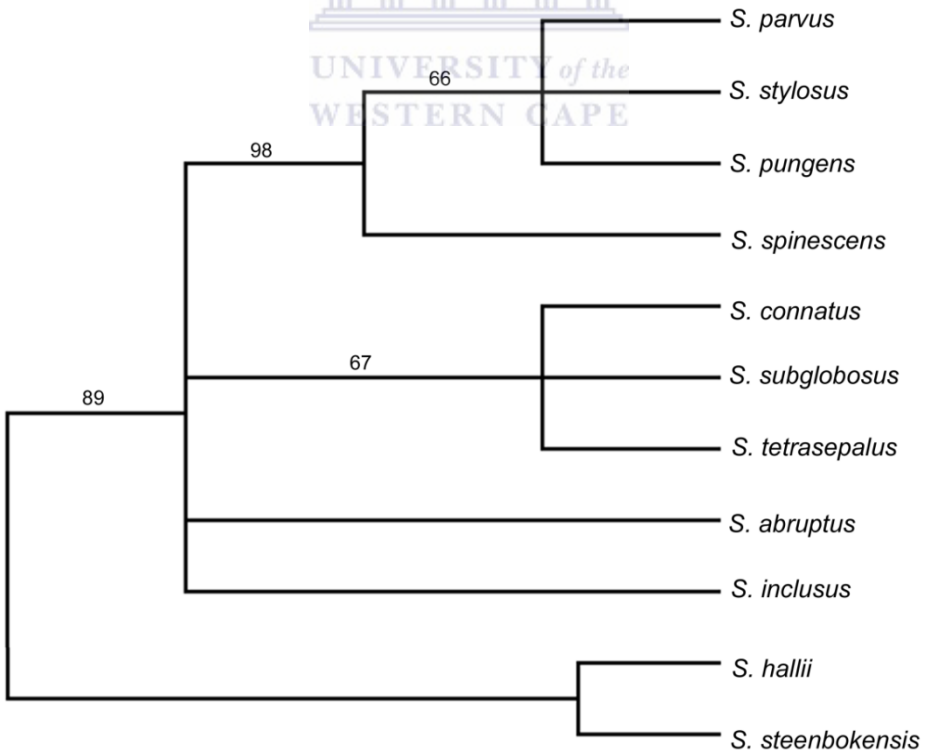

Figure 4.8. Maximum parsimony estimate based on eight coded morphological characters for species of *Schlechteranthus* subgenus *Microphyllus*. Bootstrap support values greater than 50 are indicated above the branches

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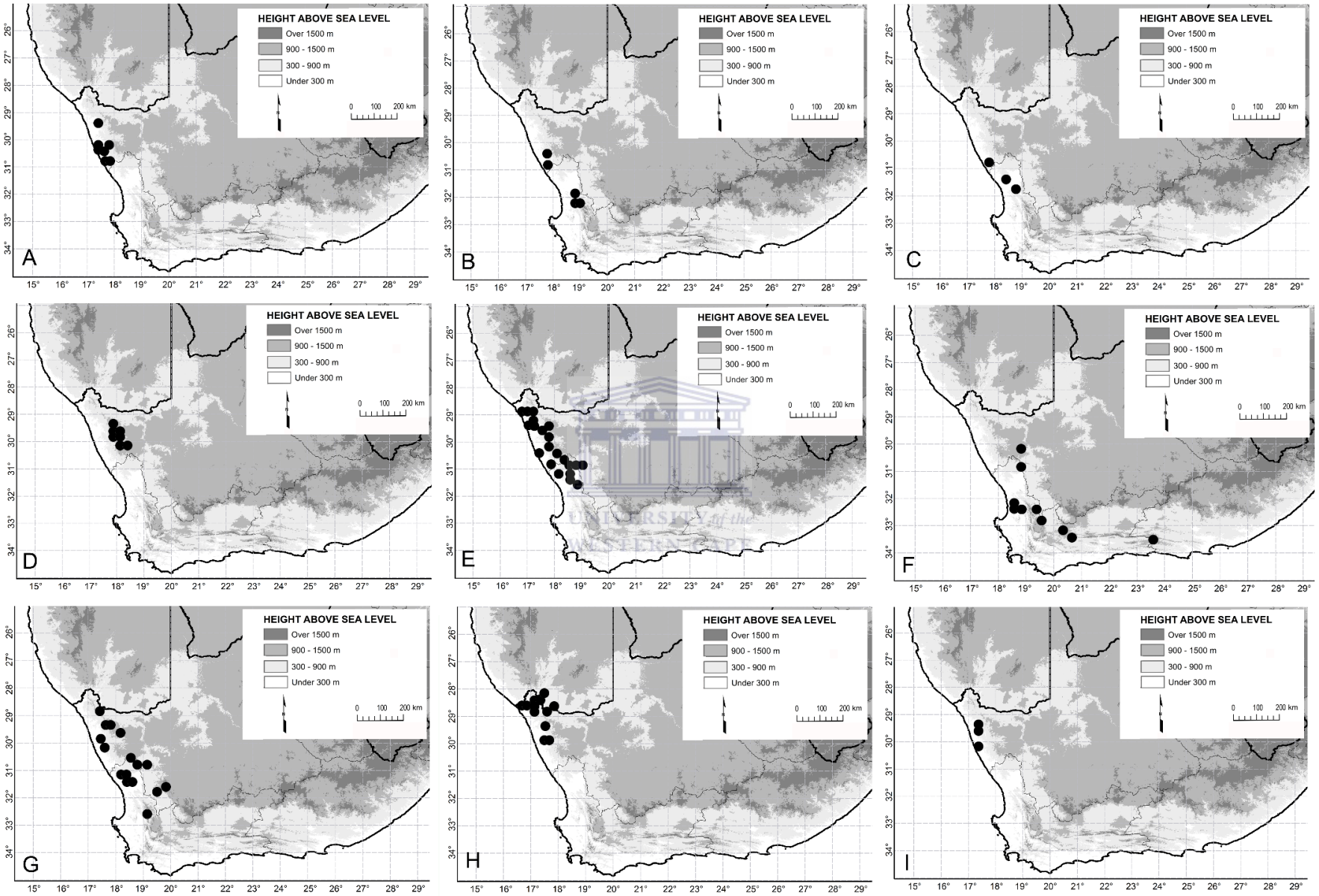

Figure 4.9. Known geographical distributions of the species in *Schlechteranthus* subgenus *Microphyllus*. (A) *Schlechteranthus inclusus*; (B) *S. abruptus*; (C) *S. tetrasepalus*; (D) *S. subglobosus*; (E) *S. connatus*; (F) *S. spinescens*; (G) *S. pungens*; (H) *S. stylosus*; (I) *S. parvus*.

4.4 TAXONOMIC TREATMENT

4.4.1 KEY TO THE SUBGENERA OF *SCHLECHTERANTHUS*:

1. Capsules > 6 mm in diameter, with 10 to 12 locules, closing bodies blocking more than half of the locule; leaves ≥ 4.5 mm wide; epidermal cells anticlinally elongated, with calcium oxalate crystals deposited on the anticlinal walls .....**subg. *Schlechteranthus***

1. Capsules < 6 mm in diameter, with 7 to 9 locules, closing bodies blocking less than half of the locule; leaves ≤ 4.5 mm wide; epidermal cells isodiameteric without crystals on the anticlinal wall.....**subg. *Microphyllus***

4.4.2 ***Schlechteranthus*** subgenus ***Microphyllus*** R.F.Powell, Taxon 65: 259: 2016. *Type*:

Schlechteranthus pungens (H.E.K.Hartmann) R.F.Powell.

Arenifera A.G.J.Herre *sensu* H.E.K.Hartmann *p.p.* excluding type, Bradleya 14:37. 1996.

Octopoma N.E.Br. *p.p*. excluding type, Gard. Chron. Ser. III. 87: 126, in clavi. 1930.

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Low-growing, compact, upright to spreading shrub, often dome-shaped, 100–400 mm tall, with sparse to dense growth. *Branches* woody, decumbent or spreading to upright, branching from the base, up to 9 (–13) mm in diameter, occasionally covered by persistent old leaves; upper branches brown, or whitish grey to white. *Leaves* triquetrous to fatly trigonous; globose, ovoid, oblong or lanceolate in outline, 1.5–20.0 x 1.5–5.0 mm; margins thickened; leaf pairs fused for $^{1}/_{5}$–¾ of length; mucronate, although mucro sometimes not prominent; slightly papillate, resulting in a slightly to prominently velvety, or rough leaf surface; bright green to deep green, sometimes yellow-green. *Flowers* solitary or in spinescent cymose inflorescences, spines formed either by old peduncles or aborted axillary buds; on central primary axis 10–55 mm long, equal to capsule length or up to 10 times longer; bracteole present or absent, sometimes cup-shaped; flowers cone-type, with stamens

collected into a cone; calyx lobes green, 4–6; petaloid staminodes ± equal, inner row sometimes shorter, white to magenta; filamentous staminodes absent or few to many, forming a complete or incomplete series around the stamens; stigmas 6 to 9, nectary in a ring. *Fruit* a loculicidal capsule, with 7 to 9 locules, top prominently to slightly convex, base funnel-shaped, sometimes cup-shaped, 2.0–6.0 x 2.0–6.0 mm; covering membranes complete; closing bodies small, blocking $^1/_3$ of locule; closing rodlet conspicuous, sometimes protruding distally; valve wings narrow, ¼ to $^1/_3$ of valve width, sometimes absent. *Seeds* obovate to pear-shaped; smooth to slightly pustulate.

Diagnostic characters:

Subgenus *Microphyllus* can be distinguished from subgenus *Schlechteranthus* by the smaller leaves (3.5–5.0 x 4–6 mm vs. 5–30 x 3.5–9.0 mm), smaller capsules (2–6 x 2–6 mm vs. 6–11 x 4–9 mm), with only 7 to 9 locules (vs. 10 to 12 locules) and small closing bodies that block $^1/_3$ of the locule (vs. larger closing bodies that block ¾ of locule). In addition, the epidermal cells are isodiametric with no crystals deposited on the anticlinal wall, whereas in subgenus *Schlechteranthus* the epidermal cells are anticlinally elongated with calcium oxalate crystal deposits on the anticlinal walls. The epidermal cells of subgenus *Microphyllus* form blunt papillae which are found throughout the epidermis, but concentrated around the stomata, while in subgenus *Schlechteranthus*, the papillate epidermal cells are only found surrounding the stomata. *Schlechteranthus holgatensis* is an exception in subgenus *Schlechteranthus,* as it is densely papillate throughout the leaf surface. Nine species are currently recognised in subgenus *Microphyllus* and six species in subgenus *Schlechteranthus* (Klak & Bruyns, 2016).

Species of subgenus *Microphyllus* may be confused with other species of Ruschioideae which also have multilocular capsules and spines, as found in *Leipoldtia*, however, species of subgenus *Microphyllus* are distinguished from these species by the

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mucronate leaves, narrow valve wings (broad in *Leipoldtia*), small or large closing bodies (always large in *Leipoldtia*) and 7–9 locular capsules (c. 10-locular capsules in *Leipoldtia*).

Distribution and ecology:

Schlechteranthus is distributed from Khubus to Clanwilliam with an eastern record near Uniondale, with hotspots around Bitterfontein, Khubus, Klienzee, Springbok and Steinkopf (Fig. 4.10A). The distribution of subgenus *Schlechteranthus* extends from north of Khubus to just north of Bitterfontein, with only two hotspots north of Kleinzee (Fig. 4.10B) (Klak & Bruyns, 2016). Subgenus *Microphyllus* has a much wider distribution range, from the border of Namibia, southwards to Clanwilliam with an eastern record at Uniondale (Fig. 4.10C). Interestingly, the highest species richness of subgenus *Microphyllus* is found around Bitterfontein (Fig. 4.10C), in contrast to subgenus *Schlechteranthus,* which does not occur as far south. There are smaller hotspots for subgenus *Microphyllus* around Bitterfontein, Steinkopf and between Steinkopf and Khubus (Fig. 4.10C).

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The species occur in a wide range of ecological habitats such as gentle rocky slopes, alluvial banks and rocky soils and on a number of geologies including calcrete, gneiss, quartz, red loamy sand, sandstone and shale. The species flower from mid-winter to spring, i.e. August to October.

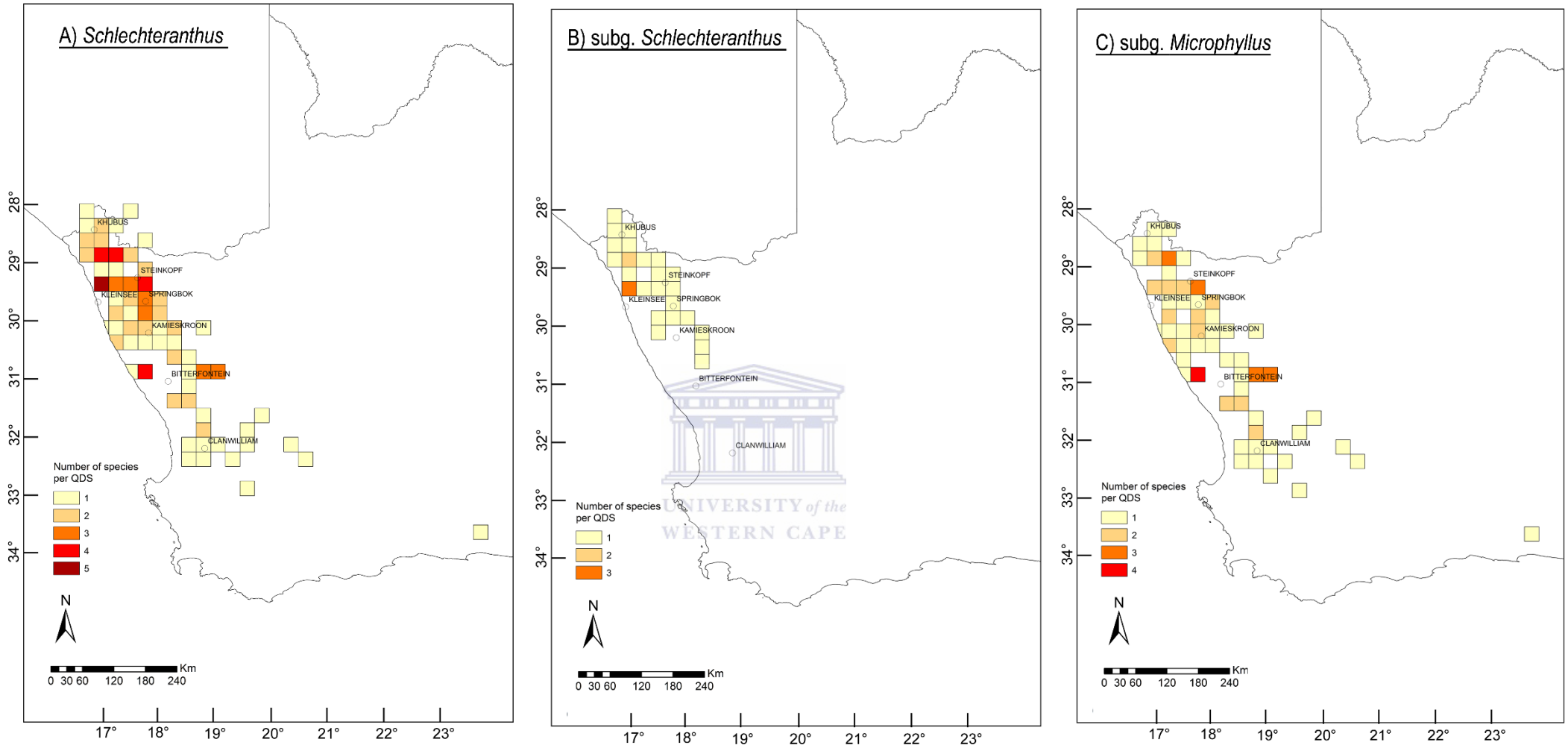

Figure 4.10. Distribution and number of species per Quarter Degree Square (QDS; Edwards and Leistner 1971) in the Greater Cape Floristic Region, South Africa for (A) the genus *Schlechteranthus*; (B) subgenus *Schlechteranthus;* (C) subgenus *Microphyllus*

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4.4.3 KEY TO THE SPECIES OF SUBGENUS *MICROPHYLLUS*:

1. Flowers solitary; primary axis bracteate, old peduncles or aborted axillary buds not forming spines; valve wings absent or very narrow, up to ¼ of valve width 2

2. Leaves markedly globose in outline; leaves positioned at regular intervals along the branch; capsules slightly convex, flattened at apex; branches upright to spreading; leaves usually bright green..... **4.4.3.4. *S. subglobosus***

2'. Leaves ovoid to lanceolate in outline; leaves crowded towards the tips of the branches or stacked tightly along the branch; capsules convex, not flattened at apex; branches decumbent or upright to spreading; leaves green to dark green, never bright green 3

3. Primary axis 1½–10 times longer than capsule length; capsules often held in a row above the terminal leaf pairs; bracteoles more than ⅓ way down the primary axis 4

4. Leaves ovoid in outline, < 5 mm long, opposite leaf pairs fused ⅔ of length; capsule bases funnel-shaped; branches upright .....**4.4.3.3. *S. tetrasepalus***

4'. Leaves lanceolate in outline, > 8 mm long, opposite leaf pairs fused ¼ of length; capsule bases rounded; branches decumbent..... **4.4.3.5. *S. connatus***

3'. Primary axis equal in length to capsule length; capsules not held in a row above terminal leaf pairs; bracteoles usually at base of capsules but always less than ¼ way down the primary axis.....5

5. Leaves decussate, with old leaves persisting on branch; opposite leaf pairs fused ¾ of length, ovoid in outline, ≤ 11 mm long.....**4.4.3.1. *S. inclusus***

5'. Leaves crowded towards the tips of the branch, with old leaves falling off branch; opposite leaf pairs fused ¼ of length, lanceolate in outline, ≥12 mm long .....

.....**4.4.3.2. *S. abruptus***

1'. Flowers in cymose inflorescences; primary axis ebracteate, old peduncles or aborted axillary buds forming spines; valve wings narrow, up to $^1/_3$ of valve width 6

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6. Blunt spines derived solely from old peduncles where capsules have fallen off; inflorescences in simple cymes.....**4.4.3.6. *S. spinescens***

6'. Sharp spines derived from aborted axillary buds; inflorescences in compound cymes....

.....7

7. Spines arranged in compound cyme on secondary axes; upper branches white; opposite leaf pairs fused for ¼ to ⅓ of length ..... **4.4.3.7. *S. pungens***

7. Spines arranged in simple cyme on secondary axes; upper branches brown; opposite leaf pairs fused for ⅓ to ¾ of length 8

8. Leaves ovoid in outline, prominently mucronate, leaf pairs fused for ≤ ½ their length; filamentous staminodes few, not forming a complete series around the stamens; large shrub to 400 mm tall .....**4.4.3.8. *S. stylosus***

8. Leaves subglobose to globose in outline, not prominently mucronate, leaf pairs fused for ≥ ½ their length; filamentous staminodes many, forming a complete series around the stamens; shrublet to 150 mm tall ..... **4.4.3.9. *S. parvus***

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4.4.3.1 ***Schlechteranthus inclusus*** (L.Bolus) R.F.Powell, Taxon 65: 259. 2016.

Mesembryanthemum inclusum L.Bolus, Ann. Bolus Herb. 4: 40. 1926.

Octopoma inclusum (L.Bolus) N.E.Br., Gard. Chron. 3: 126. 1930; Hartmann, Bradleya 16: 72. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. *Ruschia restituta* G.D.Rowley, nom. nov. non. *Ruschia inclusa* L.Bolus Natl. Cact. Succ. J. 33: 8. 1978. *Type:* South Africa, Northern Cape Province: slopes overlooking the sea, south of Hondeklip Bay, Namaqualand [3017DA], 1924, *Pillans sub BOL 17758* (lectotype: BOL!, designated by Hartmann (1998); isosyntype: K– image!).

Dense upright shrublets, to 100 mm tall. *Branches* to 8 mm in diameter; upper branches brown; leaves present along the branch, prominently stacked, with old black leaves remaining on branch. *Leaves* triquetrous, ovoid in outline; 4.0–11.0 x 3.0–3.5 mm; leaf pairs fused for ¾ of length; mucro prominent; slightly velvety; green, often blackened at base. *Flowers* solitary, on central primary axis to 11 mm long, equal proportionally to capsule length, bracteoles present less than ¼ way down axis, prominently cup-shaped, often holding capsule base; spines absent; filamentous staminodes few, forming an incomplete series around the stamens. *Fruit* 8-locular; top slightly convex, base funnel-shaped; 4.0 x 3.0 mm; closing rodlets conspicuous; valve wings absent. Figure 4.11.

Diagnostic characters:

This species is easily distinguished by the decussate leaves stacked along the branch and the old blackened leaves that remain on the branch (Fig. 4.1G). However, it may be confused with *S. tetrasepalus,* which shares a compact habit and leaf pairs with a high degree of fusion (Fig. 4.2F, G). *Schlechteranthus inclusus* is, however, distinguished by the short primary axis which is equal in length to the length of the capsule, the decussate leaves, with old leaves remaining on the branch and the cup-shaped bracteoles (central axis at least 1½ times longer than capsule, leaves crowded towards the tip of the branches, with old leaves falling off and bracteoles not cup-shaped in *S. tetrasepalus*).

Distribution and ecology:

This species has a coastal distribution, found on the west coast of South Africa between Kleinzee and the Groen River mouth (Fig. 4.9A). *Schlechteranthus inclusus* is associated with a range of geologies including calcrete, granite and gneiss, but always in shallow soil.

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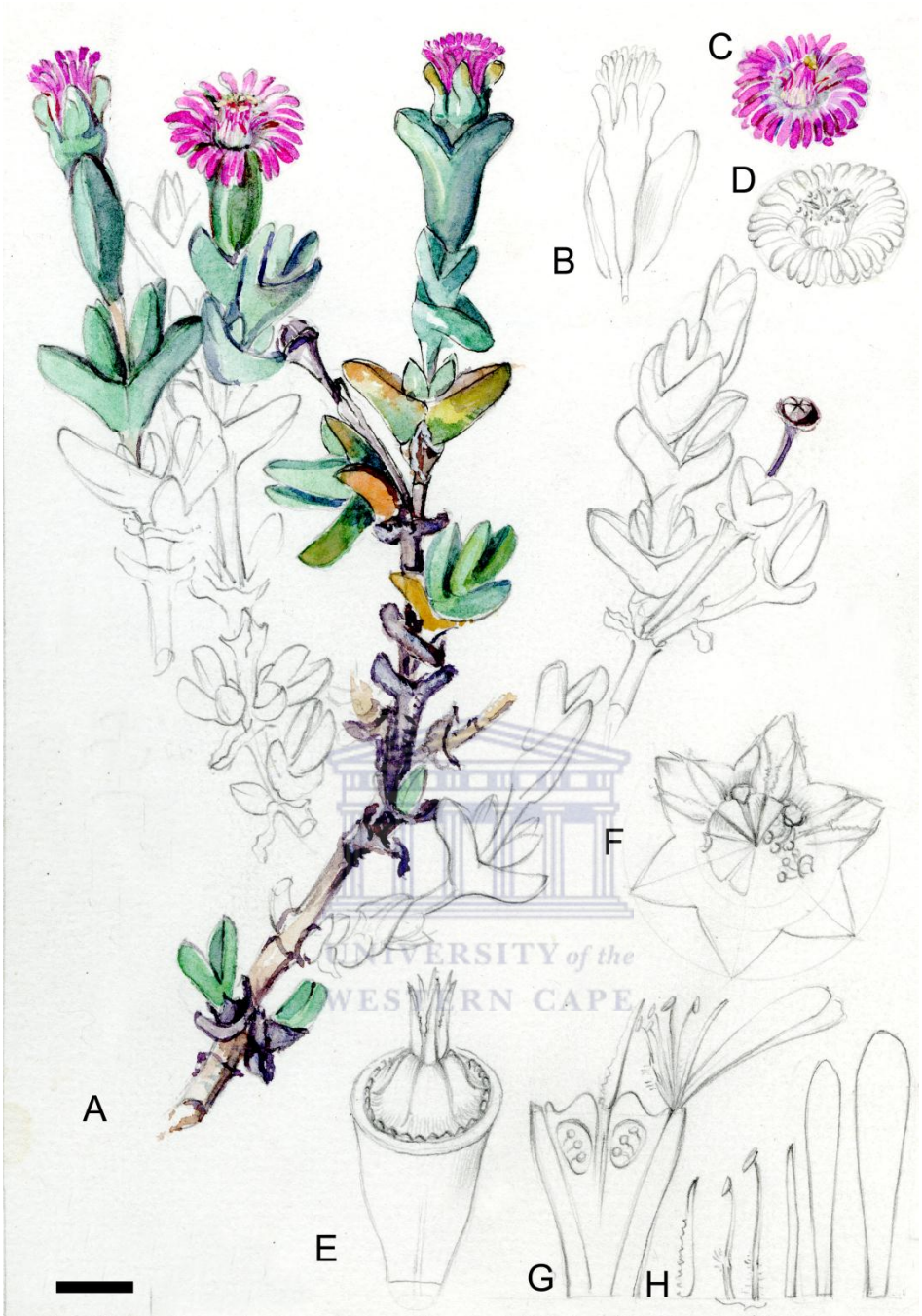

Figure 4.11. Drawing of type material for *Schlechteranthus inclusus* (*Pillans sub BOL 17758* (BOL)). (A) Habit and leaf arrangement of *S. inclusus*; (B) terminal opposite leaf pair with flower in bud; (C) flower with stamens and filamentous staminodes collected in a cone in the centre of flower, as is diagnostic for the genus; (D) flower with arrangement of stamens loosely arranged in a cone, typically found near the end of the flowering season; (E) flower with all staminodes removed showing the nectaries arranged in a ring; (F) open capsule with small closing bodies and complete covering membranes; (G) longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes; (H) floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A–D = 10 mm; E, G = 3.3 mm; F, H = 2.5 mm. Artist B. Carter.

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The distribution of this species is closely linked to alluvial habitats, often occurring in or alongside river courses. It flowers from August to September.

Additional specimens examined:

South Africa. NORTHERN CAPE: **2917 (Springbok):** Namaqualand, Kleinzee, Farm Drooge Kraal 180, lower south-eastern slopes of Naroegas se Berg, below trig beacon (–AD), *Desmet and Smale 3284* (NBG). **3017 (Hondeklipbaai):** East of Koingnaas, on south slope (–AB), *Klak 474* (BOL); South of Koingnaas along road to Hondeklip Bay, at Swartlintjiesrivier (–AB), *Klak 2359* (BOL); Close to turnoff to Hondeklipbaai from Kleinzee (–AB), *Powell 35* (NBG); Spoegrivier cave, on rocks above the river (–AD), *Klak 1836* (BOL); Nama'land, 2 miles N of Kamieskroon (–BB), *Littlewood sub KGB 584/59* (BOL); Gemsbokvlei, 10 miles east of Port Nolloth (–BC), *Van Heerde 27400* (BOL); North of Flamink Berg, Walle Kraal, Namaqualand (–BC), *Pillans 17911* (BOL); South of Groenrivier mouth, near the lighthouse (–DC), *Klak 1832* (BOL); Cape Province, Namaqualand, 6 miles north of Garies (–DD), *Hall sub NBG 1016/60* (BOL); Garies district, farm Tweerivier (–DD), *Klak 2411* (BOL). **Precise locality unknown:** South border of Little Namaqualand, *Peers sub NBG 1394/33* (BOL).

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4.4.3.2 ***Schlechteranthus abruptus*** (A.Berger) R.F.Powell, Taxon 65: 259. 2016.

Mesembryanthemum abruptum A.Berger, Bot. Jahrb. Syst. 57: 638. 1922.

Octopoma abruptum (A.Berger) N.E.Br., Gard. Chron. 3: 126. 1930; Hartmann, Bradleya 16: 72. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 189. 2001; Klak in Manning and Goldblatt, Pl. Gr. C.F.R. 1: 296. 2012.

Ruschia abrupta (A.Berger) G.D.Rowley, Natl. Cact. Succ. J. 33: 8. 1978.

Type: South Africa, Western Cape Province: Brandewynrivier, between

Clanwilliam and Calvinia [3118DD], Oct 1897, *R. Schlechter 10828* (holotype: GRA–image!; isotypes: BOL!; BR–image!, K–image!, S–image!, WAG–image!).

Ruschia rupigena L.Bolus, Notes Mesembryanthemum 3: 520. 1935. *Ruschia rupicola* L.Bolus, Notes Mesembryanthemum 2: 415. 1933, *nomen illegit*. non Schwantes Z. Sukkulentenk. 2: 187. 1926. *Octopoma rupigena* (L.Bolus) L.Bolus J. S. African Bot. 33: 306. 1967; Hartmann, Bradleya 16: 73. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Manning and Goldblatt, Pl. Gr. C.F.R. 1: 297. 2012. *Type*: South Africa, Western Cape Province: near Pakhuis, Clanwilliam Div., common on rocks between Pakhuis and Buishoekfontein [3218BB], Sep 1933, *L. Bolus sub NBG 1504/33* (holotype: BOL!; isotype: PRE–image!).

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Sparse, upright to spreading shrub, to 250 mm tall. *Branches* to 9 mm in diameter; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branch. *Leaves* triquetrous, lanceolate in outline; 12.0–20.0 x 2.5–5.0 mm; leaf pairs fused for ¼ of length; mucro prominent; prominently velvety as a result of extended papillae of epidermal cells; green. *Flowers* solitary; on central primary axis to 11 mm long, equal proportionally to capsule length, bracteoles present less than ¼ way down axis; spines absent; filamentous staminodes few, forming an incomplete series around the stamens. *Fruit* 8-locular; top slightly convex, base widely funnel-shaped; 5.0 x 3.5 mm; closing rodlets conspicuous; valve wings absent. Figure 4.12.

Diagnostic characters:

Schlechteranthus abruptus is most likely to be confused with *S. connatus* as the species share a similar leaf shape and size, but can be distinguished by the shape of the capsule, which has a funnel-shaped base (capsule base subglobose in *S. connatus*) and the primary axis which also not lengthened (prominently lengthened in *S. connatus*). The bracteole position in *S. abruptus* is also distinct, as it is almost always found at the base of the capsule, but no more than ¼ way down the primary axis (more than ½ way down the primary axis in *S. connatus*). *Schlechteranthus abruptus* can also be distinguished by its prominently velvety leaf surface, which is unique to this species in the subgenus (and otherwise only found in *S. holgatensis* Klak in subgenus *Schlechteranthus* (Klak & Bruyns, 2016)).

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Distribution and ecology:

Schlechteranthus abruptus is mainly distributed around Clanwilliam and Garies (Fig. 4.9B). The species is found on shallow rocky sandstone soils and flowers from July to August.

Additional specimens examined:

South Africa. WESTERN CAPE: **3017 (Hondeklipbaai):** North of Garies, Kys (–BD), *Klak 468* (BOL); Klip Vlei, between Kamieskroon and Garies, Namaqualand (–DD), *Thorne 49957* (SAM). **3218 (Clanwilliam):** inter Pakhuis et Nardouw (–BB), *Leipoldt s.n.* (BOL). **3219 (Wuppertal):** Klipfontein, north of Boontjieskloof (–AA), *Esterhuysen 32204* (BOL); On Pakhuis Pass, just before turnoff to Wuppertal (–AA), *Powell 5* (NBG); Pakhuis, near turnoff to Wuppertal (–AA), *Klak 433* (BOL). **Precise locality unknown:** near Clanwilliam, *L. Bolus sub BOL 23833* (BOL).

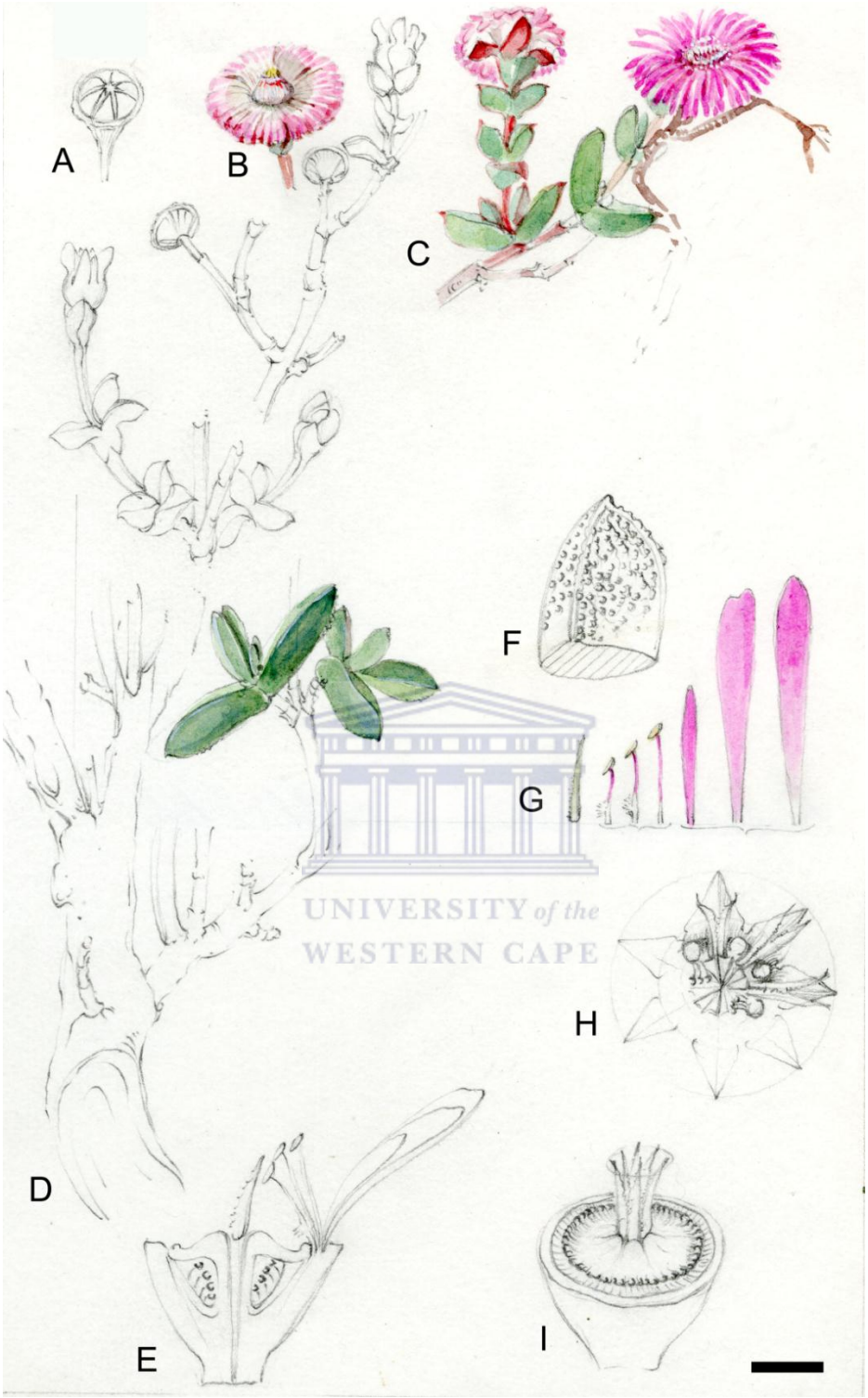

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Figure 4.12. Drawing of type material for *Schlechteranthus abruptus* (*Bolus sub NBG 1504/33* (BOL)). (A) Closed capsule of *S. abruptus*; (B) open flower with stamens and filamentous staminodes collected in a cone; (C) flowering branch of *S. abruptus*; (D) habit and leaf arrangement of *S. abruptus*; (E) longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes; (F) enlarged top part of leaf illustrating the velvety leaf surface; (G) floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes; (H) open capsule with small closing bodies and complete covering membranes. (I) flower with all staminodes removed showing the nectaries arranged in a ring. Scale: A–D = 10 mm; E–I = 2.5 mm. Artist B. Carter.

4.4.3.3 ***Schlechteranthus tetrasepalus*** (L.Bolus) R.F.Powell, Taxon 65: 259. 2016.

Ruschia tetrasepala L.Bolus, Notes Mesembryanthemum 2: 373. 1932.

Octopoma tetrasepalum (L.Bolus) H.E.K.Hartmann, Bradleya 16: 74. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. *Type*: South Africa, Western Cape Province: between the town and Sout River [3118DD], Apr 1932, *Luckhoff sub BOL 20203* (holotype: BOL!).

Dense upright shrublet, to 130 mm tall. *Branches* to 7(–13) mm in diameter; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, ovoid in outline; 3.0–5.0 x 1.5–4.0 mm; leaf pairs fused for $^{2}/_{3}$ of length; mucro prominent; slightly velvety; green. *Flowers* solitary; on central primary axis to 13 mm long, at least 1½ times longer than capsule length, bracteoles present more than $^{1}/_{3}$ way down axis; spines absent; filamentous staminodes absent. *Fruit* 7- to 8-locular; top convex, base funnel-shaped; ca. 5.5 x 4.0 mm; closing rodlets conspicuous, protruding distally; valve wings absent.

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Diagnostic characters:

This species is most similar to *S. inclusus* in its compact habit, leaves with a high degree of fusion (Fig. 4.2F, G), and solitary flowers, but is distinguished by the longer primary axis, which is at least 1½ times longer than the capsule, often resulting in the capsules held in a row above the terminal leaf pairs (primary axis equal in length to capsule and capsules not held in a row in *S. inclusus*). In *S. tetrasepalus,* the old leaves do not remain on the branch, leaving branches bare, while in *S. inclusus,* the old leaves remain on the branch.

Distribution and ecology:

This species occurs on gravel sandstone patches on alluvial banks of the Groen River (Fig. 4.9C) and in the Knersvlakte in quartz. *Schlechteranthus tetrasepalus* flowers in September.

Additional specimens examined:

South Africa. WESTERN CAPE: **3017 (Hondeklipbaai):** Tweeriviere, Groen River (–DD), *Bruyns 10856* (BOL). **3118 (Vanrhynsdorp):** Knersvlakte, Farm Moedverloor (–AD), *Klak 933, Klak 2418* (BOL).

4.4.3.4 ***Schlechteranthus subglobosus*** (L.Bolus) R.F.Powell, Taxon 65: 259. 2016.

Ruschia subglobosa L.Bolus, Notes Mesembryanthemum 1: 140. 1928.

Mesembryanthemum reductum (L.Bolus) N.E.Br., Gard. Chron. 3: 33. 1930.

Octopoma subglobosa (L.Bolus) L.Bolus, J. S. African Bot. 29: 49. 1963; Hartmann, Bradleya 16: 73. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. *Type*: South Africa, Northern Cape Province: hills on north side of O'kiep, Little Namaqualand [2917DB], Oct 1926, *Pillans 5844* (holotype: BOL!).

Sparse spreading shrub, to 200 mm tall. *Branches* to 4 mm in diameter; upper branches brown; leaves positioned at regular intervals along the branches, old leaves not remaining on the branches. *Leaves* fatly trigonous, markedly globose in outline; 2.5–9.0 x 1.5–3.0 mm; leaf pairs fused for $^{1}/_{3}$ of length; mucro very prominent as result of leaf shape; rough, as a result of stout dome-shaped epidermal cells; bright green. *Flowers* solitary; on central primary axis to 25 mm long, at least 5 times longer than capsule length,

bracteoles present more than $^{2}/_{3}$ way down axis; spines absent; filamentous staminodes few, forming an incomplete series around the stamens. *Fruit* 7- to 8-locular; top slightly convex, flattened at the apex, base narrowly funnel-shaped; ca. 4.0 x 2.5 mm; closing rodlets conspicuous; valve wings narrow, up to $^{1}/_{4}$ width of valve. Figure 4.13.

Diagnostic characters:

Schlechteranthus subglobosus is a distinct species and, although it shares a globose leaf shape with *S. parvus* (Fig. 4.2D, I), the leaf pairs are only fused for $^{1}/_{3}$ of length with a very prominent mucro (fused for ¼ of length with obscure mucro in *S. parvus*) and the flowers are solitary (flowers in cymose inflorescence in *S. parvus*). The leaves of *S. subglobosus* are regularly spaced along the branch (Fig. 4.1E), which is similar to *S. inclusus*, while the rest of the species in the subgenus have the leaves crowded towards the tips. In *S. subglobosus*, however, the leaves are positioned at regular intervals along the branch with the internodes visible between the leaves (Fig. 4.1E), while in *S. inclusus* the leaves are tightly packed and decussate, with no visible internodes (Fig. 4.1G). *Schlechteranthus subglobosus* can further be distinguished from the other species in the subgenus by its bright green leaves with a rough surface and very prominent mucro as a result of the fatly trigonous leaf shape, while the leaves in the remaining species are triquetrous green to dark green leaves, with a slightly velvety to velvety leaf surface and a prominent mucro (obscure in *S. parvus*) in the rest of the subgenus.

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Distribution and ecology:

Schlechteranthus subglobosus has a narrow distribution with a specific habitat, found only on gentle rocky slopes, usually gneiss, on hills between Springbok and Kamieskroon (Fig. 4.9D). The species flowers from August to September.

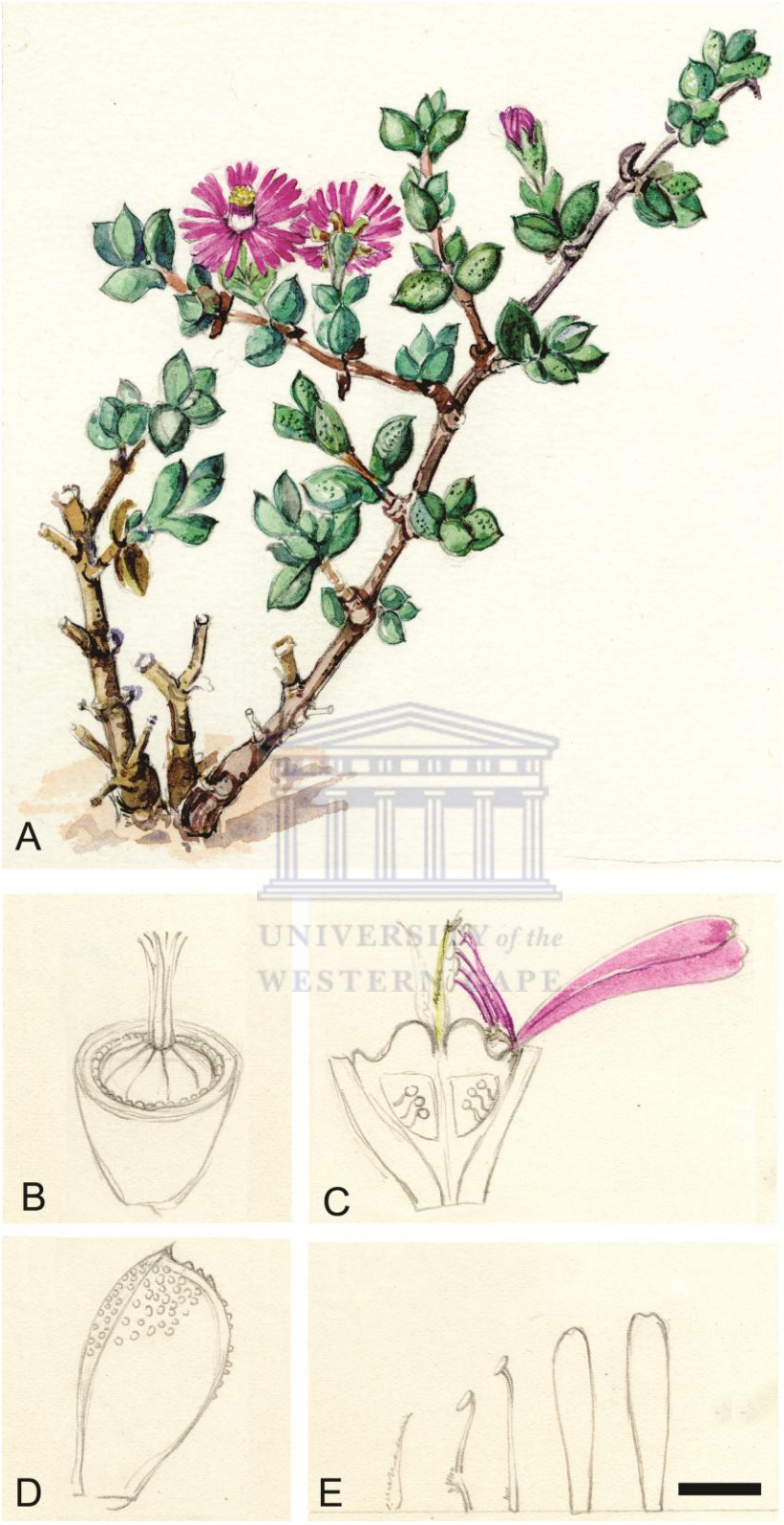

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Figure 4.13. Drawing of type material for *Schlechteranthus subglobosus* (*Pillans 5844* (BOL)). (A) Habit and leaf arrangement of *S. subglobosus*; (B) flower with all staminodes removed showing the nectaries arranged in a ring; (C) longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes; (D) enlarged leaf of *S. subglobosus* illustrating the dome-shaped epidermal cells; (E) floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A = 10 mm; B–C = 2 mm; D–E = 2.5 mm. Artist B. Carter.

Additional specimens examined:

South Africa. NORTHERN CAPE: **2917 (Springbok):** On road between Goodhouse and Concordia (–BD), *Powell 33* (NBG); Namaqualand, Ratelpoort (–BD), *Hall*, sub *NBG 1770/48* (BOL); 3 km east of Nababeep and 1.5 km north of Divide copper mine (–DB), *Hilton-Taylor 2080* (BOL); 2 km east of Springbok, O'kiep (–DB), *Klak 2418* (BOL); Namaqualand, near Springbok (–DB), *L. Bolus* sub BOL 19008 (BOL); Khamieskroon District, Ybeep (–DD), *Bruyns 7610* (BOL); Namaqualand District, Kamiesberg mountain range at pass on Droëdap road (–DD), *Burgoyne 11265* (NBG); Little Namqualand, three miles south of Mesklip (–DD), *Salter 4598* (BOL); Little Namaqualand, Mesklip (–DD), *Esterhuysen 7769* (BOL); Namaqualand, Farm De Draay 346 (–DD), *Le Roux 4126b* (NBG). **2918 (Gamoep):** Springbok District, Kweekfontein (–CA), *Bruyns 8240* (BOL); Namaqualand District, on road from Gamoep to Springbok, 2 km before farm Silverfontein (–CC), *Klak 753* (BOL); Nuwedam, Springbok (–CC), *Bruyns 9146* (BOL). **3018 (Kamiesberg):** 1 km north of Rooidoringkloof turn-off (-AA), *Klak 866* (BOL); Southeast of Hôsabees in 'Pypmaker se Poort', road to Gamoep (–AB), *Hilton-Taylor 2239* (BOL). **Precise locality unknown:** Bushmanland, *SUG 8917* (BOL).

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4.4.3.5 ***Schlechteranthus connatus*** (L.Bolus) R.F.Powell, Taxon 65: 259. 2016. *Ruschia connata* L.Bolus, Notes Mesembryanthemum 1: 139. 1928.

Mesembryanthemum connatum (L.Bolus) N.E.Br., Gard. Chron. 3: 32. 1930.

Octopoma connatum (L.Bolus) L.Bolus, J. S. African Bot. 29: 49. 1963; Hartmann, Bradleya 16: 72. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 189. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. *Type*: South Africa, Northern Cape Province: between Doornpoort and Brakfontein [2917], Jul 1927, *Pillans 5794* (holotype: BOL!).

Ruschia conjunctum L.Bolus, Notes Mesembryanthemum 2: 239. 1931. *Octopoma conjunctum* (L.Bolus) L.Bolus J. S. African Bot. 32: 128. 1966. *Type:* South Africa, Northern Cape Province: Rietkloof, Knersvlakte [3018DC], Oct 1930, *Matthews 19381* (holotype: BOL!).

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Sparse low-growing decumbent to upright shrub, to 100 mm tall. *Branches* to 7 mm in diameter; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, lanceolate in outline; 8.0–20.0 x 1.5–5 mm; leaf pairs fused for ¼ of length; mucro prominent; slightly velvety; green. *Flowers* solitary; on central primary axis to 55 mm long, at least 3 times longer than length of capsule, bracteoles present more than ½ way down axis; spines absent; filamentous staminodes few to many, forming an incomplete to complete series around the stamens. *Fruit* 8-locular; top prominently convex, base cup-shaped, resulting in capsule appearing subglobose; ca. 6.0 x 6.0 mm; closing rodlet conspicuous, protruding distally; valve wings absent. Figure 4.14.

Diagnostic characters:

Schlechteranthus connatus may be confused with *S. abruptus* as they share a similar leaf shape and size, but is distinguished by the longer primary axis, which is at least 3 times longer than the capsule (Fig. 4.1C), and bracteoles present more than ½ the way down the axis (primary axis equal to length of capsule and bracteole mostly at base of capsule in *S. abruptus*). The capsule itself is also markedly globose in *S. connatus* (Figs. 4.1C, 4.6B), as a result of the subglobose base and convex top, whereas in *S. abruptus* the base of the capsule is funnel-shaped and the top not prominently rounded. *Schlechteranthus connatus* also lacks the prominently velvety leaf surface of *S. abruptus*, and is only slightly velvety.

Distribution and ecology:

Schlechteranthus connatus is widespread in northern Namaqualand extending from the Richtersveld southwards into the Knersvlakte (Fig. 4.9E). It is generally found on rocky soil associated with gneiss or quartz and sometimes occurs in red loamy sand. The species flowers from September to October.

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Additional specimens examined:

South Africa. NORTHERN CAPE: **2816 (Oranjemund):** Richtersveld, 5 km westwards along Holgat river from Eksteenfontein turn-off, along Kuboos-Lekkersing road (–DD), *Klak 1504* (BOL). **2817 (Vioolsdrif):** 6 km south of Eksteenfontein (–CC), *Bruyns 9106* (BOL); Eksteenfontein, Pypfontein (–CC), *Bruyns 9132* (BOL); Richtersveld, 15 km east of Eksteenfontein, on road to Vioolsdrif (–CD), *Klak 1513* (BOL); Klipbokberge, Richtersveld (–CD), *Stellenbosch University Gardens 9289* (BOL); Doornriver, Richtersveld, Little Namaqualand (–CD), *Hort. Stellenbosch 8792* (BOL). **2917 (Springbok):** 24 km north of Grasvlakte on road to Eksteenfontein (–AB), *Bruyns 9104*

(BOL); 19 km south of Port Nolloth on road to Grootmis, Kwagganap River (–AC), *Klak 2397* (BOL); South-East of Naroegas (–AD), *Klak 1499* (BOL); between Bulletrap and the N7 (–BD), *Powell 10* (NBG); 2 km towards Nigramoep (–DA), *Klak 927* (BOL); Springbok, Drie Draai (–DD), *Bruyns 9152* (BOL). **3017 (Hondeklipbaai):** south of Kran se vlei, Hondeklip Bay (–AD), *Klak 899* (BOL); Grootvleipass, near Kamieskroon (–BB), *Klak 1853* (BOL); ca. 1 km north of Kamieskroon hotel (–BB), *Klak 1871* (BOL); 6 miles south of Garies, Namaqualand (–DD), *Acocks 15102* (BOL); Garies district, farm Tweerivier (–DD), *Klak 2410* (BOL); Garies dist., farm Tweerivier, near Groenrivier (–DD), *Klak 2403* (BOL). **3018 (Kamiesberg):** Kamiesberg, northern slopes of Weeskindpeak, 8 km southwest of Leliefontein (–AC), *Helme 3226* (BOL); Klipbok, Knersvlakte (–CB), *Bruyns 5372* (BOL); Garies dist., Farm Keurbos (–CB), *Klak 1263* (BOL); Rietkloof, Knersvlakte (–DC), *National Geographic-IPC Tour 119* (BOL); Garies, farm Stofkrall (–DD), *Klak 1525* (BOL). **3019 (Loeriesfontein):** Loeriesfontein, entrance to Donkiedam (–CC), *Bruyns 10859* (BOL). WESTERN CAPE: **3118 (Vanrhynsdorp):** Komkans Bitterfontein (–AA), *Bruyns 12806* (BOL); Near Goerap, along road to Rietfontein (–AA), *Klak 2299* (BOL); Knersvlakte, Kareeberg, on farm Kareeberg (–BA), *Klak 2284b* (BOL); Knersvlakte, Vanrhynsdorp div., farm Arizona (–BC), *Klak 632* (BOL); Knersvlakte, Rooiberg, south of Nuwerus (–BC), *Desmet 1805* (NBG) Farm Quaggaskop, Vanrhynsdorp (–BC), *Klak 1452* (BOL); Zandkraal, Vanrhynsdorp (–DB), *Acocks 14859* (BOL). **Precise locality unknown:** Between Garies and Kamieskroon, *L. Bolus 24220* (BOL).

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Figure 4.14. Drawing of type material for *Schlechteranthus connatus* (*Pillans 5794* (BOL)). (A) Habit and leaf arrangement of *S. connatus*; (B) floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes; (C) longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes; (D) flower with all staminodes removed showing the nectaries arranged in a ring; (E) open capsule with small closing bodies and complete covering membranes. Scale: A = 10 mm; B–D = 3.3 mm; E = 2.5 mm. Artist B. Carter.

4.4.3.6 ***Schlechteranthus spinescens*** (L.Bolus) R.F.Powell, Taxon 65: 259. 2016. *Ruschia spinescens* L.Bolus, Notes Mesembryanthemum 2: 175. 1930. *Arenifera spinescens* (L.Bolus) H.E.K. Hartmann, Bradleya 14: 38. 1996; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 1: 69. 2001; Buys in Snijman, Pl. Gr. C.F.R. 2: 178. 2013. *Type*: South Africa, Western Cape Province: Whitehill near Matjiesfontein, Laingsburg [3320BC], Oct 1929, *Compton sub BOL 19081* (holotype: BOL!).

Sparse upright to spreading shrub, to 200 mm tall. *Branches* to 8 mm in diameter; upper branches often whitish to grey; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, lanceolate in outline, 3.0–13.0 x 2.0–3.0 mm, leaf pairs fused for $^{1}/_{6}$ to $^{1}/_{5}$ of length; mucro prominent; slightly velvety; deep green. *Flowers* in simple cymose inflorescence; central primary axis to 15 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged in simple cyme, formed by old peduncles where capsules have fallen off, held at obtuse angle, blunt, mostly elevated above terminal leaf pair, but also present below terminal leaf pair; filamentous staminodes many, forming a complete series around the stamens. *Fruit* 7- to 8-locular; top convex, base narrowly funnel-shaped, 2.5 x 2.0 mm; closing rodlet conspicuous; valve wings narrow, up to $^{1}/_{3}$ width of valve. Figure 4.15.

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Diagnostic characters:

Schlechteranthus spinescens is most similar to *S. pungens* as they have similar leaf shapes in outline (Fig. 4.2B, E) and overlap in distribution (Fig. 9F, G). However, *S. spinescens* can be easily recognised by the different arrangement of spines and spine formation. In *S. spinescens*, the spines are arranged in a simple cyme with blunt spines derived from old

peduncles where the capsule have fallen off (Fig. 4.4A), while in *S. pungens* the spines are arranged in a compound cyme, with sharp spines derived from aborted axillary buds (Fig. 4.4C).

Distribution and ecology:

This species occurs in the Western Cape and Namaqualand, and is the only species in the subgenus to extend into the Little Karoo towards Uniondale (Fig. 4.9F). Plants grow in a range of soils, including quartzite, shale and sand, but generally in shallow soils. This species flowers in October.

Additional specimens examined:

South Africa. NORTHERN CAPE: **3018 (Kamiesberg):** Namaqualand, Varsche River Extension, Vanrhynsdorp, quartz lag gravel patch between Preekstoelkop and N7 (–BB), *Desmet and Ellis 1345* (NBG); 35 miles west of Loeriesfontein (–DD), *Lavis 162* (BOL). WESTERN CAPE: **3218 (Clanwilliam):** On sand flat near Calvinia road, about 2 miles out of Clanwilliam (–BA), *Galpin s.n.* (BOL); Namaqualand, Wolwenes, Knersvlakte, Heuweltjie veld to west of N7 (–BC), *Desmet and Ellis 1498* (BOL); Rondegat, Clanwilliam (–BD), *Esterhuysen s.n.* (BOL). **3219 (Wuppertal):** Syfering, west of Elandsvlei (–AD), *Klak 1370* (BOL); Cederberg, farm Ramkraal (–AD), *Klak 953* (BOL); Skitterykloof (–DC), *Klak 2424* (BOL**)**. **3320 (Montagu):** 6 km west of Matjiesfontein, farm Aasvoelbos (–AB), *Klak 1685* (BOL). **3323 (Willowmore):** along road to Willowmore, Uniondale (–DA), *Fourcade 4370* (BOL).

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Figure 4.15. Drawing of type material for *Schlechteranthus spinescens* (*Compton sub BOL 19081* (BOL)). (A) Habit and leaf arrangement of *S. spinescens*; (B) enlarged flower in bud; (C) enlarged leaf, showing opposite leaf pairs; (D) open capsule with small closing bodies and complete covering membranes; (E) flower with all staminodes removed showing the nectaries arranged in a ring; (F) longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes; (G) floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A = 10 mm; B–C = 5 mm; D = 2.5 mm; E = 3.3 mm; F–G = 2 mm. Artist B. Carter.

4.4.3.7 ***Schlechteranthus pungens*** (H.E.K.Hartmann) R.F.Powell, Taxon 65: 259. 2016.

Arenifera pungens H.E.K.Hartmann, Bradleya 14: 37. 1996; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 1: 69. 2001; Buys in Snijman, Pl. Gr. C.F.R. 2: 178. 2013. *Type*: South Africa, Western Cape Province: Vanrhynsdorp [3118], Mar 1988, *Hartmann, Dehn, Gölling, Rust and Stüber 25739* (holotype: HBG–image!).

Large upright to spreading shrub, often dome-shaped, to 400 mm tall. *Branches* to 4 mm in diameter; upper branches white; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, mostly oblong, sometimes ovoid in outline, 3.0–6.0 x 2.0–2.5 mm, leaf pairs fused for ¼ to ⅓ of length; mucro prominent; slightly velvety; dark green. *Flowers* in compound cymose inflorescences; central primary axis to 13 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged in compound cyme, secondary axes with spines arranged in compound cyme, tertiary axes with spines arranged in simple cyme; spines derived from aborted axillary buds and less frequently from old peduncles; spines branching at an angle <45°, sharp, elevated above terminal leaf pair; filamentous staminodes few to many, forming an incomplete to complete series around stamens. *Fruit* 7- to 8-locular; top convex, base funnel-shaped, 2.0–3.0 x 4.0–4.5 mm; closing rodlet conspicuous; valve wings very narrow, up to $^1/_3$ width of valve.

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Diagnostic characters:

Schlechteranthus pungens is most similar to *S. stylosus*, as both species are large shrubs (up to 400 mm tall) with spines derived from aborted axillary buds (Fig. 4.4), but *S. pungens* differs with longer (3.0–6.0 mm) mostly oblong leaves (Fig. 4.2E) in outline (shorter (1.5–2.0 mm) and ovoid (Fig. 4.2H) in *S. stylosus*). Although the spines are

derived in a similar manner to *S. stylosus*, the spines in *S. pungens* are arranged in a compound cyme on the secondary axes (Fig. 4.4C, while in *S. stylosus*, the spines are arranged in a simple cyme on the secondary axes (Fig. 4.4B).

Schlechteranthus pungens and *S. spinescens* share a similar leaf shape (Fig. 4.2B, E) and their distributions overlap (Fig. 9F, G). However, *S. pungens* is distinguished by the spines that are arranged in a compound cyme and are derived from aborted axillary buds (Fig. 4.4C) (spines arranged in simple cyme and derived from old peduncles where capsules have fallen off in *S. spinescens* (Fig. 4.4D)).

Distribution and ecology:

This species has a wide distribution, occurring from Hondeklipbaai southwards to Calvinia (Fig. 4.9G) and is found on low slopes, growing in very rocky soils. *Schlechteranthus pungens* flowers in September.

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Additional specimens examined:

South Africa. NORTHERN CAPE: **2817 (Vioolsdrif):** Namaqualand District, Richtersveld, southwest of Helskloof Valley between hills where road splits (–CD), *Burgoyne 11105* (PRE). **2917 (Springbok):** Steinkopf, west of Steinkopf (–BC), *Klak 925* (BOL); between Bulletrap and the N7 (–BD), *Powell 12* (NBG); Namaqualand District. 6 km on second Komaggas road W of Springbok (–CD), *Burgoyne 11235* (PRE). **2918 (Gamoep):** 26 km east of Springbok, farm Smorenskadu (–CA), *Klak 2358* (BOL). **3017 (Hondeklipbaai):** Namaqualand, Kookfontein, north-northeast of Soebatsfontein (–BA), *Desmet 400* (NBG). **3018 (Kamiesberg):** 23 miles east of Kliprand (–DA), *Stayner sub KGB 1208/62* (BOL); 35 miles west of Loeriesfontein, Calvinia (–DD), *Stayner 1970* (BOL). **3019 (Loeriesfontein):** Loeriesfontein, Donkiedam Farm (–CC), *Klak 1285*

(BOL); Loeriesfontein Dist., Donkiedam Farm (–CC), *Klak 1291* (BOL). WESTERN CAPE: **3118 (Vanryhnsdorp):** Bitterfontein, Komkans (–AA), *Bruyns 12804* (BOL); Nuwerus, on farm Aurora (–AB), *Bruyns 7583* (BOL); 2 km south of Nuwerus (–AB), *Bruyns 9093* (BOL); Namaqualand, Farm Moedverloor 208 (–AD), *Desmet and Ellis 1459* (NBG); Namaqualand, Droesand, Rooipan, Knersvlakte (–BC), *Desmet and Ellis 1508* (NBG); Zout Rivier Bridge (–BC), *Matthews sub NBG 282/38* (BOL); Knersvlatke, farm Arizona, behind old farmhouse shed (–BC), *Klak 1450* (BOL). **3119 (Calvinia):** Blaukrantz pass (–DB), *Pearson 3909* (BOL); South-western corner of Elandsberg (–DC), *Bruyns 9180* (BOL). **3219 (Wuppertal):** Bossiesberg, Calvinia (–BA), *Bruyns 7568* (BOL).

4.4.3.8 ***Schlechteranthus stylosus*** (L.Bolus) R.F.Powell, Taxon: 259. 2016. *Ruschia stylosa* L.Bolus, Notes Mesembryanthemum 1: 144. 1928. *Mesembryanthemum stylosum* (L.Bolus) N.E.Br., Gard. Chron. 3: 32. 1930. *Eberlanzia stylosa* (L.Bolus) L.Bolus, Notes Mesembryanthemum 3: 387. 1958. *Arenifera stylosa* (L.Bolus) H.E.K.Hartmann, Bradleya 14: 38. 1996; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 1: 70. 2001; Buys in Snijman, Pl. Gr. C.F.R. 2: 178. 2013. *Type*: South Africa, Northern Cape Province: Hills NE of Arris Drift [2816DA], Jul 1927, *Pillans 5742* (holotype: BOL!).

Ruschia armata L.Bolus, Notes Mesembryanthemum 1: 145. 1928. *Mesembryanthemum armatum* N.E.Br., Gard. Chron. 3: 32. 1930. *Eberlanzia armata* (L.Bolus) L.Bolus, Notes Mesembryanthemum 3: 387. 1958. *Type*: South Africa, Northern Cape Province: between Brakfontein and Oograbies Mt., Little Namaqualand [2917AA], Oct 1926, *Pillans 5789* (holotype: BOL!).

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Dense spreading shrub, often dome-shaped, to 400 mm tall. *Branches* to 7 mm in diameter; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, ovoid in outline, 1.5–2.0 mm x 2.0–2.5 mm, leaf pairs fused for ⅓ to ½ of length; mucro prominent; slightly velvety; yellow-green to deep green. *Flowers* in compound cymose inflorescences; central primary axis to 8 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged in compound cyme, secondary axes with spines arranged in simple cyme; spines derived from aborted axillary buds and less frequently from old peduncles; spines branching at ± 45°, sharp, elevated above terminal leaf pair; filamentous staminodes few to absent, forming an incomplete series around the stamens. *Fruit* 7 to 9-locular; top convex, base funnel-shaped, ca. 4.0 x 3.0 mm; closing rodlet conspicuous; valve wings absent. Figure 4.16.

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Diagnostic characters:

Schlechteranthus stylosus may be confused with *S. parvus* as they have a similar leaf size with a high degree of fusion (Fig. 4.2H, I) and the same spine arrangement (Fig. 4.4B). However, *S. stylosus* can be distinguished by the few filamentous staminodes which do not form a complete series around the stamens (many filamentous staminodes which form a complete series in *S. parvus*). The leaves are also ovoid in outline in *S. stylosus* (subglobose in *S. parvus*) with a very prominent mucro (mucro obscure in *S. parvus*). *Schlechteranthus stylosus* is also typically a larger shrub (up to 400 mm in height) compared to *S. parvus* (up to 150 mm in height).

Schlechteranthus stylosus may be confused with *S. pungens*, as both species are large shrubs with spines derived from aborted axillary buds (Fig. 4.4), but *S. stylosus* is distinguished by the ovoid leaves in outline (mostly oblong in *S. pungens*) and spines

arranged in a simple cyme on the secondary axes (Fig. 4.4B) (spines arranged in compound cyme on secondary axes in *S. pungens* (Fig. 4.4C)).

Distribution and ecology:

Schlechteranthus stylosus is distributed widely in northern Namaqualand, occurring from the Richtersveld to just south of Springbok (Fig. 4.9H). The species is found solely on rocky quartzite soils, generally on flats, but sometimes found on the lower slopes of hills. This species flowers from August to September.

Additional specimens examined:

South Africa. NORTHERN CAPE: **2816 (Oranjemund):** Arris drift (–DA), *Hall sub NBG 116/53* (BOL); Sendelings Drift (–DB), *Hall s.n.* (BOL). **2817 (Vioolsdrif):** On pass up the Vandersterberg towards Koeskopfontein (–AC), *Powell 75* (NBG); Grasdrif, Richtersveld (–AD), *Williamson 3267* (BOL); Richtersveld between Helskloof se Hek and Eksteenfontein (–CA), *Williamson 3304* (BOL); Gelykberg, Richtersveld (–CA), *Hall s.n.* (BOL); Lekkersing, 5 km north of Uitspanpoort (–CC), *Bruyns 9123* (BOL); Cape Prov. Nama'land, Richtersveld, Rooiberg middlemost (–CD), *NBG 423/61* (BOL); Rooiberg, Richtersveld (–CD), *NBG 432/62* (BOL); ± 15 km north of Kosies (–DC), *Bruyns 9252*. **2917 (Springbok):** About 12 km W of Bulletrap (–BC), *Klak 1255* (BOL); Springbok area on road to Komaggas; ± 5 km from Namaqua National Park gate (–CD), *Burgoyne 11799* (PRE).

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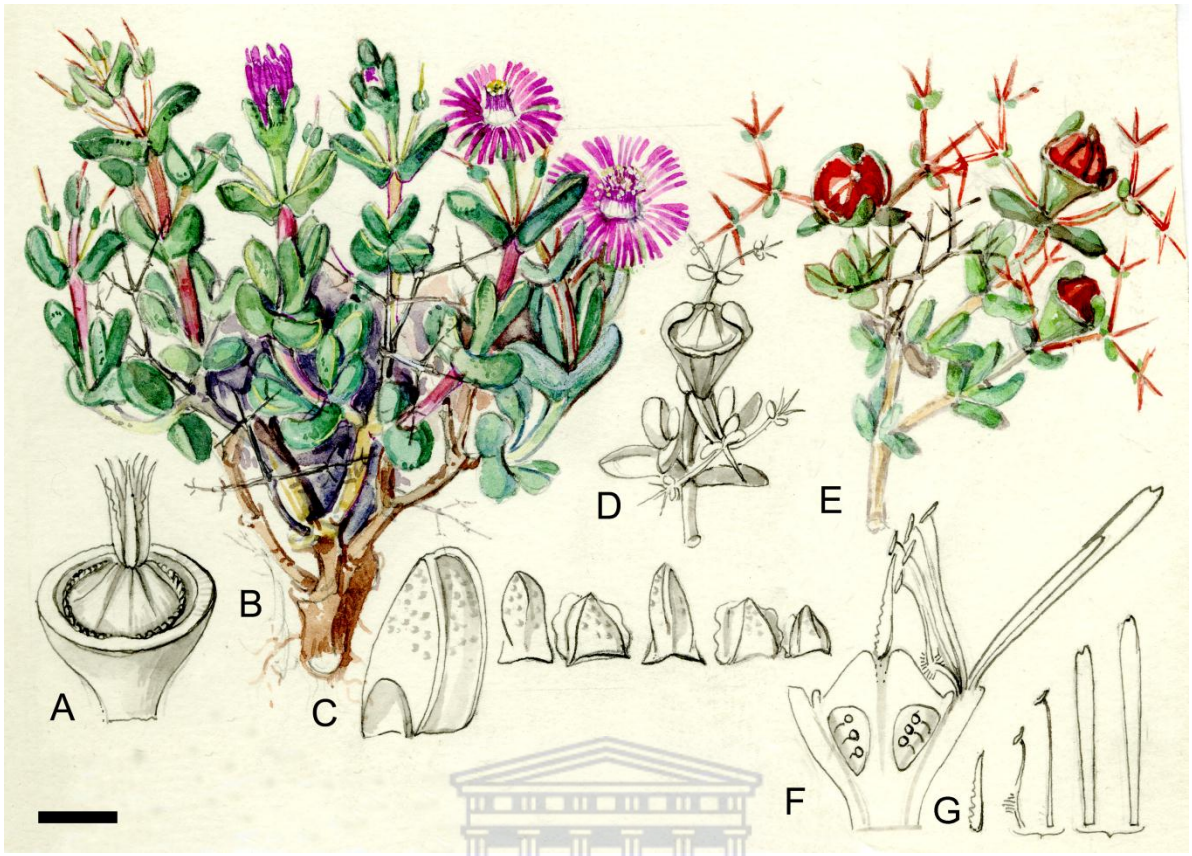

Figure 4.16. Drawing of type material for *Schlechteranthus stylosus* (*Pillans 5789* (BOL)). (A) Flower with all staminodes removed showing the nectaries arranged in a ring; (B) habit and leaf arrangement of *S. stylosus*; (C) enlarged leaf and components of calyx; (D) spine development from aborted axillary buds and cymose inflorescence; (E) new growth with early stages of development of spines and capsules; (F) longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes; (G) floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A = 2.5 mm; B= 10 mm; C = 5 mm; D–E = 10 mm; F–G = 3.3 mm. Artist M. Page.

4.4.3.9 ***Schlechteranthus parvus*** R.F.Powell & Klak, **sp. nov.** *Type*: South Africa, Northern Cape Province: Komaggas, alongside road between Koingnaas and Komaggas [3017AA], 15 May 2014, *Powell, Klak and Magee 41* (holotype: NBG!; isotype: BOL!).

Small erect to spreading dome-shaped shrublet to 150 mm tall. *Branches* to 4 mm thick; upper branches brown; leaves crowded towards tips of branches, old not remaining on the branches. *Leaves* triquetrous, globose to subglobose in outline, 2.0–4.0 x 1.5–2.0 mm, leaf pairs fused for ¾ of the length; mucro obscure; slightly velvety; yellow-

green. *Flowers* in compound cymose inflorescences; central primary axis to 4 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged in compound cyme, secondary axes with spines arranged in simple cyme; spines derived from aborted axillary buds and less frequently from old peduncles; spines branching at ± 45°, sharp, elevated above terminal leaf pair; filamentous staminodes many, forming a complete series around the stamens. *Fruit* 7- to 8-locular; top convex, base funnel-shaped, ± 3.9 x 4.7 mm; closing rodlet conspicuous; valve wings absent. Figure 4.5.

Etymology:

The species epithet refers to the small nature of the shrub and its leaves.

Diagnostic characters:

Schlechteranthus parvus is the smallest among the spiny species in subgenus *Microphyllus* and only reaches 150 mm in height. It is most similar to *S. stylosus* as they share small leaves with a high degree of fusion (Fig. 4.2H, I) and the same spine arrangement, with spines derived from aborted axillary buds (Fig. 4.4B). However, *S. stylosus* is a much larger shrub and reaches 400 mm in height.

The new species is distinguished by its many filamentous staminodes, which form a complete series around the stamens (Fig. 4.5A) (filamentous staminodes in *S. stylosus* are few or absent, forming an incomplete series around the stamens). The leaf shape can also be used to distinguish the species, with *S. parvus* possessing leaves subglobose in outline without a prominent mucro (leaves ovoid with a very prominent mucro in *S. stylosus*). *Schlechteranthus parvus* shares a globose leaf shape with *S. subglobosus* (Fig. 4.2D, I) but can be distinguished by the highly fused leaves, the obscure mucro (leaf pairs

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fused for $^{1}/_{3}$ of length and mucro very prominent in *S. subglobosus*), and also by the flowers arranged in a compound cymose inflorescence (flowers solitary in *S. subglobosus*).

Distribution and ecology:

Schlechteranthus parvus occurs along the western coast of Namaqualand (Fig. 4.9I), associated with quartz patches. Although the species has not been collected in flower, the species flowered in September in the greenhouse at Kirstenbosch.

Additional specimens examined:

South Africa. NORTHERN CAPE: **2917 (Springbok):** 19 km south of Port Nolloth on road to Grootmis, Kwaggakop River, east of road on low hillocks (–AC), *Klak 2387* (BOL); west of Wolfberg mine, along road from Kleinzee to Springbok (–CB), *Klak 2400* (BOL). **3017 (Hondeklipbaai):** Komaggas, alongside road between Koingnaas and Komaggas (–AA), *Klak 2362* (BOL).

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Chapter 5

Inclusion of *Ihlenfeldtia* and *Odontophorus* in *Cheiridopsis*, with insights into generic and subgeneric circumscriptions within the *Conophytum*-clade

5.1 INTRODUCTION

Twelve morphological groupings based mainly on capsule characters were recognised by Hartmann (1991) and generic circumscriptions of Aizoaceae were assessed within these groups, however, the weight given to capsule characters has sometimes resulted in unnatural classifications, particularly those based on continuous characters. For example, the species of *Ihlenfeldtia* H.E.K.Hartmann were separated from *Cheiridopsis* N.E.Br., and placed in a group with *Tanquana* H.E.K.Hartmann & Liede and *Vanheerdia* L.Bolus (Hartmann, 1992), based solely on the smaller closing bodies in the capsules. Subsequent phylogenetic data however places *Ihlenfeldtia* in a clade with *Cheiridopsis* and *Odontophorus* N.E.Br. (Klak *et al.*, 2013; Powell *et al.*, 2016).

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Cheiridopsis is a genus of caespitose plants, characterised by the old leaves that form a sheath enclosing the emerging leaf pair, either completely or partially (Hartmann & Dehn, 1987). The genus was revised by Hartmann & Dehn (1987), with 23 species classified into three subgenera. Subsequently, an additional 12 species were described, with several species known from only the type locality (Hammer, 1994, 1996; Hartmann, 2001; Hammer & Desmet, 2002; Klak & *et al.*, 2015). Additionally, two species of *Cheiridopsis* were separated to form the new genus, *Ihlenfeldtia* (Hartmann, 1992).

Odontophorus was included in the same capsule morphological grouping as *Cheiridopsis* (Hartmann, 1991), and was last revised by Hartmann (1976). As the generic name suggests, *Odontophorus* has distinctly toothed keels on the leaves, however, this character is shared with some species of *Cheiridopsis* (Hammer, 1996) and the similarity of

leaf and capsule morphology of these genera has been noted previously (Hammer, 1994; Klak *et al.*, 2015). In fact, *Cheiridopsis* subgenus *Odontophoroides* H.E.K.Hartmann, is named for its similarity to species of *Odontophorus* (Hartmann & Dehn, 1987).

Cheiridopsis, *Ihlenfeldtia* and *Odontophorus* all share a caespitose habit, papillate leaves that form sheaths enclosing the emerging leaf pair, radiate flowers with free petaloid staminodes and large woody, multilocular capsules (Hartmann, 2001). The genera are endemic to the arid parts of the Greater Cape Floristic Region of southern Africa, with diversity centred in the Springbok Region (Hartmann, 1976; Hartmann & Dehn, 1987; Hartmann, 1992). The genera are currently differentiated solely based on indiscrete internal capsule characters, with *Ihlenfeldtia* distinguished from *Cheiridopsis* based on the smaller closing bodies (Hartmann, 1992), while *Odontophorus* has narrower valve wings than *Cheiridopsis* (Hartmann, 2001). However, species of *Cheiridopsis* subgenus *Aequifoliae* H.E.K.Hartmann also possess narrow valve wings (Hartmann & Dehn, 1987), highlighting the overlap in characters between these genera.

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The phylogenetic analyses of the Ruschieae (Klak *et al.*, 2013), places *Cheiridopsis*, *Ihlenfeldtia* and *Odontophorus* in the *Conophytum*-clade. The genera in the clade include five of the nine morphological groups recognised by Hartmann for the Ruschieae (1991), illustrating the diversity in leaf, floral and capsule morphology of the clade. Due to this diversity in morphological characters, it has as yet not been possible to find a clear synapomorphy for the clade (Klak *et al.*, 2013). The genera do, however, share mostly multilocular capsules of the complex type, with covering membranes and closing bodies, with the exception of *Conophytum* N.E.Br., *Jensenobotrya* A.G.J.Herre and *Ruschianthus* L.Bolus, which have simple capsules without covering membranes and closing bodies (Hartmann, 2001). The leaves in the majority of the genera are papillate and in *Conophytum*, *Cheiridopsis*, *Ihlenfeldtia* and *Odontophorus*, the old leaves form a sheath that encloses the

emerging leaf pair, either completely or partially (Hartmann, 2001). The sheath almost always fully encloses the emerging leaf pair during the dormant period in *Conophytum*, with the exception of *C. herreanthus* S.A.Hammer, where the sheath remains basal (Hammer, 2002). This species is unusual and was previously recognised as a monotypic genus (viz. *Herreanthus* Schwantes), but is currently included in *Conophytum* based on the diagnostic fused petaloid staminodes that form a tube (Hammer, 1993).

Recent phylogenetic analyses of the Ruschieae (Klak *et al.*, 2013) and phylogenetic expansion of sampling within the *Conophytum*-clade, specifically of the *Schlechteranthus* Schwantes subclade (Powell *et al.*, 2016; Chapter 3), has revealed novel phylogenetic relationships between the genera and species in the clade. This phylogenetic data, in combination with morphological and leaf anatomical data, has led to the reassessment of generic circumscription within the *Schlechteranthus* subclade (Powell *et al.*, 2016; Chapter 3). Although leaf anatomy has often been used to classify taxa in the family (Reule, 1937; Ihlenfeldt & Bittrich, 1985; Klak & Linder, 1998; Landrum, 2001), pollen exine and colpi structure is seldom used. Bittrich (1987) and Dupont (1977) surveyed the pollen exine and colpi structure across the Mesembryanthemoideae and Ruschioideae, respectively, and found variation in ornamentation of the exine. In the Ruschioideae, the majority of species were tricolpate, but it was found that *Jensenobotrya* had syncolpate pollen (Dupont, 1977). Although the study also included some species of *Conophytum* (Dupont, 1977), the exine structure of the remaining genera in the *Conophytum*-clade is unknown.

In this chapter the phylogenetic sampling of *Cheiridopsis*, *Ihlenfeldtia* and *Odontophorus* is expanded to include the majority of species in these genera, while sampling in *Conophytum* is expanded to represent the large sections (eight of sixteen sections) in the genus. Generic circumscription in the *Conophytum*-clade is investigated using phylogenetic, morphological, anatomical and palynological data. The relationship between *Cheiridopsis*,

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Ihlenfeldtia and *Odontophorus* is assessed and the subgeneric circumscription of *Cheiridopsis* refined.

5.2 MATERIALS AND METHODS

5.2.1 PHYLOGENETIC DATA

Phylogenetic sampling in the *Conophytum*-clade (Klak *et al.,* 2013) was expanded by 175 new sequences for six plastid gene regions. This included 24 of the 32 species of *Cheiridopsis,* the two species of *Ihlenfeldtia,* two of the four species of *Odontophorus.* Five species of *Conophytum,* representing eight (of 16) sections in the genus: *Conophytum bilobum* (Marloth) N.E.Br. (section *Biloba* N.E.Br.), *C. bruynsii* S.A.Hammer (section *Minuscula* (Schwantes) Tischer ex S.A.Hammer), *C. calculus* (A.Berger) N.E.Br. (section *Cataphracta* Schwantes ex S.A.Hammer), *C. ficiforme* N.E.Br. (section *Conophytum*), *C. friedrichiae* Schwantes (section *Ophthalmophyllum* (Dinter & Schwantes) G.D.Rowley), *C. herreanthus* (section *Herreanthus* (Schwantes) S.A.Hammer), *C. maughanii* N.E.Br. (section *Chesire-Feles* S.A.Hammer) and *C. wettsteinii* N.E.Br. (section *Wettsteinia* (Schwantes) Tischer ex S.A.Hammer) were also sampled. Section *Herreanthus* and section *Ophthalmophyllum* represent two previously recognised genera now included in *Conophytum* (Hammer, 1993). Material was collected in the field and several rare species were obtained from greenhouse collections at the Karoo Botanical Gardens, the South African National Biodiversity Institute, Kirstenbosch as well as from the Sphaeroid Institute (California, USA). The voucher specimens for these samples processed following Section 2.1.1 of Chapter 2 are housed at the Compton Herbarium (NBG). Appendix A provides voucher information for the samples.

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Total DNA was extracted and amplified following the methods outlined in Section 2.4.1 of Chapter 2 for the six plastid gene regions *matK, rpl16, rps16, trnL–F, trnQ–rps16* and *trnS–trnG.* The new sequences, together with published sequences of the *Conophytum*-

clade from Klak *et al.* (2013) and Powell *et al.* (2016), were automatically aligned following the methods outlined in Section 2.4.2 of Chapter 2 with *Knersia diversifolia* (L.Bolus) H.E.K.Hartmann & Liede (= *Drosanthemum diversifolium* in Klak *et al.*, 2013) and *Jacobsenia vaginata* (L.Bolus) Ihlenf., selected as outgroup taxa, as they were recovered as sister to the *Conophytum*-clade in the previous analyses (Klak *et al.*, 2013; Powell *et al.*, 2016). The combined plastid dataset included a total of 4,506 characters and were analysed using Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP), following the methods outlined in Section 2.4.2 of Chapter 2.

For the BI analysis the combined plastid data were partitioned into 6 partitions. Separate matrices for the different gene regions were run in JModel test version 2.1.7 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) and the following models were suggested for the specific gene regions: HKY for *matK*, *trnL–F* and *rpl16* (Hasegawa *et al.*, 1985); F81 for *trnS–trnG* (Felsenstein, 1981) and F81+G for *rps16* and $trnQ^{UUG}$*–rps16* (Felsenstein, 1981) which were implemented accordingly. Two simultaneous runs were completed for 3,500,000 generations with a sampling frequency of 100. The standard deviation between the split frequencies stabilised below 0.01 and suboptimal trees were discarded as the "burn-in" phase. The remaining 7,002 trees were used to construct a 50% majority rule consensus tree with posterior probabilities (PP).

A topological constraint analysis was conducted following the methods of Section 2.4.3 of Chapter 2.

5.2.2 Leaf Anatomical Data

Fresh leaf material was sampled from the field and greenhouse collections for anatomical study (Appendix B). This included a representative sampling from the subgenera in *Cheiridopsis*, with seven species from subgenus *Cheiridopsis* N.E.Br., six species from

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subgenus *Aequifoliae* and six species from subgenus *Odontophoroides.* Two species of *Ihlenfeldtia*, as well as one species of *Odontophorus* were also included. Fresh leaf material for *Enarganthe octonaria* (L.Bolus) N.E.Br. was obtained from field collections, while *Jensenobotrya lossowiana* A.G.J.Herre and *Namaquanthus vanheerdei* L.Bolus were sampled from greenhouse collections at Kirstenbosch. *Ruschianthus falcatus* L.Bolus was not relocated in the field, nor was a living collection of the species available. Leaf anatomical data for *Conophytum* and *Schlechteranthus* were obtained from literature (Opel, 2005a; Powell *et al.*, 2016; Chapter 3).

Transverse leaf anatomical sections for the sampled species were prepared following the methods outlined in Section 2.5 of Chapter 2.

5.2.3 Pollen Exine and Colpi Structure

Pollen samples were collected from greenhouse collections and herbarium specimens for 18 taxa across the *Conophytum*-clade (Appendix C). These taxa were selected as they are representative of the genera in the *Conophytum*-clade, as well as representing the subgeneric classifications within the clade. This data was supplemented by previous palynological studies of the subfamily (Dupont, 1977). Pollen sampled were prepared and viewed following the methods outlined in Section 2.6 of Chapter 2.

5.2.4 Morphological Character Reconstruction

Specimens of the *Conophytum*-clade examined for morphological study included the complete collections available at BOL and NBG (including SAM).

The morphological character of sheathing (old leaves forming a sheath enclosing the emerging leaf pair) versus non-sheathing leaves, as well as sheath type, was mapped onto the BI consensus tree following methods of Section 2.3 in Chapter 2.

5.2.5 DISTRIBUTIONAL DATA

Distribution data for the species of *Cheiridopsis* s.l. (as treated in the taxonomic treatment, i.e. including *Ihlenfeldtia* and *Odontophorus*) was obtained from the 599 databased specimens in BOL, NBG (including SAM) and PRE. Species richness Quarter Degree Square (QDS) maps were produced for *Cheiridopsis* and the subgenera following the methods outlined in Section 2.2.2 of Chapter 2.

5.3 RESULTS

5.3.1 PHYLOGENETIC ANALYSES

The combined plastid matrix for the six gene regions consisted of 4506 unambiguously aligned positions with 350 variable characters and 114 parsimony informative characters. In the MP analysis, 899 trees were retained with a tree length of 540 steps (consistency index (CI)=0.90; retention index (RI)=0.81). The phylogenies produced in the BI, ML and MP analyses were all consistent with one another in topology, with slight differences in resolution and support values (Figs. 5.1–5.3). The BI analysis provided the most resolved phylogenetic tree, with the strongest support values (Fig. 5.1). The phylogenetic tree produced by the ML analysis provided a less resolved topology, but with relatively strong support values, especially within clades (Fig. 5.2). The phylogenetic tree recovered from the MP analysis was poorly resolved and showed relatively low nodal support values when compared to the other analyses (Fig. 5.3).

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Two main clades were recovered, one including the species of *Cheiridopsis*, *Conophytum*, *Ihlenfeldtia*, *Odontophorus* (PP=0.95, BS=54; Figs. 5.1, 5.2) and the other contained the species of *Schlechteranthus* (PP=1, BS=100, JK=92; Figs. 5.1–5.3). The placement of *Enarganthe* N.E.Br., *Jensenobotrya*, *Namaquanthus* L.Bolus and *Ruschianthus* remained unresolved within the *Conophytum*-clade (Figs. 5.1–5.3).

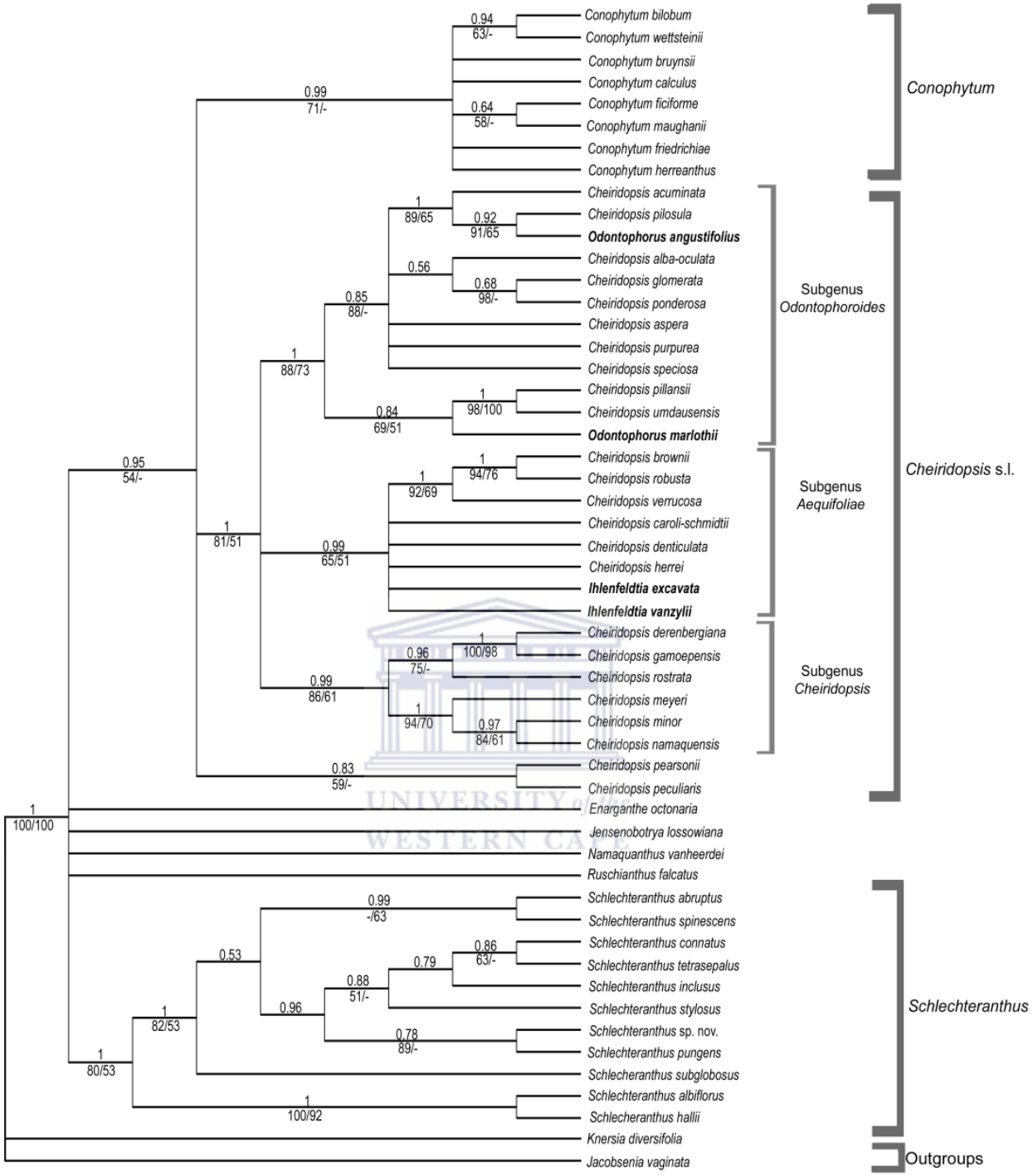

Figure 5.1. Majority Rule Consensus tree from Bayesian analysis of six plastid markers, indicating phylogenetic relationships in the *Conophytum*-clade. Posterior probability (PP) values above 0.5 are indicated above the branches. Jackknife support values (JS) and Bootstrap supports (BS) above 50 from the maximum parsimony and maximum likelihood analyses are indicated below the branches. Brackets indicate the placement of and clades discussed and species of embedded genera are indicated in bold.

Three clades were recovered within the *Conophytum–Cheiridopsis* clade, one comprising the species of *Conophytum* (PP=0.99, BS=71; Figs. 5.1–5.3), the second including species of *Cheiridopsis*, *Ihlenfeldtia*, and *Odontophorus* (PP=1, BS=81, JK=54; Figs. 5.1–5.3) and the third with *Cheiridopsis pearsonii* N.E.Br. and *C. peculiaris* N.E.Br. (PP=0.83, BS=59; Figs. 5.1–5.3).

Cheiridopsis is rendered paraphyletic by the inclusion of *Ihlenfeldtia* and *Odontophorus*, as well as by the unresolved affinity of *C. pearsonii* and *C. peculiaris* (Figs. 5.1–5.3). The sampled *Odontophorus* species were placed in a clade with the species of *Cheiridopsis* subgenus *Odontophoroides* (PP=1, BS=65, JK=73; Figs. 5.1–5.3) and the genus was recovered as polyphyletic in this clade, with *O. angustifolius* L.Bolus placed sister to *C. pilosula* L.Bolus (PP=0.92, BS=91, JK=65; Figs. 5.1–5.3) and *O. marlothii* N.E.Br. recovered as sister to *C. umdausensis* L.Bolus and *C. pillansii* L.Bolus (PP=0.84, BS=69, JK=51, Figs. 5.1–5.3). The subgenus *Odontophoroides* clade also included three species of subgenus *Aequifoliae*, i.e. *C. glomerata* S.A.Hammer, *C. pillansii* and *C. purpurea* L.Bolus (Figs. 5.1–5.3). The two species of *Ihlenfeldtia* were placed in a clade with species of subgenus *Aequifoliae*, but were unresolved within the clade (PP=0.99, BS=65, JK=51; Figs. 5.1–5.3). The remaining clade within the *Cheiridopsis* clade, is represented by species of subgenus *Cheiridopsis* (PP=0.99, BS=86, JK=61; Figs. 5.1–5.3), excluding *C. pearsonii* and *C. peculiaris*

The constrained monophyly of *Cheiridopsis* placed *Cheiridopsis pearsonii* and *C. peculiaris* directly in the subgenus *Cheiridopsis* clade, with no significant difference between the constrained and unconstrained phylogenies (Templeton: p=0.15, Winning-sites: p=0.50) and a negligible difference of 2 steps in tree length for the constrained (tree length=542 steps) and unconstrained (tree length=540 steps) trees (Table 5.1).

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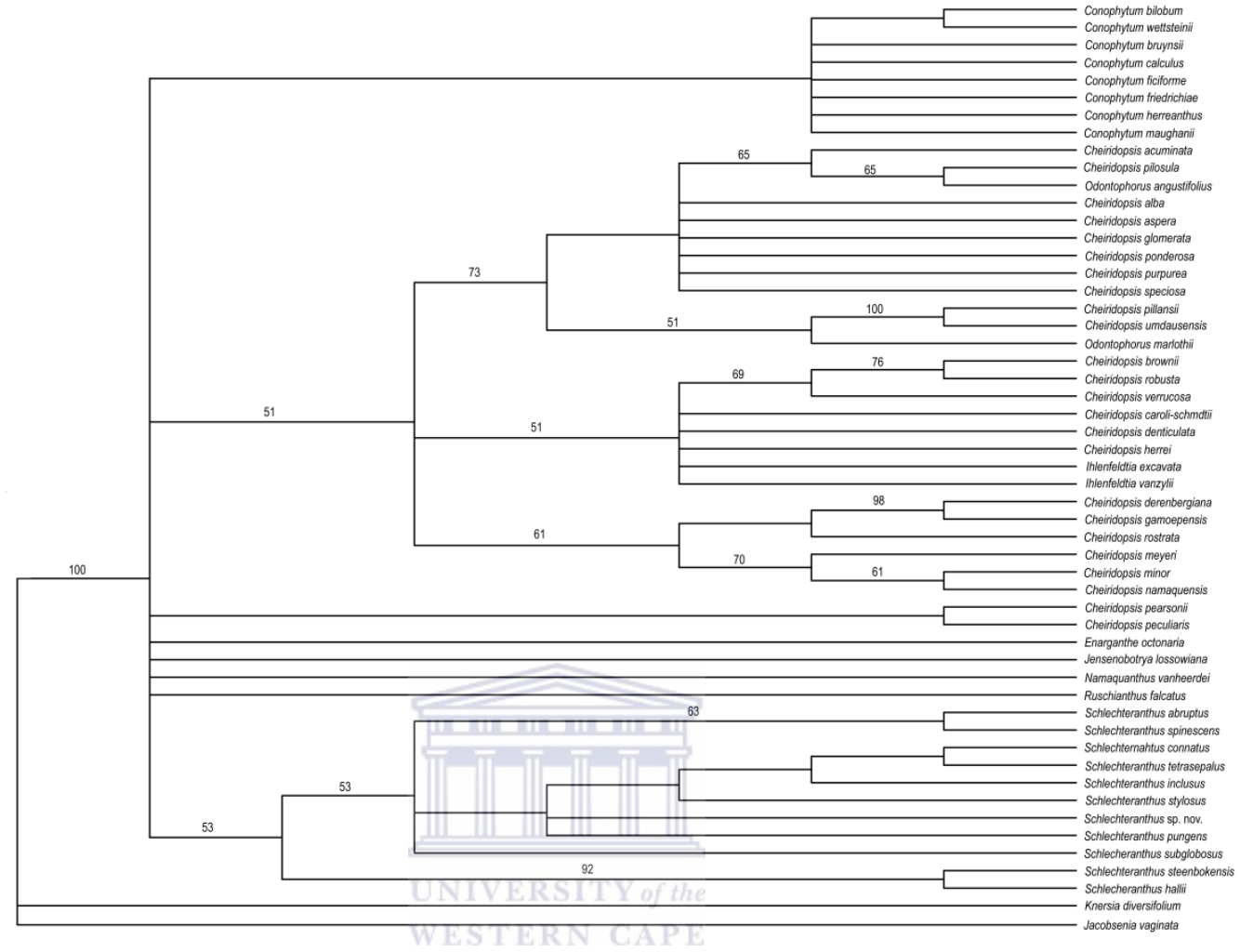

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Figure 5.2. Maximum parsimony estimate based on six plastid gene regions showing the phylogenetic relationships in the expanded *Conophytum*-clade. Jackknife support greater than 50 is indicated above the branches.

Table 5.1. Results of the topological constraint analysis, constraining *Cheiridopsis pearsonii* and *C. peculiaris* to *Cheiridopsis;* p-values reported from the Templeton-Wilcoxon test.

		Templeton				Winning-sites	
Tree	Length	Rank sums	N	z	P	Counts	P
1 (constrained topology)	542	3.0	2	-1.4142	0.1573	2	0.50
2 (unconstrained topology)	540	0.0	-	-	-	0	-

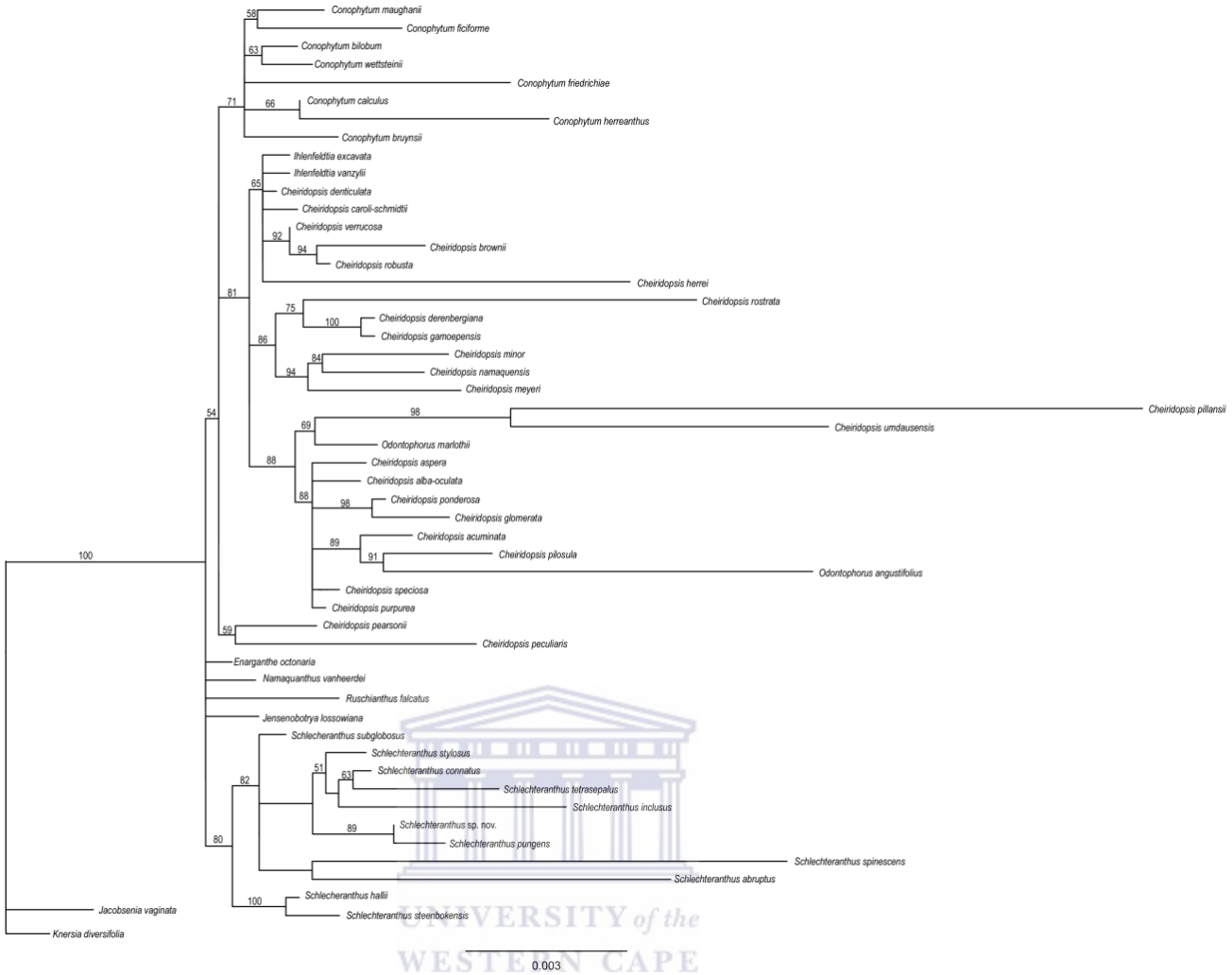

Figure 5.3. Maximum-likelihood estimate based on six plastid gene regions showing the phylogenetic relationships in the expanded *Conophytum*-clade. Bootstrap support greater than 50 is indicated above the branches.

5.3.2 LEAF ANATOMY

Leaf anatomical characters for the species examined are summarised in Table 5.2. A range of papillae formed by the epidermal cells were found across the taxa sampled, with the outer walls of epidermal cells in *Cheiridopsis aspera*, *C. caroli-schmidtii* (Dinter & A.Berger) N.E.Br., *C. gamoepensis* S.A.Hammer, *C. imitans* L.Bolus, *C. minor* (L.Bolus) H.E.K.Hartmann, *C. meyeri*, *C. namaquensis*, *C. peculiaris*, *C. umdausensis* forming blunt papillae (Fig. 5.4A), defined as low protuberances in the centre of the epidermal cell (Opel, 2005a). The epidermal cells in the remainder of the species (*Cheiridopsis acuminata* L.Bolus, *C. denticulata*, *C. herrei* L.Bolus, *C. glomerata*, *C. pilosula*, *C. ponderosa* S.A.Hammer, *C.*

purpurea and *C. verrucosa* L.Bolus, *Ihlenfeldtia vanzylii* (L.Bolus) H.E.K.Hartmann and *Odontophorus marlothii*) produced trichomes, which are defined as outgrowths of epidermal cells that are longer than broad (Opel 2005a) (Fig. 5.4B, C). *Enarganthe octonaria*, *Jensenobotrya lossowiana* and *Namaquanthus vanheerdei* had flat epidermal cells, suggesting no papillae and a glabrous leaf surface (Fig. 5.4D–F). Calcium oxalate crystals were present as deposits on the outer epidermal cell walls in all species, with no evident difference across the species. Tanniferous idioblasts were present in a ring below the epidermal cells in all the species, with raphide bundles scattered throughout the epidermis, at varying densities, across the species.

Cheiridopsis acuminata, *C. pilosula*, *C. ponderosa* and *Odontophorus angustifolius* possessed stomata sunken in the depressions, formed by the underlying tanniferous idioblasts, and protected by parastomal cells that overarch the guard cells. This type of stomatal protection was identified and described by Ihlenfeldt & Hartmann (1982) and referred to as Form II (Fig. 5.4H, I). Form I (Ihlenfeldt & Hartmann, 1982) was found in the remainder of the species, with the stomata placed in depressions, formed by the underlying tanniferous idioblasts below the epidermis, which sculpt the leaf surface into elevation and depressions (Fig. 5.4G).

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5.3.3 Pollen Exine Structure

Three types of pollen exine and colpi structure were found within the *Conophytum*-clade: (1) tricolpate pollen with poorly-defined colpi, (2) tricolpate pollen with well-defined colpi, and (3) syncolpate pollen (summarised in Table 5.2). Tricolpate pollen grains with poorly-defined colpi were the most common, observed in *Cheiridopsis*, *Conophytum*, *Ihlenfeldtia*, *Namaquanthus*, *Odontophorus* and *Schlechteranthus* (Table 5.2), although the exine in *Namaquanthus* was unique in that it was extensively micro-recticulate. Tricolpate pollen with

well-defined colpi was found only in *Enarganthe*. Similarly, syncolpate pollen grains with numerous colpi were found only in *Jensenobotrya* (Table 5.2; Dupont, 1977).

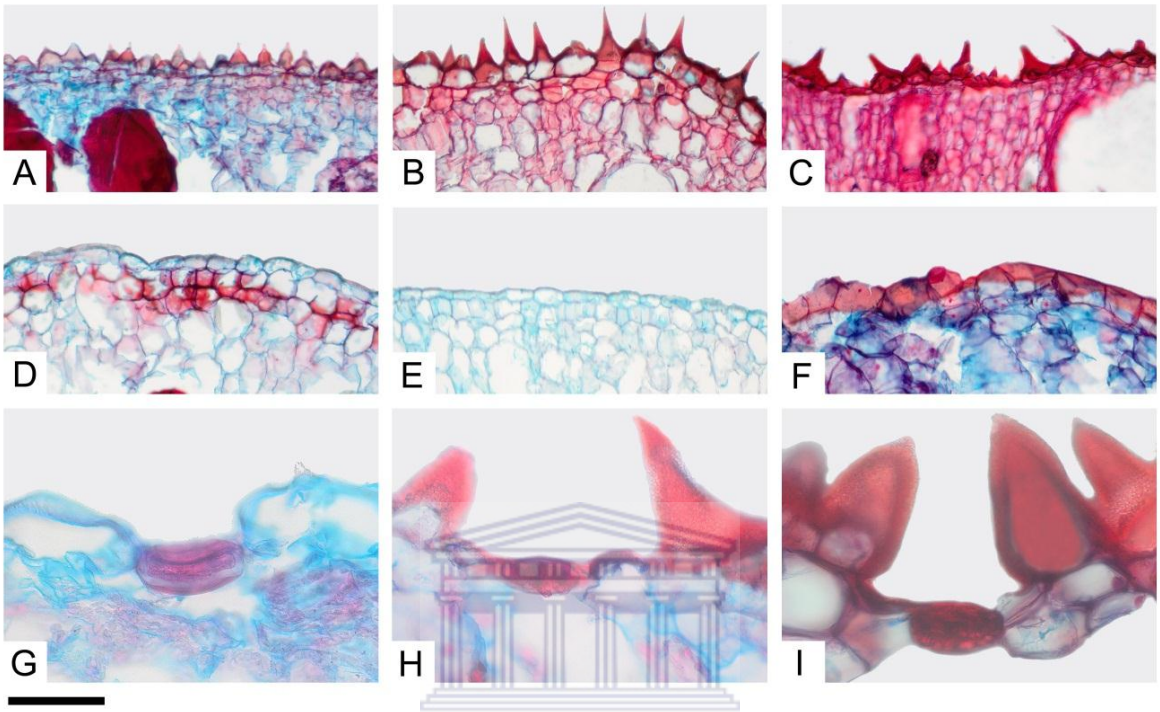

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Figure 5.4. Transverse sections through the leaves of taxa in the *Conophytum*-clade illustrating characters of taxonomic importance. (A) Epidermal cells forming blunt papillae in *Cheiridopsis caroli-schmidtii*; (B) epidermal cells forming trichomes in *Cheiridopsis verrucosa*; (C) trichomes in *Ihlenfeldtia vanzylii*; (D) glabrous epidermis of: (D) *Namaquanthus vanheerdei*; (E) *Enarganthe octonaria*; (F) *Jensenobotrya lossowiana;* (G) stomata in depression, not sunken or hidden in *Cheiridopsis robusta*; (H) stomata in depression, sunken and hidden by parastomal cell in *Cheiridopsis acuminata*; (I) sunken stomata in *Odontophorus angustifolius*. Vouchers: (A) *Powell 105* (NBG); (B) *Powell 99* (NBG); (C) *KBG222/98* (KBG); (D) *van Jaarsveld 2475* (NBG); (E) *Powell 45* (NBG); (F) *SUG 12618* (NBG); (G) *Powell 66* (NBG); (H) *Powell 68* (NBG); (I) *EVJ 106/87* (NBG). Scale: A–F = 200µm; G–I = 50 µm.

5.3.4 Morphological Character Reconstruction

The selected morphological characters (sheathing and sheathing type) were traced with 1 step onto the BI consensus tree. Old leaves which forms sheaths enclosing the emerging leaf pair during dormancy, was reconstructed as a synapomorphy for the clade including *Cheiridopsis, Conophytum, Ihlenfeldtia* and *Odontophorus* (Fig. 5.5A). The complete sheath type, where

the sheath fully encloses the leaves during dormancy, was recovered as a synapomorphy for *Conophytum*, with a loss in *C. herreanthus.* However, due to the poor resolution within this genus, it is unclear if the loss of this sheathing type represents a reversal in *C. herreanthus.* This sheath type was also found in *Cheiridopsis meyeri*, *C. minor*, *C. namaquensis* and *C. peculiaris* (Fig. 5.5B). The majority of *Cheiridopsis* taxa however, had sheaths that do not fully enclose the leaves during dormancy, only partially protecting the leaves, and this character was recovered as a synapomorphy for the genus (Fig. 5.5B).

5.3.5 DISTRIBUTIONAL DATA

The distribution of *Cheiridopsis* follows the winter-rainfall pattern and extends from southern Namibia to Langebaan on the south-western coast of South Africa and eastwards to Pofadder (Fig. 5.6A). Species diversity is centred in the Springbok-Steinkopf Region, with 14 (Springbok) to 15 (Steinkopf) species per QDS. *Cheiridopsis* subgenus *Cheiridopsis* is confined to South Africa and has the most southern distribution, extending to Langebaan in the Western Cape (Fig. 5.6B). The centre of diversity for the subgenus is in the Springbok Region, with 5 species within the QDS (Fig. 5.6B). *Cheiridopsis* subgenus *Odontophoroides* has the smallest distribution range, with species extending from the South Africa-Namibia border, southwards to Garies. Steinkopf is the largest hotspot for the subgenus, with 5 species within the QDS (Fig. 5.6C) and a smaller hotspot in the Richtersveld, containing 4 species in the QDS (Fig. 5.6C). *Cheiridopsis* subgenus *Aequifoliae* is the only subgenus that extends into Namibia, with the distribution of the subgenus extending southwards to Clanwilliam and eastwards to Pofadder (Fig. 5.6D). Subgenus *Aequifoliae* has two centres of diversity, the Springbok-Steinkopf Region (5 species within the QDS) and the Richtersveld (3 species within the QDS) (Fig. 5.6D).

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Table 5.2: Summary of important generic anatomical, morphological and palynological characters in genera of the *Conophytum*-clade.

	Schlechteranthus	***Conophytum***	***Cheiridopsis* s.s.**	***Ihlenfeldtia* s.s.**	***Odontophorus s.s.***	***Enarganthe***	***Jensenobotrya***	***Namaquanthus***	***Ruschianthus***
Habit	Woody shrub	Caespitose	Caespitose	Caespitose	Caespitose, sometimes mat-forming	Woody shrub	Woody shrub	Woody shrub	Caespitose
Leaf shape	Triquetrous to trigonous	Globose to ovoid, rarely square	Triquetrous to trigonous	Triquetrous to trigonous	Triquetrous to trigonous	Clavate to trigonous	Shortly clavate to almost globose	Finger-shaped	Sickle-shaped
Degree of leaf length fused	1/6 to ¾ fused	Usually completely fused, sometimes top ¼ free, rarely completely free	1/6 to 5/6 fused	1/5 to ¼ fused	1/5 to ¼ fused	Leaf pairs not fused	1/5 fused	1/6 fused	1/6 fused
Old leaf forming sheath	No	Yes	Yes	Yes	Yes	No	No	No	No
Epidermal papillae*	Blunt papillae	Blunt papillae to trichomes	Blunt papillae to trichomes	Blunt papillae to trichomes	Blunt papillae to trichomes	Flat epidermal cells, no papillae	Flat epidermal cells, no papillae	Flat epidermal cells, no papillae	Epidermal cells very slightly elevated
Stomatal protection†	Form I and II	Form I and II, and stomata sometimes elevated	Form I and II	Form I	Form I and II	Form I	Form I	Form I	Form I
Petaloid staminodes	Free at base, never forming a tube	Fused, forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube
Flower colour	White to magenta	White, cream, yellow, orange, red or magenta	White, cream, yellow, orange or magenta	Yellow, often purplish suffused, rarely creamy white, red with age	Yellow, often cream-coloured, sometimes white or basally white	Bright reddish-purple	Pink	Bright purple	Green
Filamentous staminodes	Present	Present or absent	Present	Absent	Absent	Absent	Absent	Present	Present
Pollen exine and colpi structure	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi, distinctly micro-reticulate	Syncolpate	Tricolpate with well-defined colpi	Unknown
No of locules	7–12	3–8	8–18	10–15	10	8	5	(8–)12(–17)	5
Covering membranes	Present	Absent	Present	Present	Present	Present	Absent	Present	Absent
Closing bodies	Present	Absent	Present	Present	Present	Absent, placenta sometimes forms hood over locule	Absent	Absent	Absent

Footnotes:
* as defined by Opel, (2005): blunt papillae are low protuberances in the centre of the epidermal cell and trichomes are defined as outgrowths of epidermal cells that are longer than broad.
† Form I – stomata placed in depressions formed by underlying tanniferous idioblasts; Form II – stomata sunken below epidermal surface, in depressions and further protected by overarching parastomal cells (Ihlenfeldt & Hartmann, 1982).

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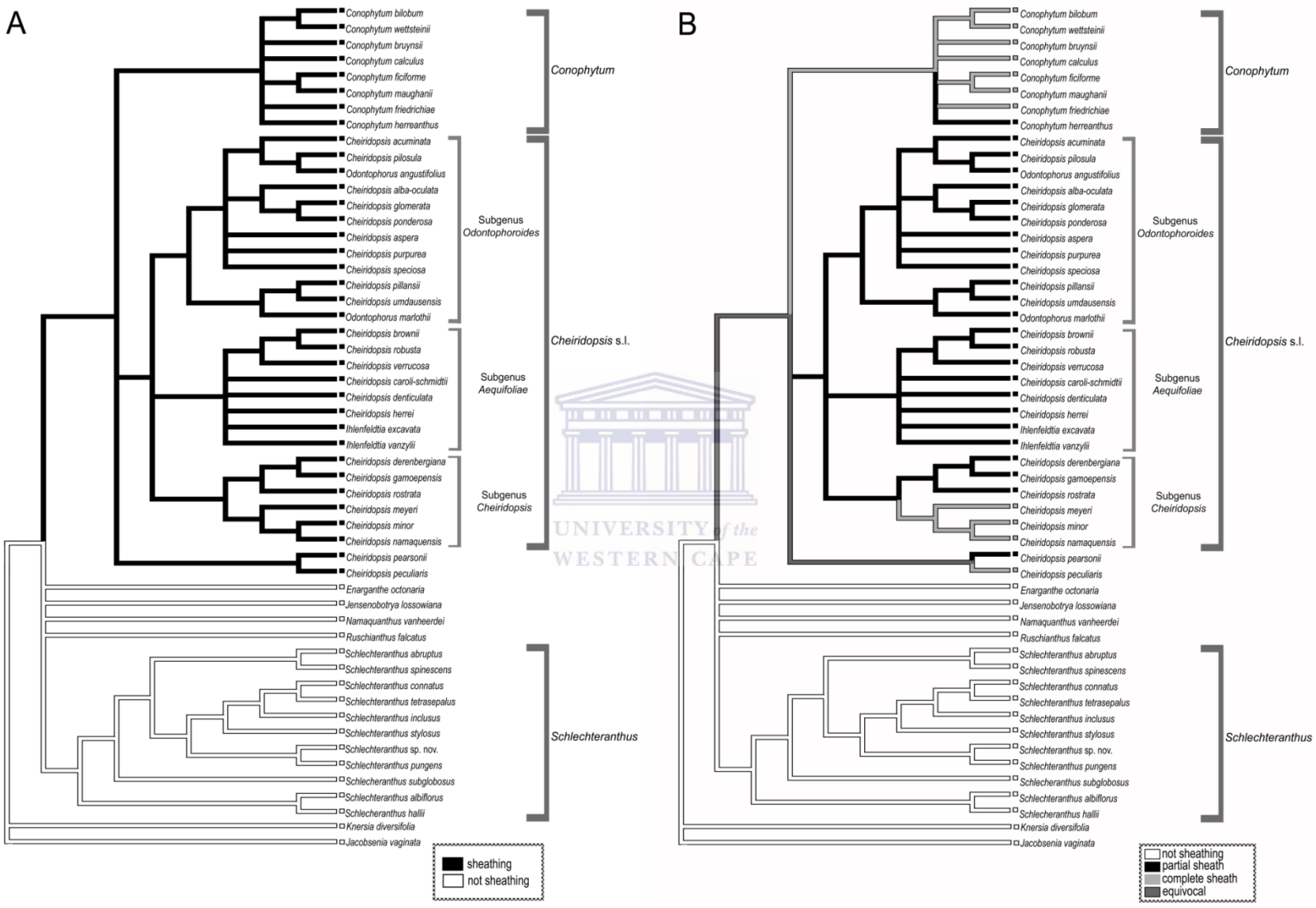

Figure 5.5. Character reconstruction of sheathing and sheathing type on the Bayesian inference consensus tree of the *Conophytum*-clade. (A) Sheathing genera of the *Conophytum*-clade, where old leaves enclose the emerging leaf pair, indicated by the black branches; (B) sheath type mapped onto the Bayesian inference consensus tree, complete sheath refers to species were the sheath fully encloses the emerging pair during the dormant period (black branches) and in the partial sheath, the sheath only encloses part of the emerging leaf pair during dormancy (grey branches). The white branches indicate species that do not sheath.

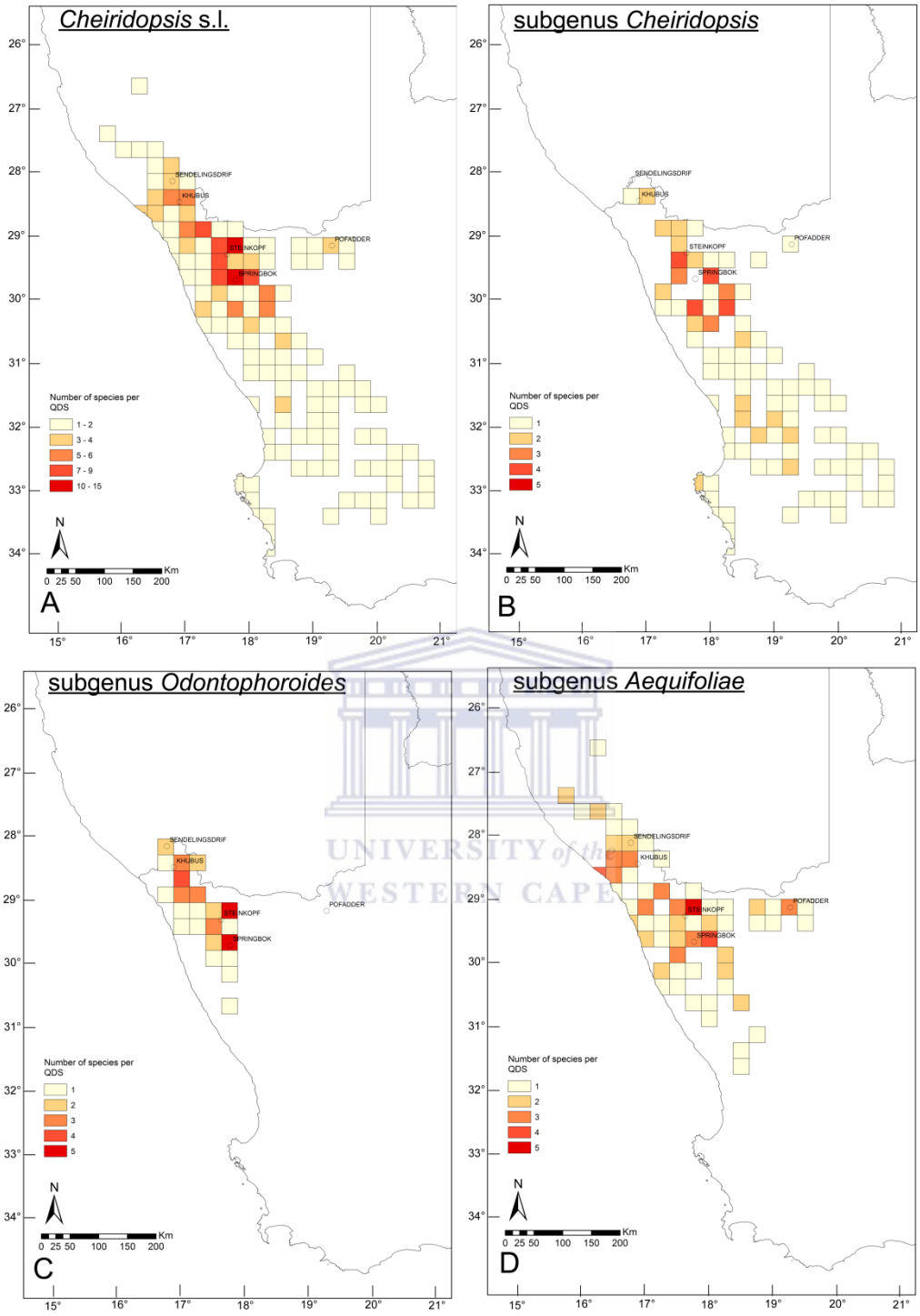

Figure 5.6. Number of species per Quarter Degree Square (QDS) (Edwards & Leistner 1971) and distribution of *Cheiridopsis* s.l. and subgenera (as treated in the taxonomic treatment) in the Greater Cape Floristic region, South Africa. (A) Distribution and number of species per QDS for the genus *Cheiridopsis* s.l.; (B) distribution and number of species per QDS in subgenus *Cheiridopsis*; (C) distribution and number of species per QDS in subgenus *Odontophoroides* (including species of the previously recognised *Odontophorus*)*;* (D) distribution and number of species per QDS in subgenus *Aequifoliae* (including species of previously recognised *Ihlenfeldtia*).

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5.4 DISCUSSION

5.4.1 TAXONOMICALLY INFORMATIVE CHARACTERS IN THE *CONOPHYTUM*-CLADE

Expanded phylogenetic sampling has often been shown to improve phylogenetic signal, providing comprehensive results from which to assess generic circumscription (Hillis, 1998; Pick *et al.,* 2010; Echternacht *et al.,* 2014). This was again found in the present study, where the expanded sampling of the *Conophytum*-clade, which included 35 of the 48 (73%) species in the clade (excluding the large genus *Conophytum,* which was representatively sampled), uncovered novel phylogenetic relationships, improved support for existing relationships and allowed for assessment of monophyly of the genera (Fig. 5.1, Klak *et al.,* 2013; Powell *et al.,* 2016). *Cheiridopsis* and *Conophytum* were recovered as sister taxa (PP=0.95; Fig. 5.1) and although this relationship was recovered previously (Powell *et al.,* 2016), the expanded sampling in the present study improved support for the relationship (Powell *et al.,* 2016, PP=0.84), while in the subfamilial phylogeny Klak *et al.* (2013) recovered these genera in separate clades.

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The greatly expanded sampling of *Cheiridopsis* (including 75% of the currently recognised species) showed for the first time *Ihlenfeldtia* and *Odontophorus* embedded within *Cheiridopsis* (PP=1, BS=81, JK=54; Fig. 5.1). Despite the expanded phylogenetic sampling, the position of the four monotypic genera, i.e. *Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus,* remained unresolved in the *Conophytum*-clade (Figs. 5.1–5.3). In Klak *et al.* (2013), *Enarganthe* was sister to *Conophytum, Namaquanthus* was sister to *Schlechteranthus,* while *Jensenobotrya* and *Ruschianthus* were unresolved. The phylogenetic placement of *Enarganthe* and *Namaquanthus* in Klak *et al.* (2013) was unsupported by morphological characters, as these genera, as well as *Jensenobotrya* and *Ruschianthus,* display a combination of unique morphological characters that distinguish them from all other genera in the subfamily. In addition, *Jensenobotrya* and *Ruschianthus* are

geographically isolated from other members of the subfamily, with *Jensenobotrya* only found on gneiss cliffs off the coast of Namibia, at Dolphin head, surrounded by Namib dunes (Burgoyne, 1998) and *Ruschianthus* is known only from limestone outcrops at high altitudes (1200–1400 m) on the summit of mountains in the Rosh Pinah area in Namibia (BOL records). The unresolved placement, morphological and anatomical data illustrates the uniqueness of these monotypic genera and as such, *Enarganthe*, *Jensenobotrya*, *Namaquanthus* and *Ruschianthus* are retained as monotypic genera. Although monotypic genera may be regarded as artificial, reflecting incomplete exploration (Bartlett, 1940; Atran, 1990), these four monotypic genera are morphologically distinct and based on our current knowledge have no allied species. Suitable generic concepts are based on predictability and stability (Humphreys & Linder, 2009) and therefore the predictability of these monotypic genera, based on the unique combination of characters discussed, as well as the generic stability, with no known allies, supports the argument to maintain these monotypic genera. The diversity in capsule morphology within the *Conophytum*-clade can be seen by the inclusion of genera from five of the twelve morphological groups recognised by Hartmann (1991). When assessing the capsule morphology across the *Conophytum*-clade, two main capsule morphologies were present, (1) a simple capsule without covering membranes and closing bodies (Fig. 5.7A, B) and (2) a complex capsule with covering membranes and closing bodies (Fig. 5.7C, D) (Klak *et al.*, 2013). The simple capsule type corresponded with a lower locule number, as the genera with simple capsules, i.e. *Jensenobotrya*, *Ruschianthus* and *Conophytum*, possessed 5 locules; although locule number in *Conophytum* ranges from 3 to 8 locules. The majority of genera in the *Conophytum*-clade are, however, multilocular (>6 locules) with complex capsules (Fig. 5.7C, D) that include covering membranes and closing bodies (Hartmann, 2001), although in *Enarganthe* and *Namaquanthus* closing bodies are diagnostically absent (Bolus, 1927b, 1954). In addition, the size of the closing bodies has also

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been used to define genera within the *Conophytum*-clade; however, this is often variable, even within a genus. In *Schlechteranthus,* both large closing bodies (blocking ¾ of the locule) and small closing bodies (blocking $^{1}/_{3}$ of the locule) are present (Powell *et al.*, 2016). The smaller closing bodies were used to distinguish *Ihlenfeldtia* from *Cheiridopsis*, leading to the hypothesis of a close phylogenetic relationship between *Ihlenfeldtia, Tanquana* and *Vanheerdia* (Hartmann, 1992) rather than *Ihlenfeldtia* with *Cheiridopsis.* However, *Ihlenfeldtia* shares a compact caespitose habit, with leaves that form sheaths enclosing the emerging leaf pair, and large woody, multilocular capsules (Table 5.2) with *Cheiridopsis* (Hammer, 1996; Hartmann, 2001). This relationship is supported by the phylogenetic results of the present study, which show that *Ihlenfeldtia* is embedded within *Cheiridopsis* (PP=1, BS=81, JK=54; Figs. 5.1–5.3). Valve wings are also often used as a diagnostic character for distinguishing genera in the Ruschieae (Hartmann, 2001), but were shown to be variable in the *Conophytum*-clade (ranging from absent to broad, with the full range of variation found in *Schlechteranthus*), as well as across the subgenera in *Cheiridopsis* (Hartmann & Dehn, 1987; Powell *et al.*, 2016). The width of valve wings has been used to distinguish *Odontophorus* from *Cheiridopsis*, with *Odontophorus* possessing narrow valve wings (Hartmann, 2001). However, species in *Cheiridopsis* subgenus *Aequifoliae* also possess narrow valve wings (Hartmann & Dehn, 1987), and therefore this character is not suitable to differentiate these genera. *Cheiridopsis* and *Odontophorus* also share a mostly caespitose habit, with leaves forming sheaths enclosing the emerging leaf pair and large multilocular, woody capsules (Hammer, 1994; Hartmann, 2001). Although capsule characters have proven to be informative in the *Conophytum*-clade, continuous indiscrete internal capsule characters are not useful, as they are often variable within a genus. The sole reliance on these indiscrete characters to distinguish genera, such as *Cheiridopsis* from *Ihlenfeldtia* and *Odontophorus*, has previously been shown to be unsuitable (Klak & Bruyns, 2016; Powell *et al.*, 2016) and is

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further supported here by the phylogenetic placement of *Ihlenfeldtia* and *Odontophorus* in *Cheiridopsis* (Figs. 5.1–5.3). Valve wings can however sometimes be useful in distinguishing genera, for examples, in *Jensenobotrya*, the valve wings are unusual, as they are only distally attached to the capsule and so spread like ribbons when the capsule is opened (Herre, 1951; Hartmann, 2001).

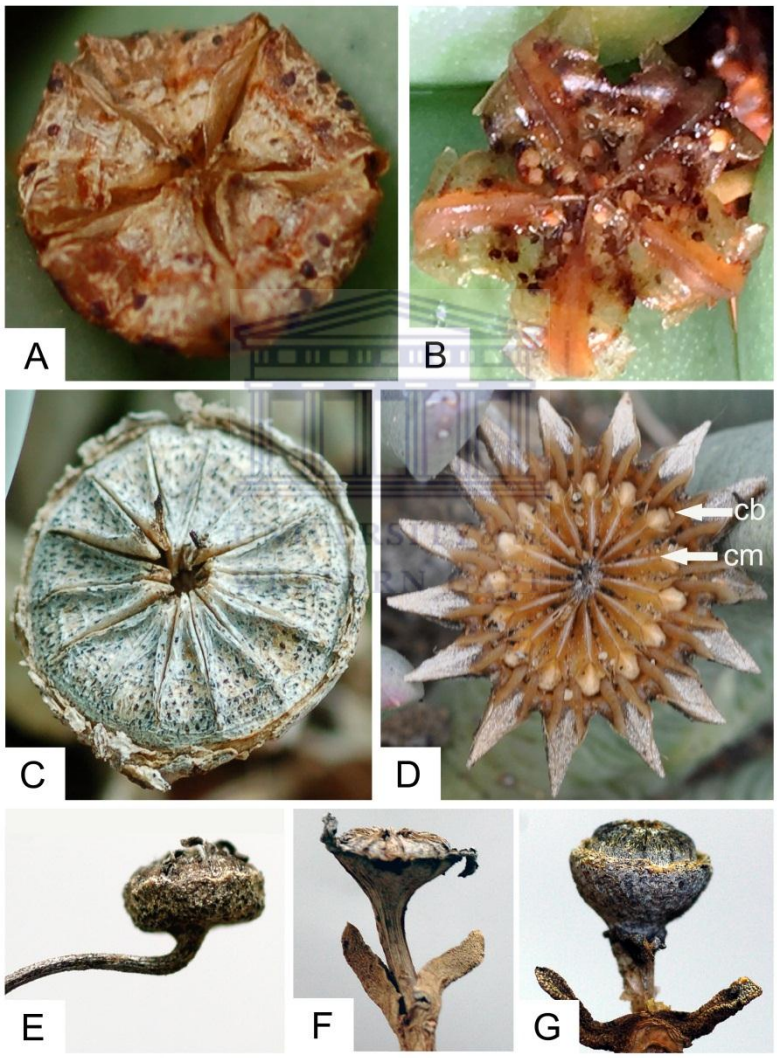

Figure 5.7. Capsules in *Conophytum* and *Cheiridopsis*. (A) Closed simple capsule of *Conophytum wettsteinii*; (B) open simple capsule of *Conophytum wettsteinii* showing the absence of covering membranes and closing bodies; (C) closed multi-locular capsule of *Cheiridopsis denticulata;* (D) open capsule of *Cheiridopsis denticulata* showing the complex internal structures, i.e. covering membranes (cm) and closing bodies (cb), indicated by the white arrows; (E) decumbent capsules of *Cheiridopsis* subgenus *Cheiridopsis,* in *Cheiridopsis namaquensis;* (F) erect capsule of *Cheiridopsis denticulata* with flat to centrally elevated tops as in *Cheiridopsis* subgenus *Aequifoliae;* (G) *Cheiridopsis pilosula* illustrating the erect capsule with rounded tops, typical of *Cheiridopsis* subgenus *Odontophoroides.*

The development of sheaths that protect the emerging leaves during the summer dormant period was recovered as a synapomorphy for the *Cheiridopsis–Conophytum* clade (Fig. 5.5A). In most species of *Cheiridopsis* (as well as in *Conophytum herreanthus*) the sheath only partially encloses the emerging leaf pair during the dormant period (Figs. 5.5B, 5.8E; Hammer, 1996, 2002; Hartmann, 2001). In *Conophytum* (and secondarily in a few species of *Cheiridopsis,* viz. the *Cheiridopsis meyeri-C. namaquensis* clade and *Cheiridopsis peculiaris*), the sheath completely envelops the emerging leaf pair (Figs. 5.5B, 5.8C, D; Hammer, 1996). Sheathing is also found in other genera in the subfamily, such as *Antimima* N.E.Br. and *Mitrophyllum* Schwantes (Hartmann, 2001), as well as in the Mesembryanthemoideae (Bittrich, 1987; Klak *et al.,* 2013). However, in *Conophytum* and *Cheiridopsis,* the protective sheath is retained despite the genera possessing xeromorphic leaves (Fig. 5.4A, D; Klak *et al.,* 2013).

Leaf anatomical characters have been used to inform generic circumscription in the family (Reule, 1937; Ihlenfeldt & Bittrich, 1985; Klak & Linder, 1998), as well as subgeneric classification in the *Conophytum*-clade (Opel, 2005a; Powell *et al.,* 2016). Although epidermal shape was mostly uniform across the clade, cell shape was used to distinguish the subgenera in *Schlechteranthus* (Powell *et al.,* 2016). The epidermal cells are papillate, ranging from blunt papillae to trichomes (Fig. 5.4A–C), with the exception of *Enarganthe*, *Jensenobotrya* and *Namaquanthus,* where leaf surfaces are glabrous (Fig. 5.4D–F). The stomata across the *Conophytum*-clade were found in depressions (Fig. 5.4G), while in seven *Schlechteranthus* species and three *Cheiridopsis* species (*Cheiridopsis acuminata–Odontophorus angustifolius* clade, Fig. 5.1), stomata were sunken and protected by parastomal cells (Fig. 5.4H, I; Powell *et al.*, 2016). Although this stomatal type was variable within *Schlechteranthus,* in *Cheiridopsis* the species with this stomatal protection formed a strongly supported clade (PP=0.92, BS=91, JK=65; Fig. 5.1).

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Surveys of pollen exine and colpi structure in Ruschioideae (Dupont, 1977) and Mesembryanthemoideae (Bittrich, 1987) showed that there was variation in this character in both subfamilies. However, the sampling did not include surveys of all the genera in the subfamilies, and with the absence of a phylogenetic hypothesis, patterns in pollen structures could not be sufficiently assessed. However, in the present study, pollen exine and colpi structure were comprehensively surveyed for all the genera in the *Conophytum*-clade as well as for the subgeneric groupings (Table 5.2), which allowed for assessment of the variation across the clade. Although the exine and colpi structures were mostly uniform (tricolpate with poorly-defined colpi) in the *Conophytum*-clade, differences were found in *Enarganthe* (tricolpate with well-defined colpi) and *Jensenobotrya* (syncolpate), which distinguished these genera from others in the clade (Table 5.2).

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5.4.2 *CHEIRIDOPSIS*, *IHLENFELDTIA* AND *ODONTOPHORUS*

Ihlenfeldtia and *Odontophorus* are strongly recovered within a paraphyletic *Cheiridopsis* in all of the phylogenetic analyses (Figs. 5.1–5.3). This is hardly surprising as they share several morphological characters, and are currently distinguished solely on the basis of continuous indiscrete internal capsule characters, i.e. the size of closing bodies or the width of valve wings. Based on the phylogenetic, morphological and anatomical evidence, we here include the species of *Ihlenfeldtia* and *Odontophorus* into an expanded *Cheiridopsis* s.l. In its new circumscription, *Cheiridopsis* s.l. is easily recognised by the mostly compact, caespitose habit, leaves that form sheaths that enclose the emerging leaf pair (either partially or fully) and large woody multilocular capsules, with 8 to 18 locules. The three subgenera currently recognised are largely recovered in the phylogenetic analyses (Figs. 5.1–5.3), although some minor adjustments are required.

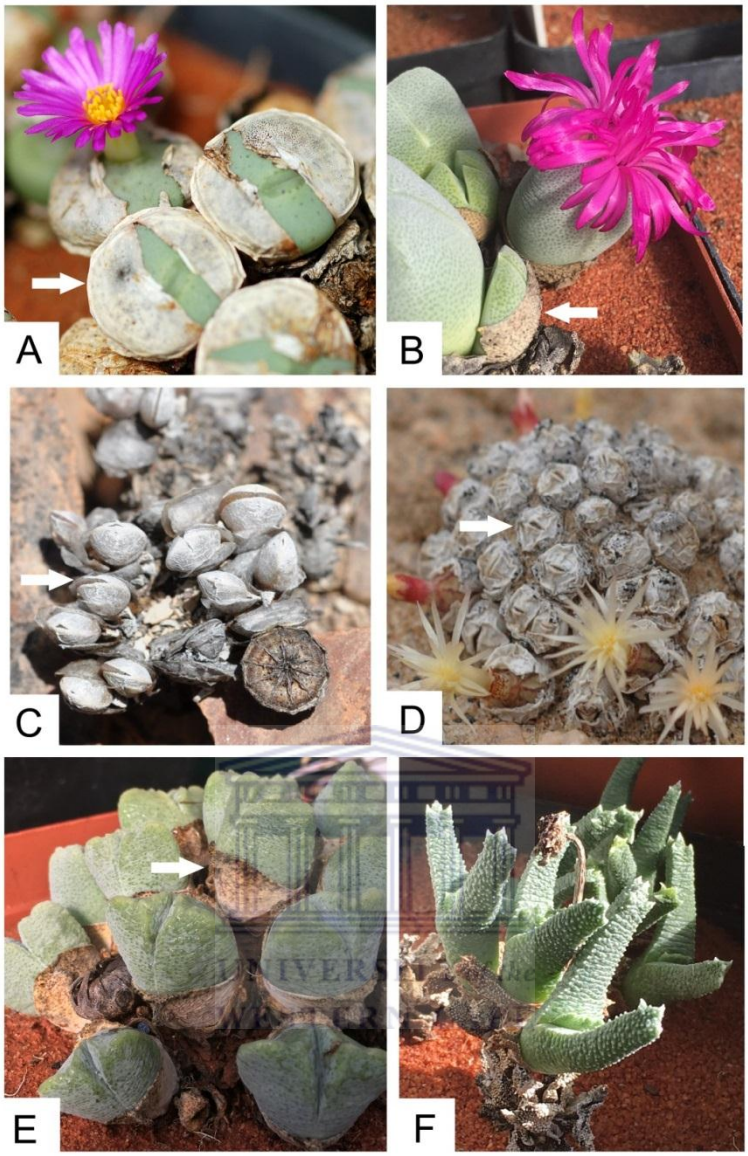

Figure 5.8. Leaf and floral characters of the sheathing genera, *Cheiridopsis* and *Conophytum*. (A) New leaves of *Conophytum wettsteinii* breaking out of their papery sheath, with magenta flower; (B) partial sheath enclosing the emerging leaf pair, with a magenta flower, in *Cheiridopsis glomerata;* (C) leaves of *Cheiridopsis meyeri* completely enclosed by a white papery sheath, during the dormant period, indicated by the arrow; (D) leaves of *Conophytum uviforme* completely enclosed in a sheath during the dormant period, indicated by the arrow; (E) *Cheiridopsis purpurea* with the partial sheath, common to many *Cheiridopsis* species which only encloses part of the leaves during the dormant period; (F) *Cheiridopsis aspera* with the prominent rough leaf surface often found in species of subgenus *Odontophoroides.*

5.4.2.1 *Cheiridopsis* subgenus *Cheiridopsis*

With the exception of *Cheiridopsis pearsonii* and *C. peculiaris*, all of the species with decumbent capsules (Fig. 5.7E) and heterophyllous leaves were recovered as a monophyletic group (Figs. 5.1–5.3) corresponding to Hartmann & Dehn's (1987) concept of subgenus *Cheiridopsis*. When the genus is constrained to monophyly, *Cheiridopsis pearsonii* and *C. peculiaris* are included in the subgenus *Cheiridopsis* clade, with a constrained phylogenetic tree only 2 steps longer and not significantly different (p=0.15) from the unconstrained tree. The fact that *C. pearsonii* and *C. peculiaris* are placed directly in the subgenus, without specifically constraining them to the subgenus, provides strong support for their inclusion in subgenus *Cheiridopsis. Cheiridopsis peculiaris* is somewhat unusual, as the name eludes, due to the strange shell-shaped leaves, but shares decumbent capsules and heterophyllous leaves with subgenus *Cheiridopsis* (Hartmann & Dehn, 1987). The sheath is also unusual within *Cheiridopsis*, in that it completely encloses the emerging leaf pair during the dormant period. However, similar sheaths can also be found in other species within the subgenus, i.e. the species of the *C. meyeri–C. namaquensis* clade (Figs. 5.5B, 5.8C). *Cheiridopsis pearsonii* shares the decumbent capsules with undulate covering membranes and large closing bodies with the rest of the species in the subgenera, and is most similar to *C. namaquensis* in leaf shape (Hartmann, 2001).

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5.4.2.2 *Cheiridopsis* subgenus *Odontophoroides*

Species recovered in the *Cheiridopsis* subgenus *Odontophoroides* clade all share erect capsules (Fig. 5.7G), with rounded tops (Figs. 5.1–5.3). This clade includes the two species of *Odontophorus* sampled, and the placement of these species is supported by the similar capsule characters and the isophyllous leaves (Hartmann, 2001). *Odontophorus* is recovered as polyphyletic (Figs. 5.1–5.3), with *O. angustifolius* included in the *C. acuminata–O.*

angustifolius clade, characterised by sunken stomata hidden by parastomal cells (Fig. 5.4I). This complex stomatal protection was only found in these three species in the *Cheiridopsis* s.l. clade (PP=0.92, BS=91, JK=65; Fig. 5.1).

Three species (*Cheiridopsis glomerata, C. pillansii, C. purpurea*), previously treated in subgenus *Aequifoliae*, were recovered within the subgenus *Odontophoroides* clade in all of the phylogenetic analyses (Figs. 5.1–5.3). These species were placed by Hartmann & Dehn (1987) in subgenus *Aequifoliae* based on the internodes, keeled leaves and awned valve wings. However, they share the erect capsules with rounded tops, diagnostic of subgenus *Odontophoroides* (Fig. 5.7G), and so we transfer them here to subgenus *Odontophoroides*. As a result, the magenta flowering, *C. glomerata* (Fig. 5.8B) and *C. purpurea*, are now placed together with the only other magenta flowered species in the genus, *C. speciosa.*

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5.4.2.3 *Cheiridopsis* subgenus *Aequifoliae*

The *Cheiridopsis* subgenus *Aequifoliae* clade is characterised by species that possess erect capsules with flat to centrally elevated tops (Fig. 5.7F) and isophyllous leaves (Figs. 5.1–5.3). This clade differs from Hartmann & Dehn's (1987) concept of *C.* subgenus *Aequifoliae* by the exclusion of *C. glomerata, C. pillansii, C. purpurea* (discussed earlier) and the inclusion of both species of *Ihlenfeldtia* (Figs. 5.1–5.3). The placement of *Ihlenfeldtia* in subgenus *Aequifoliae* is further supported by the shared presence of trichomes (Fig. 5.4C) which are found in all species of subgenus *Aequifoliae* (Fig. 5.4B).

5.5 KEY TO THE GENERA OF THE *CONOPHYTUM*-CLADE

1. Old leaves do not form a sheath that encloses the emerging leaf pair during dormancy 2

2. Capsules 5-locular, without covering membranes 3

3. Flowers green, never opening fully as restricted by adjacent leaf pair; leaves sickle-shaped; pollen unknown; caespitose, with leaves held upright..... ***Ruschianthus***

3. Flowers white to pink, opening fully; leaves shortly clavate to almost globose; pollen syncolpate; shrub, with leaves hanging down cliffs like bunches of grapes ..... ***Jensenobotrya***

2. Capsules multilocular (>6 locules), with covering membranes..... 4

4. Leaves not fused at the base, trigonous to club-shaped; flowers without filamentous staminodes.....***Enarganthe***

4. Leaves fused at least $^{1}/_{3}$ of their length at the base, leaves finger-shaped, triquetrous to trigonous; flowers with or without filamentous staminodes..... 5

5. Capsules without closing bodies; opposite leaf almost forming a U-shape .....

.....***Namaquanthus***

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5. Capsules with closing bodies; leaves triquetrous to trigonous ..... ***Schlechteranthus***

1. Old leaves form sheaths that encloses the emerging leaf pair during dormancy..... 6

6. Petaloid staminodes fused forming a tube; capsules soft and papery, 3–8-locular, without covering membranes and closing bodies .....***Conophytum***

6. Petaloid staminodes free, not forming a tube; capsules hard and woody, 8–18-locular, with covering membranes and closing bodies .....***Cheiridopsis***

5.6 TAXONOMIC TREATMENT

5.6.1 ***Cheiridopsis*** N.E.Br., Gard. Chron 3: 412. 1925, emend. nov. R.F.Powell *Type: C. rostrata* N.E.Br.

Ihlenfeldtia H.E.K.Hartmann, Bot. Jahrb. Syst. 114. 1992, **syn. nov.** *Type: I. excavata* (L.Bolus) H.E.K.Hartmann (= *Cheiridopsis excavata* L.Bolus).

Odontophorus N.E.Br., Gard. Chron 3: 12. 1927, **syn. nov.** *Type: O. marlothii* N.E.Br. (= *Cheiridopsis marlothii* (N.E.Br.) R.F.Powell).

Succulent, perennial shrubs, compact to caespitose or rarely mat-forming, 50–300 mm in height. *Leaves* isophyllous, with subsequent leaf pairs equal along stem or heterophyllous, with subsequent leaf pairs unequal along stem; triquetrous to trigonous, sometimes rhombic, 4–150 mm long; leaf surface slightly velvety or smooth, sometimes rough, formed by elevations above subhypodermal idioblasts; mucronate, sometimes with dentate margins or keels, epidermis papillate, with epidermal cells forming blunt papillae or trichomes; old leaves forming sheaths which usually only partially enclose the emerging leaf pair (fully enclose the emerging leaf pair in only a few species, i.e. *C. meyeri, C. minor, C. namaquensis, C. peculiaris*). *Flowers* solitary; peduncles 30–90 mm; radiate, 30–70 mm in diameter; petaloid staminodes free, cream to yellow, rarely orange, red, or magenta, sometimes magenta-suffused. *Capsules* woody, peduncles decumbent or erect, top flat to centrally elevated or rounded, base funnel-shaped to semi-globose; 8 to 18 locules; covering membranes undulate in radial direction, often with elevations in subcentral position, sometimes as prominent bulges, humps or tubes, very rarely flat; closing bodies usually large, blocking at least ¾ of locule, seldom smaller, blocking at least ½ of locule; valve wings broad, equal in width to valve, or narrow, $^1/_3$ width of valve, sometimes reduced to awns. *Seeds* flat to round, light to darker brown, smooth to papillate.

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Diagnostic characters and distribution:

Cheiridopsis now includes 38 species classified into three subgenera, *Cheiridopsis, Aequifoliae* and *Odontophoroides.* The genus is characterised by the old leaves that form sheaths enclosing the emerging leaf pair partially, but sometimes fully (Fig. 5.8C, E) and the large multilocular woody capsules, with 8 to 18 locules (Fig. 5.7C, D). The species of *Cheiridopsis* are also compact to caespitose, rarely mat-forming. The diversity of the genus is centred in the Springbok-Steinkopf region (Fig. 5.6A) and distribution follows the winter-rainfall region of southern Africa. The distribution extends from southern Namibia, southwards to Langebaan and eastwards to Pofadder (Fig. 5.6A).

5.6.2 KEY TO THE SUBGENERA IN *CHEIRIDOPSIS*

1. Penduncle decumbent, leaves heterophyllous.....

..... **5.6.3.1. *Cheiridopsis* subgenus *Cheiridopsis***

1. Peduncle erect; leaves isophyllous..... 2

2. Capsule top rounded ..... **5.6.3.2. *Cheiridopsis* subgenus *Odontophoroides***

2. Capsule top flat to centrally elevated.....**5.6.3.3. *Cheiridopsis* subgenus *Aequifoliae***

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5.6.3 TAXONOMIC TREATMENT OF THE SUBGENERA

5.6.3.1 ***Cheiridopsis*** subgenus ***Cheiridopsis***. *Type: C. rostrata*.

Compact perennial shrubs, sometimes mat-forming. *Leaves* heterophyllous, with subsequent leaf pairs unequal along stem; surface smooth, with no elevations; epidermal cells forming blunt papillae; sheaths partially or fully enclosing the emerging leaf pair during dormancy. *Flowers* cream to yellow, rarely orange or red. *Capsules* with decumbent peduncles; top flat to rounded, base funnel-shaped; valve wings narrow, $^{1}/_{3}$ width of valve or rarely rectangular, often narrowing in an awn distally; covering membranes prominently

undulate, often with additional protrusions in subcentral position; closing bodies large, blocking at least ¾ of the locule. *Seeds* mostly smooth, rarely papillate.

Diagnostic characters and distribution:

Cheiridopsis subgenus *Cheiridopsis* includes 14 species that are distinguished by their decumbent capsules (Fig. 5.7E) and heterophyllous leaves. Some species (*C. meyeri, C. minor, C. namaquensis, C. peculiaris*) can be distinguished by the sheath that fully encloses the emerging leaf pair during the dormant period (Fig. 5.8C). The species occur from the Richtersveld, southwards to Langebaan, with the centre of diversity in the Springbok-Steinkopf Region (Fig. 5.6B).

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Recognised species:

Cheiridopsis amabilis S.A.Hammer, *C. delphinoides* S.A.Hammer, *C. derenbergiana* Schwantes, *C. gamoepensis* S.A.Hammer, *C. imitans* L.Bolus, *C. meyeri* N.E.Br., *C. minor* (L.Bolus) H.E.K.Hartmann, *C. namaquensis* (Sond.) H.E.K.Hartmann, *C. pearsonii* N.E.Br., *C. peculiaris* N.E.Br., *C. rostrata* (L.) N.E.Br., *C. schlechteri* Tischer, *C. turbinata* L.Bolus, *C. umbrosa* S.A.Hammer & Desmet.

5.6.3.2 ***Cheiridopsis*** subgenus ***Odontophoroides*** H.E.K.Hartmann, Biblioth. Bot. 136: 59. 1983. *Type: C. acuminata* L.Bolus.

Compact perennial shrubs, sometimes mat-forming. *Leaves* isophyllous, with subsequent pairs equal along stem; surface often rough with elevations above the subhypodermal idioblasts or smooth; epidermal cells forming blunt papillae or trichomes; sheath always only partially enclosing the emerging leaf pair during dormancy. *Flowers* cream, yellow, magenta, or magenta-suffused. *Capsules* on erect peduncles; top rounded,

base semi-globose; valve wings broad, as wide as valve, to narrow, $^1/_3$ width of valve, sometimes ending in an awn; covering membranes undulate; closing bodies large, blocking at least ¾ of locule. *Seeds* sparsely to densely papillate.

Nomenclatural changes:

Cheiridopsis angustifolia (L.Bolus) R.F.Powell, **comb. nov.** *Odontophorus angustifolius* L.Bolus, Notes Mesembryanthemum 3: 128. 1938. *Type*: South Africa, Northern Cape Province: Kosies, *Van Heerde 21845* (holotype: BOL!).

Cheiridopsis marlothii (N.E.Br.) R.F.Powell, **comb. nov.** *Odontophorus marlothii* N.E.Br., Gard. Chron. 3:12. 1927. *Type*: South Africa, Northern Cape Province: Ezelsfontein, between Spektakel and O'kiep, *Marloth 6947* (lectotype: BOL!).

Cheiridopsis nana (L.Bolus) R.F.Powell, **comb. nov.** *Odontophorus nanus* L.Bolus Notes Mesembryanthemum 2: 59. 1929. *Type*: South Africa, Northern Cape Province: Eenriet, upper slopes, about 7 miles N.E. of Steinkopf, Little Namaland [Namaqualand], *Pearson 4071* (holotype: BOL!).

Cheiridopsis pusilla (S.A.Hammer) R.F.Powell, **comb. nov.** *Odontophorus pusillus* S.A.Hammer Piante Grasse 15(4): 86. 1996. *Type*: *Van Jaarsveld 11461* (holotype: BOL!).

Diagnostic characters and distribution:

Cheiridopsis subgenus *Odontophoroides* is the largest subgenus with 16 species and is characterised by the isophyllous leaves (equal subsequent pairs) and the erect capsules with rounded tops (Fig. 5.7G). Often the leaves are also characterised by a rough surface (Fig. 5.8F), but this is not found in all species in the subgenus. Although this subgenus has the largest number of species, it has the most restricted distribution from the northern border of South Africa at Sendelingsdrif, in the Richtersveld, to Wallekraal, with hotspots in the Springbok-Steinkopf Region and the Richtersveld (Fig. 5.6C).

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Recognised species:

Cheiridopsis acuminata L.Bolus, *C. alba-oculata* Klak & Helme, *C. angustifolia* (L.Bolus) R.F.Powell, *C. aspera* L.Bolus, *C. glomerata* S.A.Hammer, *C. marlothii* (N.E.Br.) R.F.Powell, *C. nana* (L.Bolus) R.F.Powell, *C. pillansii* L.Bolus, *C. pilosula* L.Bolus, *C. ponderosa* S.A.Hammer, *C. purpurea* L.Bolus, *C. pusilla* (S.A.Hammer) R.F.Powell, *C. rudis* L.Bolus, *C. speciosa* L.Bolus, *C. umdausensis* L.Bolus, *C. velox* S.A.Hammer.

5.6.3.3 ***Cheiridopsis*** subgenus ***Aequifoliae*** H.E.K.Hartmann, Biblioth. Bot. 136: 59. 1983. *Type: C. denticulata* (Haw.) N.E.Br.

Compact perennial shrubs. *Leaves* isophyllous, with subsequent pairs equal along stem; surface smooth with no elevations, epidermal cells forming trichomes, sheath always only partially enclosing the emerging leaf pair during dormancy. *Flowers* cream to yellow, rarely orange or red. *Capsules* on erect peduncles; top flat to centrally elevated, but never rounded, base funnel-shaped; valve wings reduced to awns; covering membranes undulate with low distal rims; closing bodies usually large, blocking at least ¾ of locule, seldom smaller, blocking at least ½ of locule. *Seeds* smooth.

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Nomenclatural changes:

Cheiridopsis excavata L.Bolus, Notes Mesembryanthemum 3: 48. 1937. *Ihlenfeldtia excavata* (L.Bolus) H.E.K.Hartmann Bot. Jahrb. Syst. 114(1): 48. 1992. *Type*: South Africa, Northern Cape Province: South of Breekpoort, Namaland, *van Heerde 21836* (holotype: BOL!).

Cheiridopsis vanzylii L.Bolus, Notes Mesembryanthemum 2: 116. 1929. *Ihlenfeldtia vanzylii* (L.Bolus) H.E.K.Hartmann, Bot. Jahrb. Syst. 114(1): 49. 1992. *Type*: South Africa, Northern Cape Province: Pofadder, *Fuller 4* (holotype: BOL!).

Diagnostic characters and distribution:

Cheiridopsis subgenus *Aequifoliae* is the smallest subgenus in *Cheiridopsis*, with eight species. The subgenus shares the isophyllous leaves (equal subsequent pairs) and erect peduncles with subgenus *Odontophoroides* but differs by the capsule tops that are flat or centrally elevated on the top (Fig. 5.7I). The subgenus has the most northern distribution extending from southern Namibia to Clanwilliam and eastwards to Pofadder, with two centres of diversity in the Springbok-Steinkopf Region and the Richtersveld (Fig. 5.6D).

Recognised species:

Cheiridopsis brownii Schick & Tisch., *C. caroli-schmidtii* (Dinter & A.Berger) N.E.Br., *C. denticulata* (Haw.) N.E.Br., *C. excavata* L.Bolus, *C. herrei* L.Bolus, *C. robusta* (Haw.) N.E.Br., *C. vanzylii* L.Bolus, *C. verrucosa* L.Bolus.

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Chapter 6

Phylogenetic relationships and character evolution in the highly speciose genus *Conophytum* (Ruschioideae; Aizoaceae)

6.1 INTRODUCTION

Conophytum N.E.Br. is the largest genus in the *Conophytum*-clade, with approximately 106 species and a distribution centred in the arid parts of the Greater Cape Florist Region (GCFR) (Hammer, 2002; Young & Desmet, 2016). The genus has a unique floral structure in Aizoaceae, with fused petaloid staminodes forming a tubular flower (Fig. 6.1; Haas, 1976; Hartmann, 1991). This floral structure is diagnostic for the genus, with other members of the subfamily possessing radiate flowers with free petaloid staminodes (Hartmann, 1991). The variation of floral morphology within *Conophytum* is also remarkable, with variation in structure, colour (Fig. 6.1) and scent (Liede & Hammer, 1990; Hammer, 2002; Jürgens & Manning, 2004). Other floral traits, such as anthesis and phenology, are also variable within the genus (Hammer, 2002).

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Species of *Conophytum* flower mainly in autumn (March to May), earlier than the majority of the members in the Aizoaceae, which flower from July to September (Hartmann, 2001; Hammer, 2002). Although this shift in flowering phenology is uncommon in the Aizoaceae, it is also found in *Argyroderma* N.E.Br., which flowers in late autumn (Hartmann, 2001; Hammer, 2002) and *Erepsia* which flowers in summer (Liede, 1990). In some species of *Conophytum*, the flowering season has shifted either to winter or spring, or in some cases to summer (Hammer, 2002).

The opening and closing time of flowers (anthesis) in *Conophytum* also varies from diurnal, afternoon, late afternoon (crepuscular) to nocturnal (Hammer, 2002). Although afternoon and nocturnal flowering is unusual in the family, there are a few summer-rainfall genera that

flower in the afternoon, closing shortly after nightfall, such as *Bergeranthus* Schwantes, *Deilanthe* N.E.Br., *Hereroa* (Schwantes) Dinter & Schwantes, *Nananthus* N.E.Br. and *Stomatium* Schwantes (Peter *et al.,* 2004; Hartmann, 2001). True nocturnal flowering species as found in *Conophytum,* are however very rare in the family, specifically in the winter-rainfall region. However, *Mesembryanthemum noctiflorum* L. is another example of a true nocturnal flowering species and is included in a group of species (subgenus *Phyllobolus* (N.E.Br.) Klak section *Vesperifolia* Haw. (Klak & Bruyns, 2013)), which also display a range in anthesis (Gerbaulet, 1996), as found in *Conophytum.* Similarly, *Drosanthemum* subgenus *Vespertina* H.E.K.Hartmann also includes nocturnal flowering species (*D. duplessiae* L.Bolus and *D. vespertinum* L.Bolus), as well as the afternoon-flowering *D. crassum* L.Bolus and *D. lique* Schwantes, while the remainder of the species in the subgenus are diurnal (Hartmann, 2007)

Floral characters have been used in combination with leaf characters to circumscribe species and sections within *Conophytum* (Hammer, 1993, 2002). These leaf characters include markings (spots and lines), blisters, warts and windows (Fig. 6.1, Hartmann, 2001; Hammer, 2002, Opel, 2005a) *Conophytum* includes the full range of papillation found in genera of the *Conophytum*-clade (Hartmann, 2001; Powell *et al.,* 2016, submitted b; Chapters 3 & 5), from blunt papillae to trichomes, while papillae is absent (no epidermal extensions) in some species (Opel, 2005a). In addition, leaf anatomical characters are also variable in the genus, with variation in the presence of a hypodermis, calcium oxalate crystals and tanniferous idioblasts (Opel, 2005a). These leaf anatomical characters, together with floral and capsule characters, were used to produce a morphological phylogeny for the genus (Opel, 2005b). This phylogeny recovered some monophyletic clades that supported the current sectional classification of the genus proposed by Hammer (2002), while some of the larger and more diverse sections were recovered as paraphyletic or polyphyletic (Opel, 2005b).

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Figure 6.1. Variation in leaf and floral characters in *Conophytum.* (A) Yellow-flowered *C. bilobum* with short calyx and corolla tube and reflexed petaloid staminodes with anthers at mouth of tube; (B) structure B2 flower (Liede & Hammer, 1990), with recessed anthers in *C. pellucidum;* (C) *C. luckhoffii* with magenta flower and intricate markings on the leaves; (D) prominent windowed leaves in *C. lithopsoides;* (E) leaf markings on *C. uviforme* with fading yellow flowers; (F) *C. stephanii* densely covered in trichomes; (G) ornately marked leaves of *C. obcordellum;* (H) *C. pellucidum* with cryptic leaf markings occupying a granite grit pan; (I) couplings of *C. flavum,* displaying the chalky leaf epidermis resulting from dense calculate oxalate crystals, also present in species of sect. *Cataphracta;* (J) leaf surface covered in trichomes *C. depressum*; (K) large magenta flowers and spotted leaf epidermis of *C. wettsteinii;* (L) minute soft-bodied *C. maughanii* with closed white flowers partly submerged below the surface amongst quartz stones on a quartz patch.

Conophytum was shown to be monophyletic in Powell *et al.* (submitted b; Chapter 5), however, only a representative sampling of the sectional classification was included, with eight species sampled. This chapter presents an expanded sampling of *Conophytum*, with a comprehensive sampling across the sections and diversity of the genus. The aims of this chapter were to provide insight into phylogenetic relationships within the genus for the first time based on DNA sequence data, as well as test the monophyly of the sections proposed by Hammer (2002) and Opel (2005b). In addition, selected leaf and floral characters are reconstructed onto the phylogeny to investigate the role these characters may have played in the evolution and diversification of the genus.

6.2 MATERIALS AND METHODS

6.2.1 TAXONOMIC SAMPLING

Sampling of *Conophytum* was expanded to include 59 of the 106 species (56%) and includes members from 15 of the 16 currently recognised sections (excluding monotypic sect. *Batrachia*) in the genus (Hammer, 2002).

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Material was collected in the field as well as from living collections housed at the Karoo Botanical Garden (Worcester, RSA), South African National Biodiversity Institute, Kirstenbosch Garden (Cape Town, RSA) and the Sphaeroid Institute (California, USA) and processed following Section 2.1.1 of Chapter 2.

6.2.2 PHYLOGENETIC DATA

Phylogenetic data was produced following the methods described in Section 2.4.1 of Chapter 2 for six plastid gene regions: *matK, rpl16, rps16, trnL–F, trnQ*^{UUG}*–rps16* and *trnS–trnG.* The 304 new sequences, together with published sequences of the *Conophytum*-clade from Klak *et al.* (2013) and Powell *et al.* (2016; submitted b), were automatically aligned using the

Clustal W function in MEGA version 6 (Higgins *et al.*, 1994; Tamura *et al.*, 2013). *Knersia diversifolia* (L.Bolus) H.E.K.Hartmann & Liede (= *Drosanthemum diversifolium* in Klak *et al.*, 2013) and *Jacobsenia vaginata* (L.Bolus) Ihlenf., were selected as outgroup taxa, as they were recovered as sister to the *Conophytum*-clade in the previous analyses (Klak *et al.*, 2013; Powell *et al.*, 2016, submitted b). The combined plastid dataset included a total of 4,497 characters and were analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) following the methods outlined Section 2.4.2 in Chapter 2. Support values for these analyses are abbreviated as follows: Posterior probability values (PP), Bootstrap support values (BS) and Jackknife support values (JK).

The data were partitioned into six partitions for the ML and BI analyses. The matrices for the different gene regions were run in JModel test version 2.1.7 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) and the following models were suggested for the specific gene regions: HKY for *matK*, *trnL–F* and *rpl16* (Hasegawa *et al.*, 1985); F81 for *trnS-trnG* (Felsenstein, 1981) and F81+G for *rps16* and $trnQ^{UUG}$*-rps16* (Felsenstein, 1981) and these models were implemented accordingly in the BI analyses. Two simultaneous runs were completed for 1.5 x 10^7 generations with a sampling frequency of 100. The standard deviation between the split frequencies stabilised below 0.01 and suboptimal trees were discarded as the "burn-in" phase. The remaining 22,502 trees were used to construct a 50% majority rule consensus tree with posterior probabilities (PP).

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6.2.3 MORPHOLOGICAL CHARACTER RECONSTRUCTION

Selected leaf, floral and capsule morphological characters were coded from the database produced in the digital DELTA Key for *Conophytum* (Section 2.1.3 of Chapter 2; Appendix E4) and from Hartmann (2001) for the remaining genera in the clade. The characters were

mapped individually onto the BI consensus tree following the methods outlined in Section 2.3 of Chapter 2. The following characters and character states were coded:

1) Papillae type, specifically the absence of epidermal extensions (0), blunt papillae (1), defined as low protuberances in the centre of the epidermal cell, and trichomes (2) which were outgrowths of epidermal cells that are longer than broad (Opel, 2005b).

2) The presence (1) or absence (0) of windowed leaves, defined as leaf apices with a translucent to transparent area underlain by clear mesophyll that lacks chloroplast (Opel, 2005b).

3) Leaf type, specifically the absence (0) or presence (1) of soft-bodied leaves, which form when there is a reduction and loss of the epidermal crystal layer in combination with the formation of bladder cells in the leaves (Opel, 2005b), resulting in a soft leaf body.

4) Flowering season (phenology), the peak season when flowers emerge and bloom in a species, and included autumn (1), winter (2), spring (3) and summer (4).

5) Flowering time (anthesis), to the time of day or night when the flowers open in a species. This included diurnal (1) flowering (10 am–5 pm), afternoon flowering (2), with flowers open after midday (12 pm), closing after a few hours; late afternoon flowering (3) with flowers opening shortly before dusk and closing shortly after nightfall and nocturnal flowers (4), which open after nightfall and close shortly before dawn (Liede *et al.*, 1991; Hammer, 2002).

6) Floral types in *Conophytum* were coded following Liede & Hammer (1990), while the remaining genera in the clade possess radiate flowers (0). The different floral types in *Conophytum* include the variation in degree of fusion of the petaloid staminodes and fusion of the stigmas (types A and B) as well as the position of the style and anthers (Fig. 6.2; Liede & Hammer, 1990). The floral types are defined as follows: Type A1 (1): the calyx and corolla tube are short, with reflexed petaloid staminodes and anthers and the free style-branches

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projecting beyond the mouth of the tube; Type A2 (2): calyx and corolla-tube short, with reflexed petaloid staminodes and with anthers and style-branches at mouth of tube, and the style-branches only fused at the base; Type B1(3): calyx-tube short, with long corolla tube and reflexed petaloid staminodes, with anthers well-exposed and the styles fused into a long column with short branches; Type B2 (4): short calyx-tube and long corolla-tube, petaloid staminodes forming a funnel, filaments very short and hidden near the base of the tube, styles brief; Type C: Calyx-tube very short (tepals membranaceous, very reduced); corolla-tube very long with the petaloid staminodes fused for most of their length; anthers remaining within the corolla-tube and exceeding the only basally fused style-branches (Fig. 6.2; Liede & Hammer, 1990). Although the definition of type C is included here, *C. acutum*, the only representative of this floral structure (Liede & Hammer, 1990), was not included in this study.

7) Capsule locule number was coded as a range, as the number of locules typically varies within a species (e.g. 7–9 locules) (Hartmann, 2001; Powell *et al.*, submitted a), this character was coded as capsules with <5 locules (1), 5–6-locular capsules (2) and multilocular capsules, with 7–9 locules (3) or >9 locules (4).

The coded matrix is presented in Table 6.1, while Appendix D2 includes the description of characters and character states.

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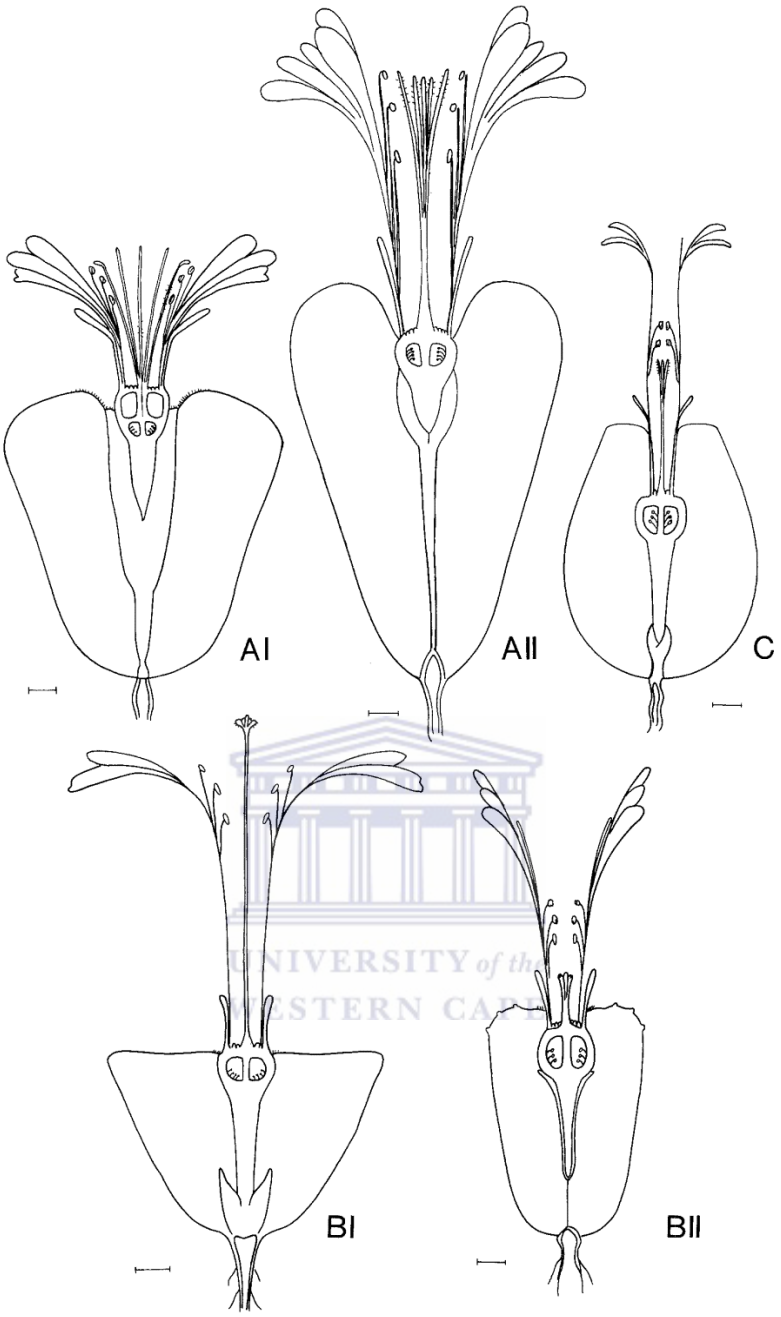

Figure 6.2. From Liede & Hammer (1990). Floral types in *Conophytum.* Type *AI* Calyx and corolla tube short, petaloid staminodes reflexed; anthers and style-branches projecting beyond the mouth of the tube; style-branches free (based on *C. friedrichiae).* Type *AII* Calyx and corolla-tube short, petaloid staminodes reflexed; anthers and style-branches shorter than petaloid staminodes, well exposed; style-branches fused only at the very base (based on *C. bilobum).* Type *BI* Calyx-tube short, corolla-tube long, petaloid staminodes reflexed; anthers well exposed, styles fused into a long column with branches very short (based on *C. wettsteinii).* Type *BII* Calyx-tube short, corolla-tube long, petaloid staminodes infundibuliform; filaments very short and hidden near the base of the tube, styles brief (based on *C. minusculum).* Type *C* Calyx-tube very short (tepals membranaceous, very reduced); corolla-tube very long with the petaloid staminodes fused for most of their length; anthers remaining within the corolla-tube and exceeding the only basally fused style-branches (based on *C. acutum*, the only representative of this type). Scale bar = 1 mm

Table 6.1: Leaf, floral and capsule morphological character matrix for *Conophytum* and the *Conophytum*-clade, mapped individually onto the Bayesian inference tree (Figs. 6.6–6.8). Description of characters and character states are included in Appendix D2. The character matrix for *Conophytum* is presented on the left, and continued for the other species in the clade on the right.

	1	2	3	4	5	6	7
Conophytum achabense	0	1	1	1	2	3	1
Conophytum angelicae	1	0	0	1	4	2	1/2/3
Conophytum auriflorum	1	0	0	1	1	3	1
Conophytum bilobum	1	0	0	1	1	2	2/3
Conophytum bolusiae	2	0	0	3/4	1	3	1/2
Conophytum breve	1	0	0	1	4	2	1/2
Conophytum bruynsii	?	0	0	1	1	3	1
Conophytum burgeri	0	1	1	1	3	2	2
Conophytum calculus	1	0	0	1	4	2	2
Conophytum caroli	1	1	1	1	1	1	2
Conophytum chauvinae	2	0	0	1	1	1	1
Conophytum comptonii	0	0	0	1	4	2	1/2
Conophytum concavum	2	1	1	1	3	2	1/2
Conophytum concordans	?	1	1	1	1	1	2
Conophytum depressum	2	0	0	1	4	2	1/2
Conophytum ectypum	1	0	?	1	1	3/4	1/2
Conophytum ernstii	2	0	0	1	3	2	1/2
Conophytum ficiforme	1	0	0	1	4	3	1/2
Conophytum flavum	1	0	0	1	1	3	1/2/3
Conophytum friedrichiae	2	1	1	1	2	1	2
Conophytum frutescens	1	0	0	3/4	1	3	1/2
Conophytum fulleri	1	0	0	1	1	2	?
Conophytum globosum	1	0	0	1	2	3/4	1/2
Conophytum hermarium	?	0	1	1/4	1	2	1/2
Conophytum herreanthus	1	0	0	1	1	1	2/3
Conophytum hians	2	0	0	1	4	3	?
Conophytum joubertii	1	0	0	1	4	1	1/2
Conophytum jucundum	1	0	0	1	1	2	1
Conophytum khamiesbergensis	0	0	1	2/3	1	1	1
Conophytum klinghardtense	2	0	0	1	4	2	1/2
Conophytum limpidum	1	1	1	1	1	1	2
Conophytum lithopsoides	1	1	1	1	1	4	1/2
Conophytum longum	2	1	1	1	1	3	2/3
Conophytum luckhoffii	0	0	0	1	1	4	1/2
Conophytum marginatum	2	0	0	1	1	3	1/2
Conophytum maughanii	1	1	1	1	4	2	2/3
Conophytum meyeri	2	0	0	1	1	3	1/2
Conophytum minutum	1	0	0	1	1	4	1/2
Conophytum obcordellum	1	0	0	1	4	2	1/2
Conophytum pageae	1	0	0	1/4	4	2	1/2/3
Conophytum pellucidum	1	1	1	1/4	1	4	1/2
Conophytum phoeniceum	2	1	1	1	4	3	1/2
Conophytum piluliforme	1	0	0	1	4	2	1
Conophytum quaesitum	1	0	0	1	4	3	1/2/3
Conophytum ratum	1	1	1	1	2	1	2
Conophytum regale	2	0	0	1	2	2	2
Conophytum roodiae	1	0	1	1	2	3	1/2
Conophytum saxetanum	1	0	0	1	4	3	1/2
Conophytum smorenskaduense	1	1	1	2	2	2	1/2
Conophytum stephanii	2	0	0	1	4	2	1/2
Conophytum subfenestratum	2	1	1	1	1	1	1/2/3
Conophytum swanepoelianum	1	0	0	1	1	4	1/2
Conophytum tantillum	1	0	0	1	1	3	1/2
Conophytum truncatum	1	0	0	1	4	2	1/2
Conophytum uviforme	2	0	0	1	4	2	1/2
Conophytum vanheerdei	1	0	1	1	1	3	1/2
Conophytum velutinum	2	0	0	1	1	3	1/2
Conophytum violaciflorum	1	0	0	1	1	3	1/2
Conophytum wettsteinii	1	0	0	1	1	3	1/2

	1	2	3	4	5	6	7
Cheiridopsis acuminata	2	0	0	2	1	0	4
Cheiridopsis alba-oculata	?	0	0	2	1	0	4
Cheiridopsis angustifolia	?	0	0	2	1	0	4
Cheiridopsis aspera	1	0	0	2	1	0	4
Cheiridopsis brownii	?	0	0	2	1	0	4
Cheiridopsis caroli-schmidtii	1	0	0	2	1	0	4
Cheiridopsis denticulata	2	0	0	2	1	0	4
Cheiridopsis derenbergiana	?	0	?	2	1	0	4
Cheiridopsis excavata	?	0	0	2	1	0	4
Cheiridopsis gamoepensis	1	0	0	2	1	0	4
Cheiridopsis glomerata	2	0	0	2	1	0	4
Cheiridopsis herrei	2	0	0	2	1	0	4
Cheiridopsis marlothii	2	0	0	2	1	0	4
Cheiridopsis meyeri	1	0	0	2	1	0	4
Cheiridopsis minor	1	0	0	2	1	0	4
Cheiridopsis namaquensis	1	0	0	2	1	0	4
Cheiridopsis pearsonii	?	0	0	2	1	0	4
Cheiridopsis peculiaris	1	0	0	2	1	0	4
Cheiridopsis pillansii	?	0	0	2	1	0	4
Cheiridopsis pilosula	2	0	0	2	1	0	4
Cheiridopsis ponderosa	2	0	0	2	1	0	4
Cheiridopsis purpurea	2	0	0	2	1	0	4
Cheiridopsis robusta	?	0	0	2	1	0	4
Cheiridopsis rostrata	1	0	0	2	1	0	4
Cheiridopsis speciosa	?	0	0	2	1	0	4
Cheiridopsis umdausensis	1	0	0	2	1	0	4
Cheiridopsis vanzylii	2	0	0	2	1	0	4
Cheiridopsis verrucosa	2	0	0	2	1	0	4
Enarganthe octonaria	0	0	0	2	1	0	2
Jensenobotrya lossowiana	0	0	0	2	1	0	2
Namaquanthus vanheerdei	0	0	0	2	1	0	3/4
Ruschianthus falcatus	0	0	0	2	1	0	2
Schlechteranthus abruptus	1	0	0	2	1	0	3
Schlechteranthus albiflorus	1	0	0	3	1	0	4
Schlechteranthus connatus	1	0	0	2/3	1	0	3
Schlechteranthus hallii	1	0	0	2	1	0	4
Schlechteranthus inclusus	1	0	0	2/3	1	0	3
Schlechteranthus parvus	1	0	0	3	1	0	3
Schlechteranthus pungens	1	0	0	3	1	0	3
Schlechteranthus spinescens	1	0	0	3	1	0	3
Schlechteranthus stylosus	1	0	0	2/3	1	0	3
Schlechteranthus subglobosus	1	0	0	2/3	1	0	3
Schlechteranthus tetrasepalus	1	0	0	2	1	0	3
Knersia diversifolia	1	0	0	2	1	0	2
Jacobsenia vaginata	?	0	0	2	1	0	2/3/4

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6.3 RESULTS

6.3.1 PHYLOGENETIC ANALYSES

Due to the poor resolution of single plastid gene trees, the data was analysed in combination following Klak *et al.* (2013) and Powell *et al.* (2016, submitted b; Chapters 3 & 5). The combined plastid matrix for the six gene regions consisted of 4,497 unambiguously aligned positions with 463 variable characters and 254 parsimony informative characters. In the MP analysis, 158 trees were retained with a tree length of 1,065 steps (consistency index (CI)=0.72; retention index (RI)=0.63). The phylogenies produced in the BI (Fig. 6.3), MP (Fig. 6.4) and ML (Fig. 6.5) analyses were consistent with one another in topology, with the exception of the phylogenetic placement of *C. marginatum* Lavis and *C. roodiae* N.E.Br.. In the BI phylogenetic tree, *C. marginatum* and *C. roodiae* were recovered in a clade with *C. minutum* N.E.Br. and *C. swanepoelianum* Rawé (Fig. 6.3), while in the ML analyses *C. marginatum* was placed sister to *C. burgeri* L.Bolus and *C. roodiae* sister to *C. hermarium* (S.A.Hammer) S.A.Hammer and *C. pellucidum* Schwantes (Fig. 6.5). The placement of these two species was unresolved in the MP analyses (Fig. 6.4). The different analyses also produced differences in resolution and support for the recovered clades of *Conophytum* (Figs. 6.3–6.5). The phylogenetic tree recovered from the MP analysis was poorly resolved with the phylogenetic placement of the majority of species unresolved, however, the few recovered clades were moderately to strongly supported (Fig. 6.4). The phylogenetic tree produced by the ML analysis provided a well resolved topology, however, support for the clades, especially for the larger recovered clades was very poor or absent (Fig. 6.5). The BI phylogenetic tree provided a number of well-resolved clades with strong support values (Fig. 6.3).

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Conophytum was recovered as monophyletic in all the analyses, albeit with only poor support in the BI analyses (PP=0.72) and no support in the MP and ML analyses (Figs. 6.3–

6.5). Although the backbone resolution of *Conophytum* was poor, six main clades were recovered within the genus (clades A–F; Fig. 6.3).

Clade A: This clade was recovered with strong support (PP=1, BS=100, JK=100) and included mainly members of sect. *Ophthalmophyllum* (Dinter & Schwantes) G.D.Rowley, as well as *C. achabense* S.A.Hammer of sect. *Chesire-Feles* S.A.Hammer and *C. lithopsoides* L.Bolus from section *Pellucida* (Schwantes) Tischer ex S.A.Hammer (Figs. 6.3–6.5).

Clade B: This clade (PP=0.54) included species from various sections including sects. *Biloba* N.E.Br., *Conophytum*., *Herreanthus* (Schwantes) S.A.Hammer, *Minuscula* (Schwantes) Tischer ex S.A.Hammer, *Saxetana* (Schwantes) S.A.Hammer and *Wettsteinia* (Schwantes) Tischer ex S.A.Hammer (Fig. 6.3). Within this clade, *C. klinghardtense* Rawé and *C. quaesitum* N.E.Br., both of sect. *Saxetana* were recovered as a strongly supported subclade (PP=1, BS=68; Figs. 6.3, 6.5).

Clade C: This clade included most of the species sampled from section *Biloba* and was recovered with *C. ectypum* N.E.Br. and *C. violaciflorum* Schick & Tisch. from section *Minuscula* (PP=0.7; Fig. 6.3). The species from section *Biloba* (*C. bilobum* (Marloth) N.E.Br., *C. frutescens* Schwantes, *C. meyeri* N.E.Br., *C. velutinum* Schwantes) formed a strongly supported subclade (PP=0.98, BS=69; Figs. 6.3, 6.5).

Clade D: This clade was recovered in both the BI and ML analyses with strong nodal support (PP=1, BS=85; Figs. 6.3, 6.5). The species were further separated into two strongly supported subclades; one including *C. breve* N.E.Br., *C. flavum* N.E.Br. and *C. calculus* (A.Berger) N.E.Br. (PP=1, BS=94), and a second clade with *C. depressum* Lavis, *C. regale* Lavis and *C. stephanii* Schwantes (PP=1, BS=98, JK=85; Figs. 6.3–6.5). Both *C. breve* and *C. calculus* are classified in sect. *Cataphracta* Schwantes ex S.A.Hammer, with *C. flavum* included in sect. *Wettsteinia*. *Conophytum depressum* and *C. stephanii* were the only species

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sampled from sect. *Barbata* Schwantes ex S.A.Hammer, while *C. regale* was one of three species sampled from sect. *Herreanthus.*

Clade E: This large clade (PP=0.71) included species from sections. *Conophytum, Pellucida, Subfenestrata* Tischer ex S.A.Hammer and *Verrucosa* Schwantes ex S.A.Hammer (Fig. 6.3). The species included in sect. *Conophytum* formed a strongly supported subclade (PP=1, JK=85; Figs. 6.3, 6.4) which was recovered as sister to *C. subfenestratum* Schwantes, the type species of sect. *Subfenestrata,* and together these species formed a strongly supported clade (PP=0.92, BS=53; Figs. 6.3, 6.5). *Conophytum hermarium* (sect. *Verrucosa*) and *C. pellucidum* (sect. *Pellucida*) were recovered as sister taxa (PP=0.92, BS=69; Figs. 6.3, 6.5), as well as *C. smorenskaduense* de Boer and *C. vanheerdei* Tisch. from sect. *Verrucosa* (PP=1, BS=97, JK=92; Figs. 6.3–6.5).

Clade F: This clade included *C. marginatum* (sect. *Herreanthus*), *C. minutum* (sect. *Wettsteinia*), *C. swanepoelianum* (sect. *Minuscula*) and *C. roodiae* (one of two species sampled from sect. *Cylindrata* Schwantes ex S.A.Hammer) (PP=0.88, Figs. 6.3, 6.4). Within this clade, *C. minutum and C. swanepoelianum* were recovered as sister taxa (PP=1, BS=71, JK=82), while the phylogenetic relationships of *C. marginatum* and *C. roodiae* was unresolved (Figs. 6.3–6.5).

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Some additional species were recovered as sister pairs, but the phylogenetic relationships of these species within *Conophytum* was unresolved (Fig. 6.3). These sister pairs included *C. angelicae* N.E.Br. (sect. *Costata* Schwantes ex S.A.Hammer) and *C. luckhoffii* Lavis (sect. *Minuscula*) (PP=0.89, BS=73; Figs. 6.3, 6.5); *C. bruynsii* S.A.Hammer (sect. *Minuscula*) and *C. pageae* N.E.Br. (sect. *Cataphracta*) (PP=0.98; Fig. 6.3) and *C. maughanii* N.E.Br. and *C. ratum* S.A.Hammer (both from sect. *Chesire-Feles*) (PP=0.99, BS=70; Figs. 6.3, 6.5).

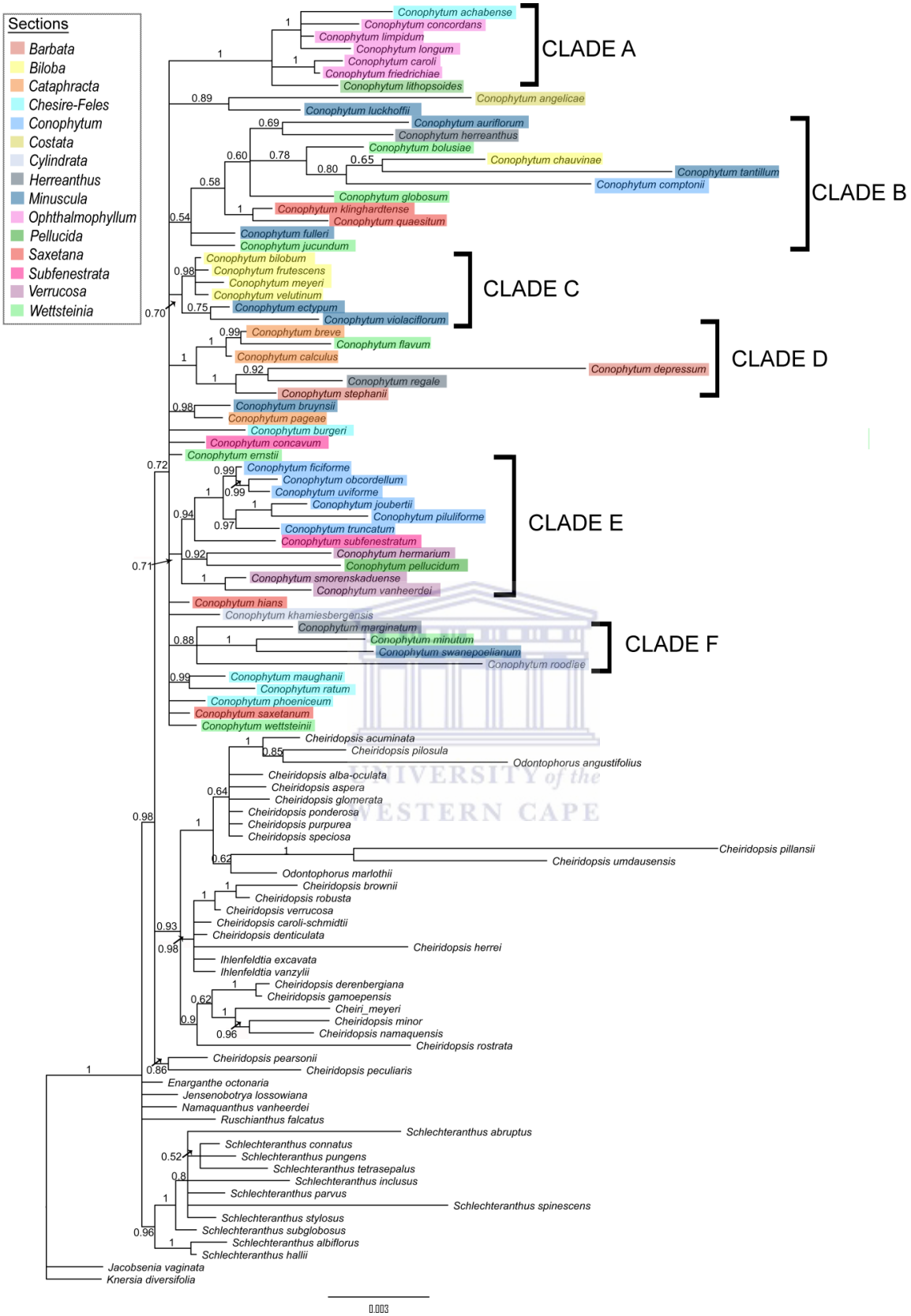

Figure 6.3. Majority Rule Consensus tree from Bayesian analysis of six plastid markers, indicating phylogenetic relationships in *Conophytum* and the *Conophytum*-clade. Posterior probability (PP) values above 0.5 are indicated above the branches. Subgeneric sections of *Conophytum* are marked with different colours, and the main clades are identified.

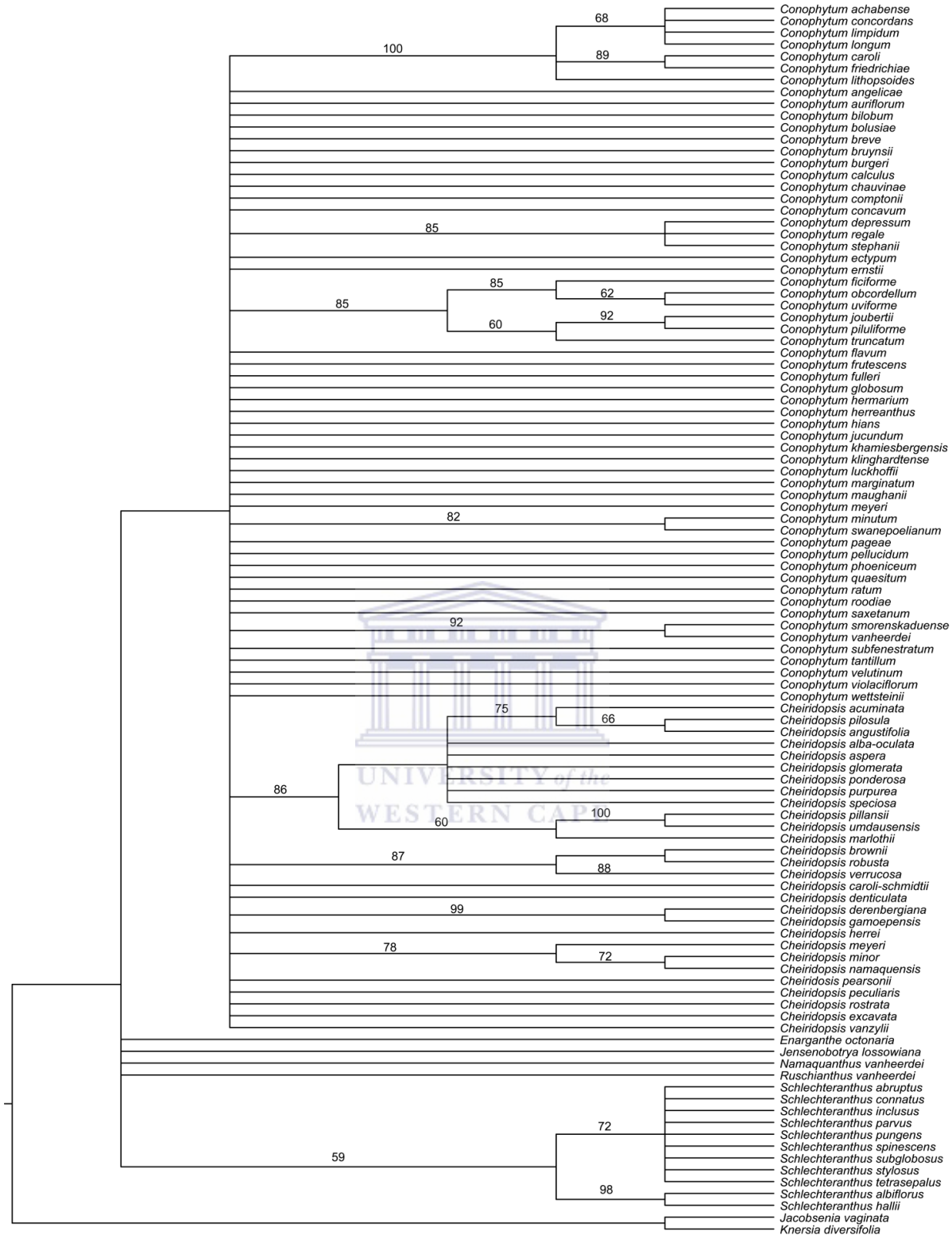

Figure 6.4. Maximum parsimony estimate based on six plastid gene regions, showing the phylogenetic relationships in *Conophytum* and the *Conophytum*-clade. Jackknife support greater than 50% is indicated above the branches.

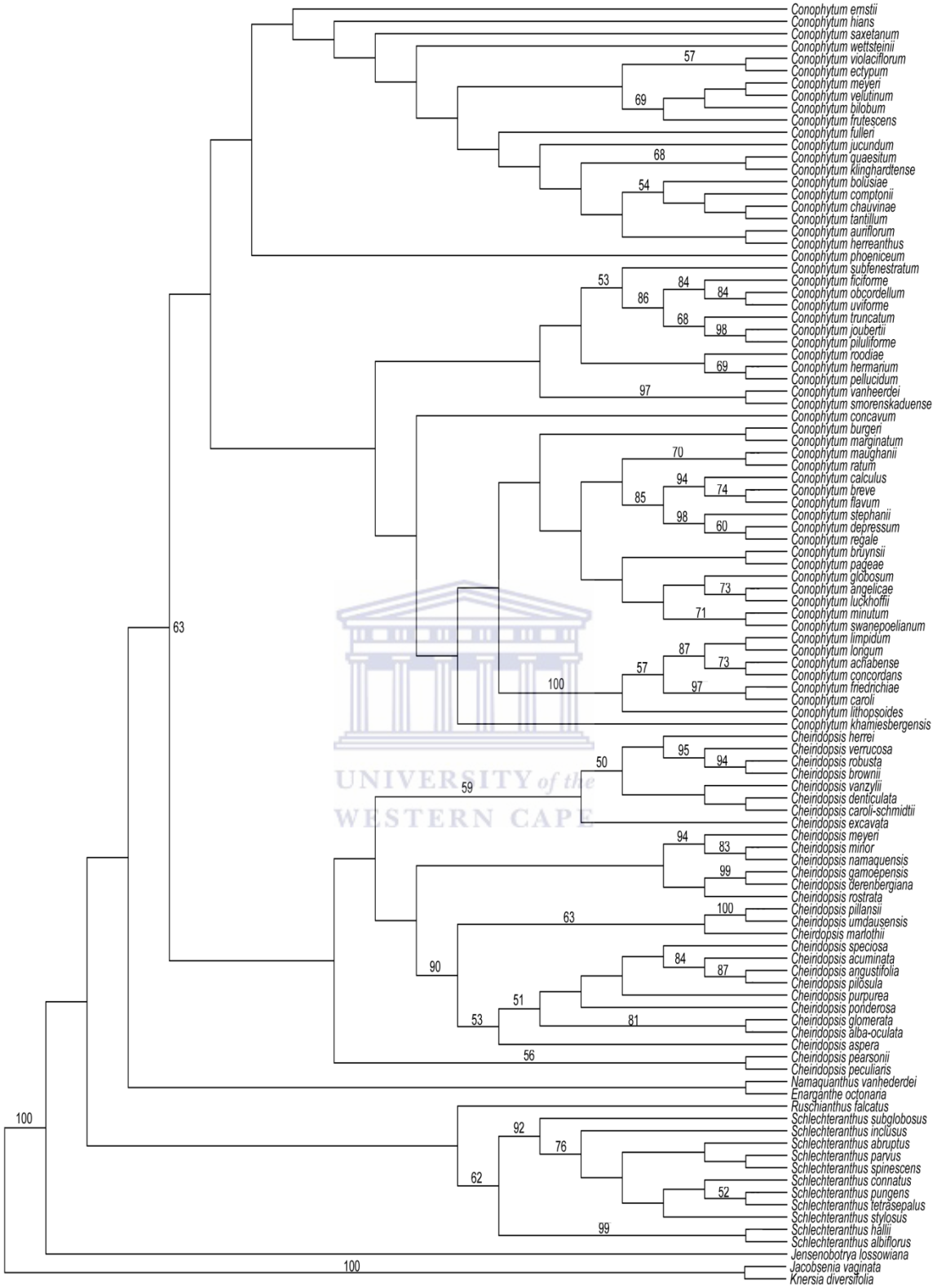

Figure 6.5. Maximum-likelihood estimate based on six plastid gene regions showing the phylogenetic relationships in *Conophytum* and the *Conophytum*-clade. Bootstrap support greater than 50% is indicated above the branches.

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The phylogenetic relationship of eight species was unresolved in *Conophytum* (Fig. 6.3). These species included *C. burgeri* and *C. phoeniceum* S.A.Hammer both from sect. *Chesire-Feles, C. khamiesbergensis* (L.Bolus) Schwantes (sect. *Cylindrata*), *C. hians* N.E.Br. and *C. saxetanum* N.E.Br. from sect. *Saxetana, C. concavum* L.Bolus (sect. *Subfenestrata*) and *C. ernstii* S.A.Hammer and *C. wettsteinii* N.E.Br. from section *Wettsteinia.*

6.3.2 Evolution of Leaf, Floral and Capsule Morphological Characters

6.3.2.1 Leaf epidermal papillae

Papillae were found in the majority of genera in the *Conophytum*-clade were was rarely absent (absent in *Conophytum achabense, C. burgeri, C. comptonii* N.E.Br., *C. khamiesbergensis, Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus*) (Fig. 6.6A). There were two types of papillae present in the genera, either blunt papillae or trichomes (Fig. 6.6A).

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Schlechteranthus Schwantes only included species with blunt papillae, supporting the recovered clade of these species, while in *Cheiridopsis* and *Conophytum,* there were blunt papillae, trichomes or no epidermal extensions (i.e. absent, only in *Conophytum*) (Fig. 6.6A). Subgenus *Cheiridopsis* only included blunt papillae, providing support for this subgenus, while in *Conophytum* blunt papillae, trichomes and absence of epidermal extensions evolved numerous times independently, not providing support for any specific clades within the genus (Fig. 6.6A).

Although papillae type was variable in the *Conophytum*-clade, it was found to be constant within a species, i.e. there were no intermediate species which displayed a range of epidermal extensions or variability between presence and absence of epidermal extensions (Fig. 6.6A).

Although the majority of the clade included papillae, the recovered ancestral state for the *Conophytum*-clade was recovered as an absence of epidermal extensions (Fig. 6.6A). The absence of epidermal extensions was found in a few genera in the *Conophytum*-clade, but this absence could only be interpreted as a loss in some species of *Conophytum* (*C. achabense, C. comptonii, C. luckhoffii*), while the remaining species without epidermal extensions (*C. burgeri, C. khamiesbergensis, Enarganthe octonaria* (L.Bolus) N.E.Br., *Jensenobotrya lossowiana* A.G.J.Herre, *Namaquanthus vanheerdei* L.Bolus, *Ruschianthus falcatus* L.Bolus) were unresolved.

6.3.2.2 Leaf epidermal windows

Leaf epidermal windows were found only in species of *Conophytum*. Although this character is not often present across the genus it appears to have evolved independently several times (Fig. 6.6B). The presence of windows was recovered as a character for clade A, while it was also found in three species of clade E (Fig. 6.6B). Leaf windows were also shown to be present in the sister species *C. maughanii* and *C. ratum* as well as the unresolved taxa *C. burgeri, C. concavum* and *C. phoeniceum* (Fig. 6.6B).

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6.3.2.3 Soft-leaved bodies

Leaves which form soft bodies were unique to *Conophytum* within the *Conophytum*-clade, and although this character was shown to have evolved numerous times, it provided morphological support for some clades (Fig. 6.6C). Soft-leaved bodies were found in all species of clade A as well as in the sister species *C. maughanii* and *C. ratum* (Fig. 6.6C). This character was also recovered as the ancestral state for clade E (Fig. 6.6C). *Conophytum roodiae* in clade F also possessed soft-leaved bodies, as well as the unresolved taxa *C. burgeri, C. concavum* and *C. phoeniceum* (Fig. 6.6C).

6.3.2.4 Flowering season (phenology)

Autumnal flowering was recovered as a synapomorphy for *Conophytum* (Fig. 6.7A). Within the genus shifts to other flowering seasons were found in only seven of the sampled *Conophytum* species (Fig. 6.7A). In three of these species, *C. hermarium, C. pageae* and *C. pellucidum,* the flowering season begins in summer and continues into autumn (Fig. 6.7A). The flowering season in *C. bolusiae* Schwantes and *C. frutescens* has completely shifted, with the species flowering from spring into summer (Fig. 6.7A). *Conophytum smorenskaduense* only flowers in winter, while *C. khamiesbergensis* flowers from winter into spring (Fig. 6.7A).

6.3.2.5 Flowering time (anthesis)

The majority of taxa in the *Conophytum*-clade are diurnal flowering, while *Conophytum* displays a variation of flowering times (Fig. 6.7B). Nocturnal flowering has evolved numerous times in the genus (Fig. 6.7B), and characterises clade D and a subclade in clade E (*C. ficiforme–C. truncatum*) (Fig. 6.7B). Nocturnal flowering was also found in the sister taxa *C. klinghardtense* and *C. quaesitum,* as well as in *C. angelicae, C. comptonii, C. pageae, C. hians, C. maughanii, C. phoeniceum* and *C. saxetanum* (Fig. 6.7B). Afternoon flowering (open midday and close a few hours after) was only present in a few species in the genus, but was shown to have evolved a number of times across the genus (e.g. *C. achabense, C. friedrichiae* Schwantes, *C. globosum* N.E.Br., *C. regale, C. smorenskaduense, C. roodiae* and *C. ratum* (Fig. 6.7B)). Late afternoon flowering (open a few hours before dusk and close shortly thereafter) was very rare in the genus and was only found in three unresolved species (*C. burgeri, C. concavum, C. ernstii*) in the phylogenetic tree (Fig. 6.7B).

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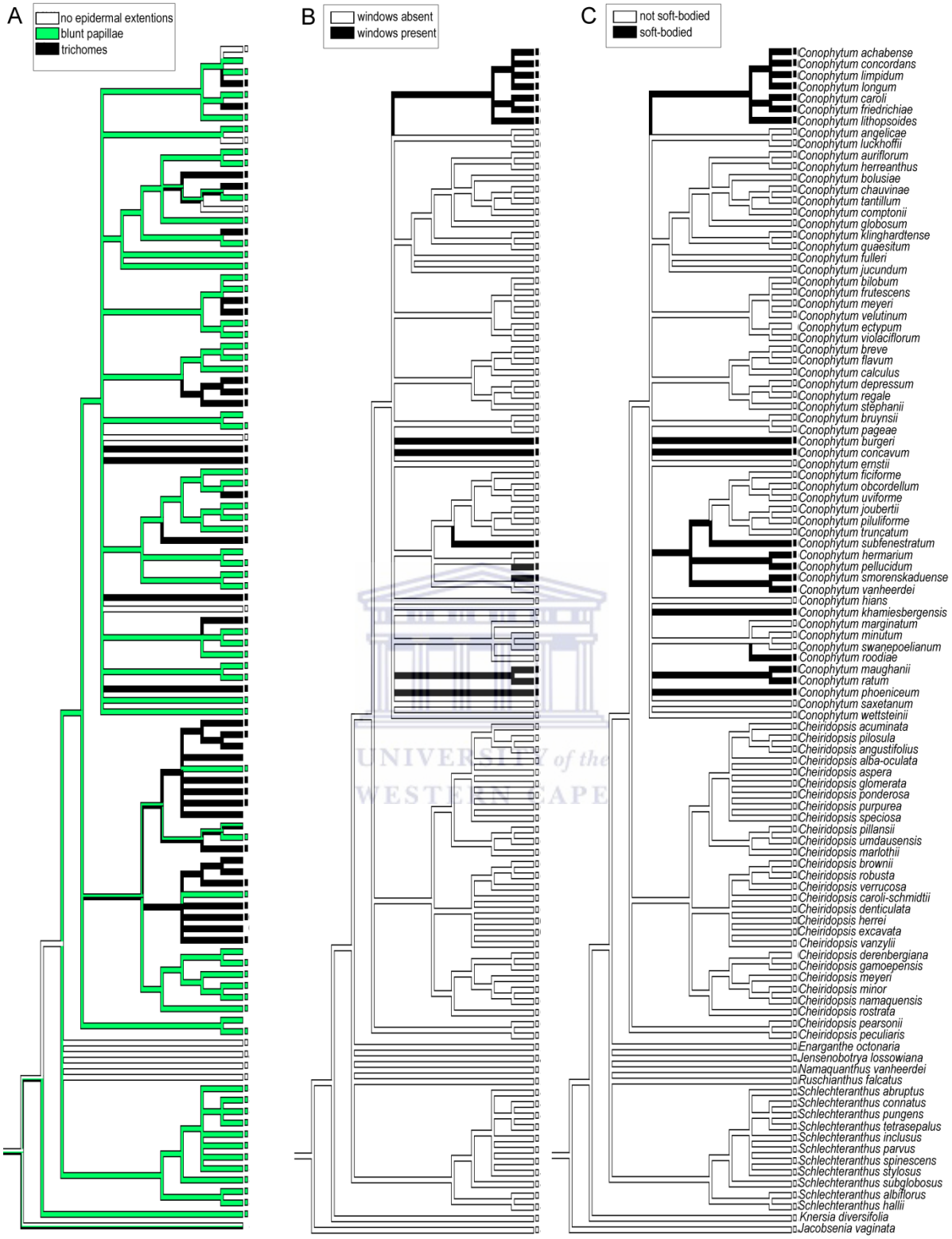

Figure 6.6. Character reconstruction of leaf epidermal characters (Appendix D2) in the *Conophytum*-clade, mapped onto the Bayesian inference consensus tree: (A) papillae type; (B) presence of leaf epidermal windows; (C) soft-leaved bodies.

6.3.2.6 Floral structure

The different structure types as defined by Liede & Hammer (1990), where shown to have evolved and shifted multiple times (Fig. 6.7C). Structure A2 (Fig. 6.2) was the most common structure in the sampled *Conophytum* species, while Structure A1 (Fig. 6.2) was uncommon across the sampled taxa, and was recovered as the ancestral state for clade A (Fig 6.7C). Structure B1 (Fig. 6.2) was the ancestral state for (Clade C, with different floral structures found in *C. bilobum* and *C. ectypum* (Fig. 6.7C). Similarly, the floral structure A2 (Fig. 6.2) characterised clade D, with only *C. flavum* displaying structure type B1 (Fig. 6.7C). Structure B2 (Fig. 6.2) was found to be rare, presented in *C. lithopsoides, C. pellucidum* and sister species *C. minutum and C. swanepoelianum* (Fig. 6.7C). *Conophytum ectypum* and *C. globosum* displayed both structure B1 and B2, with B1 recovered as the ancestral state (Fig. 6.7C).

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6.3.2.7 Locule number

The number of locules mostly supported generic and subgeneric groupings within the *Conophytum-clade* (Fig. 6.8). Capsules with more than nine locules was present in all *Cheiridopsis* species, while locule number (7–9 and 10–12 locules) in *Schlechteranthus* supported the recovered subgeneric clades (Fig. 6.8; Chapter 3). Locule number was more variable within *Conophytum*, but the reconstruction showed that clade B included mainly species with less than 5 locules, while clades A, C, D and F, as well as the majority of the recovered species pairs and some unresolved species, were characterised by capsules with 5–6 locules (Fig. 6.8). The morphological analysis indicated that Clade F, as well as the species pair *C. bruynsii* and *C. pageae*, were polymorphic with less than 5 or 5–6 locules (Fig. 6.8).

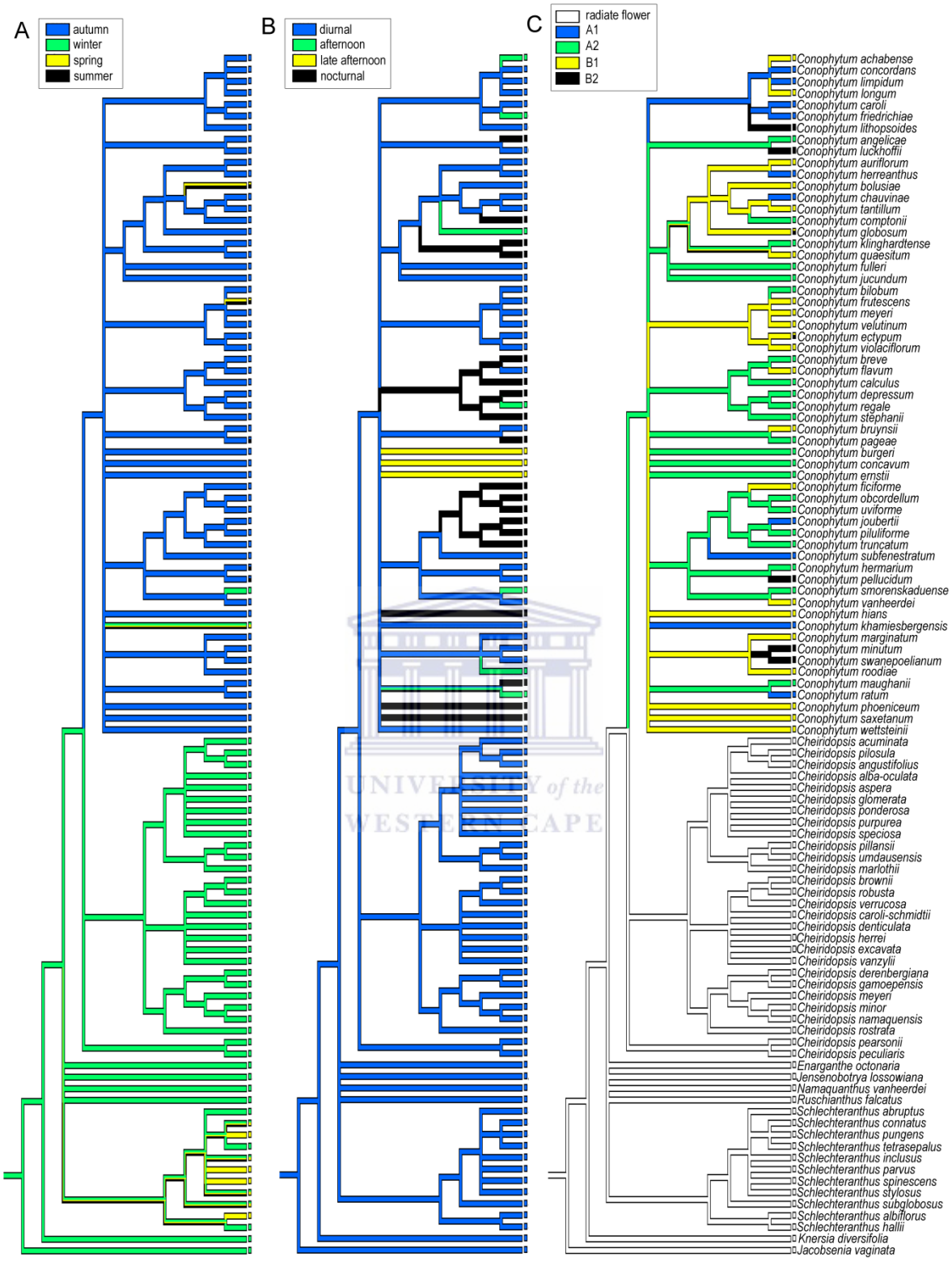

Figure 6.7. Character reconstruction of floral morphological traits (Appendix D2) in the *Conophytum*-clade, mapped onto the Bayesian inference consensus tree: (A) flowering season (phenology); (B) flowering time (anthesis); (C) floral structure (Liede & Hammer, 1990; Fig. 6.2).

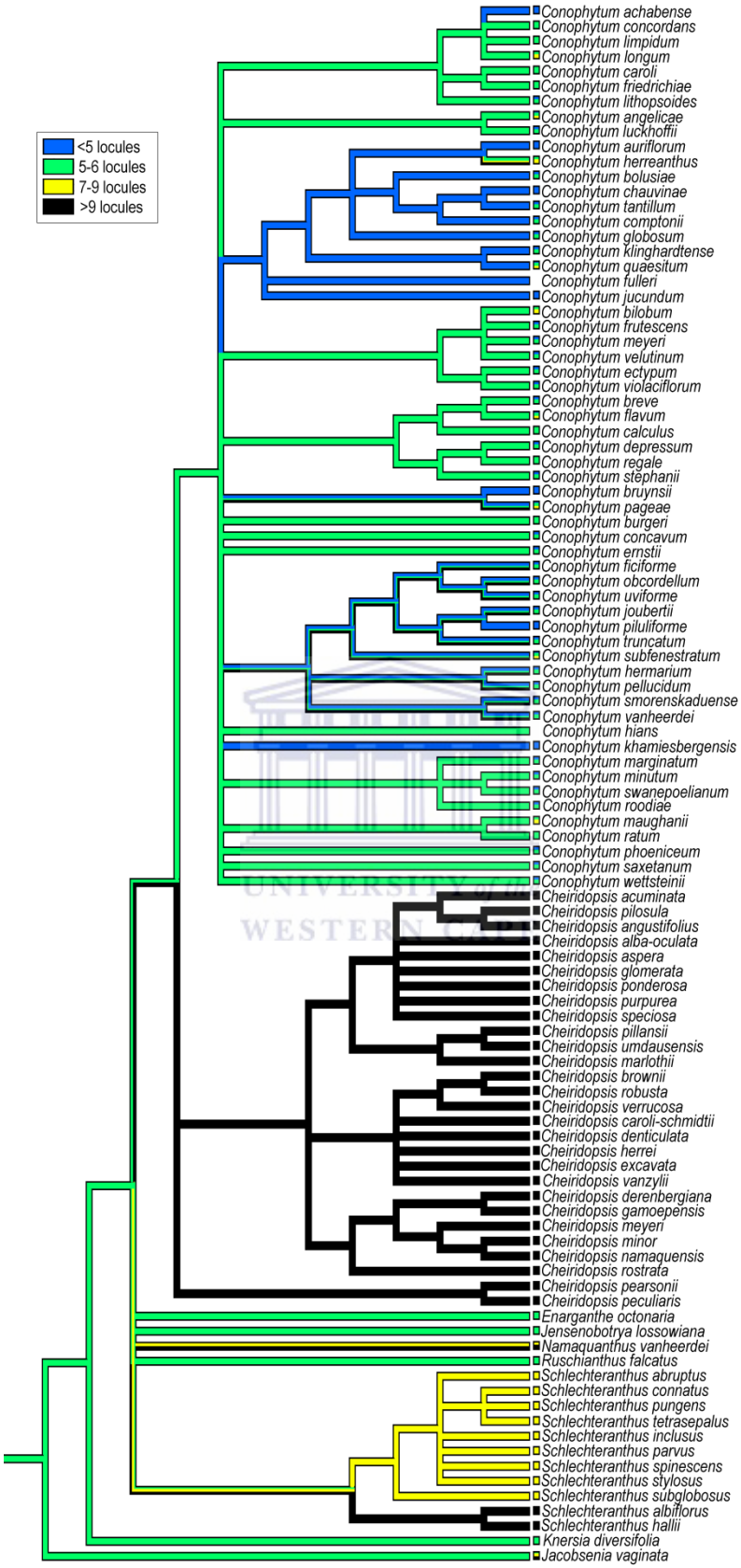

Figure 6.8. Character reconstruction of locule number (Appendix D2) in capsules of the *Conophytum*-clade, mapped onto the Bayesian inference consensus tree.

6.4 DISCUSSION

6.4.1 MONOPHYLY OF *CONOPHYTUM*

Conophytum was recovered as monophyletic, with the majority of species in the recovered in well-supported clades (Fig 6.3). However, despite the expanded sampling of the genus, backbone resolution in *Conophytum* remains poor (Fig. 6.3), which may be attributed to the recent and rapid radiation of this genus, with low levels of sequence divergence (Klak *et al.*, 2004).

The genus includes species that were previously recognised as separate genera. These include species of sect. *Ophthalmophyllum* (=*Ophthalmophyllum* Dinter & Schwantes), *C. herreanthus* (=*Herreanthus* Schwantes) and *C. khamiesbergensis* (=*Berrisfordia* L.Bolus). The recovery of these species in the monophyletic *Conophytum* (Fig. 6.3) supports the inclusion of these taxa in the genus by Hammer (1993).

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6.4.2 SECTIONAL CLASSIFICATION AND RECOVERED CLADES IN *CONOPHYTUM*

6.4.2.1 Current sectional classification of *Conophytum* and previously recovered clade

None of the sections of *Conophytum* (Hammer, 2002) were recovered as monophyletic, although the phylogenetic analyses did recover a few clades (Clades A, C, E) that more or less corresponded to the current sectional classification. A number of large clades including species from a range of section (sects. *Biloba, Conophytum, Herreanthus, Minuscula, Saxetana* and *Wettsteinia*) were also recovered (Fig. 6.3).

All the species included in section *Ophthalmophyllum* were recovered in a strongly supported clade (PP=1, BS=100, JK=100; Figs. 6.3–6.5), which was supported by the shared characters of soft-leaved bodies and windowed leaves (Fig. 6.6B, C).

Almost all species sampled of sect. *Biloba* are included in clade C, with the exception of *C. chauvinae* (Figs. 6.3, 6.4) which is placed in clade B. Species of sect. *Biloba* are robust,

developing stems at maturity with lobed leaves that are usually red-keeled (Hammer, 2002). The majority of species form a strongly supported clade (PP=0.98, BS=69; Figs. 6.3, 6.5) and mainly share diurnal autumnal flowers (flowers of *C. frutescens* appear from spring to summer) with the B1 floral structure (Fig. 6.2; Liede & Hammer, 1990). *Conophytum chauvinae* shares these morphological characters with the species of sect. *Biloba* and therefore appears to be misplaced in the phylogeny, with no characters supporting its current placement in clade C.

A strongly supported subclade corresponding to sect. *Cataphracta* (*C. breve* and *C. calculus*) is recovered within clade D (PP=1, BS=94; Figs. 6.3, 6.5) and the species share the chalky epidermis which characterises the section (Hammer, 2002). The other subclade recovered in clade D (PP=1, BS=98, JK=85) includes the species of sect. *Barbata* (*C. depressum* and *C. stephanii*) which are characterised by the presence of trichomes on the leaves (Hammer, 2002; Fig. 6.6A). Both these clades were recovered in the analysis of Opel (2005b). Furthermore, when papillae characters were excluded from the morphological analysis by Opel (2005b), these two clades were recovered as sisters, corresponding to the molecular phylogenetic results (PP=1, BS=85; Figs. 6.3, 6.5).

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Section *Conophytum* is characterised by nocturnal, strongly scented flowers (Hammer, 2002) and the species of this section (with the exception of *C. comptonii*) were recovered in a strongly supported clade (PP=1, JK=85). These species were also recovered as a monophyletic clade in the study by Opel (2005b). In the clade including species of sect. *Conophytum*, flower colour ranges from white to bright pink, however, in *C. piluliforme* (N.E.Br.) N.E.Br., the flowers are red-orange (Hammer, 2002). Although the distribution of species in this section extends across the range of *Conophytum*, some species occupy the most south-eastern extent of the distribution, into the Little Karoo, where the distribution of the species seldom overlaps (Young & Desmet, 2016). The close phylogenetic relationship

between these species and the south-eastern extension of their distribution suggests a successional colonisation and migration of these species into the Little Karoo, with subsequent allopatric speciation (Coyne & Orr, 2004).

The species of sect. *Verrucosa* do not form a monophyletic clade, as found in Opel (2005b), but rather *C. smorenskaduense* is recovered sister to *C. vanheerdei,* while *C. hermarium* is recovered sister to *C. pellucidum* from sect. *Pellucida* (Fig. 6.3).

The sects. *Cylindrata, Herreanthus, Minuscula, Pellucida* and *Wettsteinia* were found to be placed grossly polyphyletic with species of these sections placed in different clades throughout the phylogeny (Fig. 6.3). Although species are included in these section based on shared morphological characters, the leaf and floral characters which were used to circumscribe the sections (e.g. warty leaves, cylindrical leaf body, prominent leaf markings etc.) do not support any groupings in the phylogenetic analysis (Fig. 6.3).

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6.4.2.2 Novel phylogenetic relationships in *Conophytum*

Clade A: Although this clade is mainly characterised by species of sect. *Ophthalmophyllum,* it includes species of the morphological similar sect. *Chesiere-Feles* (*C. achabense*) and *C. lithopsoides* from sect. *Pellucida.* Sections *Ophthalmophyllum* and *Chesiere-Feles* are mainly distinguished from one another by the fusion of the leaf lobes (unfused in *Ophthalmophyllum* and fused in *Chesire-Feles*), but are morphologically similar, sharing soft-leaved bodies and windowed leaves (Fig. 6.6B, C; Hammer, 2002). Based on the morphological similarity of the species, and the phylogenetic data, the results suggest an expansion of sect. *Ophthalmophyllum* to include those of sect. *Chesire-Feles*. *Conophytum lithopsoides* is included in sect. *Pellucida* in the sectional classification of Hammer (2002) based on the prominent leaf markings. However, the phylogenetic results suggest that this species should rather be included in an expanded sect. *Ophthalmophyllum,* with which it shares the soft-

leaved bodies and windowed leaves (Figs. 6.1D, 6.3, 6.6B, C). Due to the unresolved phylogenetic placement of the other sampled members of sect. *Chesire-Feles*, (*C. burgeri, C. maughanii* and *C. ratum*; Fig. 6.3) the monophyly of such an expanded section to include these unresolved species remains unclear.

Clade A also includes species which share the most north-eastern extent of the distribution of the genus, into Bushmanland (Hammer, 2002; Young & Desmet, 2016) and the species all occupy the quartz patch habitat which is common throughout this region (Desmet, 2000).

Clade B: This clade is an example of the great variation in morphological characters in *Conophytum* (Fig. 6.7), including a variety of floral structures, phenology and anthesis. Clade B includes a large number of species from an array of sections with no apparent morphological apomorphy.

Clade C: The placement of *C. ectypum* and *C. violaciflorum*, species from sect. *Minuscula*, as a sister clade to the species of sect. *Biloba* is supported by the bilobed leaves, with species of sect. *Minuscula* described as "miniature bilobes" with ornately marked leaf surfaces (Fig. 6.1C) by Hammer (2002). In addition, the placement of *C. violaciflorum* in sect. *Minuscula* was questioned by Hammer (2002) due to its possible affinity with species of sect. *Biloba*. The phylogenetic analyses and shared morphological characters of the species in clade C suggest an expansion of sect. *Biloba* to include *C. ectypum* and *C. violaciflorum.*

Two of the most widespread and variable species in the genus, *C. bilobum* (Fig. 6.1A) and *C. ectypum* (Fig. 6.3, Hammer, 2002) are included within clade C. *Conophytum bilobum* and *C. ectypum* also have a different floral structure than the remaining species of clade C, with the A2 structure (Fig. 6.2) in *C. bilobum* (Fig. 6.1A), and some populations of *C. ectypum* including the B2 structure (Figs. 6.2, 6.7C).

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Clade D: This clade includes species of sects. *Barbata, Cataphracta, Herreanthus* and *Wettsteinia.* Flowers of species in Clade D have an unusual floral syndrome, i.e. red-orange, strongly scented, nocturnal flowers, with the exception of *C. flavum* and *C. regale*, which have switched back to the ancestral state of diurnal flowering (Fig. 6.7B). In *C. regale,* the flowering time has only shifted a little earlier to afternoon flowering (Fig. 6.7B), and the flowers are pink, but have maintained a strong scent. In *C. flavum,* flowering time has switched to diurnal flowering (Fig. 6.7B), with yellow flowers and no scent, and so therefore exhibiting a complete shift in floral syndrome.

Species of sect. *Barbata* are characterised by the presence of trichomes. *Conophytum regale,* currently classified in sect. *Herreanthus* based on the softly keeled lobed leaves (Hammer, 2002), is however rather recovered within the clade of sect. *Barbata.* Such a placement is supported by the shared presence of trichomes in this species.

Another novel relationship recovered in clade D is the placement of *C. flavum* (currently included in sect. *Wettsteinia*), embedded in a clade with species of sect. *Cataphracta, C. breve* and *C. calculus.* These species all share leaves with a chalky epidermis (Fig. 6.1I; Hammer, 2002), and supports the inclusion of this species rather in sect. *Cataphracta*.

Clade E: Clade E is a large clade and includes species of sects. *Conophytum, Subfenestrata, Verrucosa* and *Wettsteinia.* The species of sect. *Conophytum* are recovered sister to *C. subfenestratum* (sect. *Subfenestrata*) and together these species represent the scented species in clade E (PP=0.92, BS=53; Figs. 6.3, 6.5).

The remaining species in clade E are included in sect. *Verrucosa,* with the exception of *C. pellucidum* which is from sect. *Pellucida.* Although *C. pellucidum* is currently classified in a different section based on the markings on the leaves (Fig. 6.1H), i.e. the shared

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characters of soft-bodied and windowed leaves (Fig. 6.6B, C) as well as the shift in flowering season with *C. hermarium*, supports the phylogenetic placement of this species in this clade.

Clade F: Species in this clade are included in a number of sections, specifically sects. *Cylindrata, Herreanthus, Minuscula* and *Wettsteinia,* and no apparent synapomorphy for the species in the clade could be ascertained. The sister taxa *Conophytum minutum* and *C. swanepoelianum* (PP=1, BS=71, JK=82, Figs. 6.3–6.5) do, however, share the rare B2 floral structure (Figs. 6.2, 6.7C).

6.4.3 Evolution of Locule Number in *Conophytum*

Capsule characters are often used to distinguish genera and subgenera in the Aizoaceae (Ihlenfeldt, 1960; Hartmann, 2001), and have been used to circumscribe subgenera in the *Conophytum*-clade (Powell *et al.*, 2016, submitted b; Chapters 3 & 5). Locule number has previously been used, specifically to define subgenera in *Schlechteranthus* (Powell *et al.*, 2016, submitted b; Chapters 3 & 5) with locule number ranging from 10 to 12 in subgenus *Schlechteranthus*, and 7 to 9 in subgenus *Microphyllus*, while the capsules of *Cheiridopsis* include more than 9 locules (Fig. 6.8). Although locule number is not typically relied on to distinguish the subgeneric sections in *Conophytum,* the ancestral trait reconstruction (Fig. 6.8) does recover a relationship between locule number and the recovered phylogenetic clades. However, the coded locule ranges occur numerous times across the genus (Fig. 6.8). The majority of clades (clades A, C, D, F) as well as a number of species pairs and unresolved species include 5–6-locular capsules (Fig. 6.8). Capsules with less than 5 locules were included in clades B and E, with locule number in capsules of clade E also sometimes less than 5 (Fig. 6.8). Locule number in the species, as well as across the genus was also very variable (Fig. 6.8).

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6.4.4 Evolution of Leaf Epidermal Characters in *Conophytum*

Papillae type (Fig. 6.6A), presence of windowed leaves (Figs. 6.1D, 6.6B) and soft-leaved bodies (Figs. 6.1 D, L; 6.6C) were shown to have evolved numerous times across the *Conophytum*-clade as well as in *Conophytum*. Although papillae type (as well as presence or absence of papillae) was variable across the clade, blunt papillae supported the *Schlechteranthus*-clade and the *Cheiridopsis* subgenus *Cheiridopsis*-clade (Fig. 6.6A). In *Conophytum,* the presence of trichomes supported a subclade in clade D which included *C. depressum, C. regale* and *C. stephanii* (Fig. 6.6D). The presence of soft-leaved bodies were particularly informative in *Conophytum,* grouping the species of clade A as well as a subclade in clade E (Fig. 6.6C). Some of the clades which included soft-leaved bodies were further supported by the presence of windows (specifically clade A), despite the fact that this character was recovered as a convergent character (Fig. 6.6B). Although a few clades were characterised by leaf epidermal characters, the majority of clades in *Conophytum* included a range of epidermal characters.

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6.4.5 Evolution of Floral Characters and Pollination as a Possible Driver of Floral Diversity in *Conophytum*

Floral traits are particularly diverse in *Conophytum,* including variation in phenology, anthesis and structure, and the ancestral trait reconstruction indicates that the different character states have evolved numerous times across the genus (Fig. 6.7). Floral anthesis, particularly nocturnal flowering, was shown to have evolved multiple times (Fig. 6.7B), which was also recovered in the morphological phylogeny of Opel (2005b), while other diurnal anthesis, such as afternoon and late afternoon flowering, also occurred numerous times across the genus (Fig. 6.7B). In addition, a shift back to diurnal flowering is recovered in clade D, with *C. regale* flowering in the afternoon, while the flowers of *C. flavum* are diurnal (Fig. 6.7B). These shifts in anthesis illustrate the plasticity of the floral characters in

Conophytum and suggest that there are numerous drivers of change across the genus. Similarly, floral structure and phenology show no clear phylogenetic pattern across the species of *Conophytum* (Fig. 6.7A, C). The floral diversity displayed in *Conophytum* as well as the evolution of these characters multiple times across the genus, suggests that these shifts in floral syndromes may be driven by pollination (Boberg *et al.,* 2014; De Jager & Ellis, 2014; Forest *et al.,* 2014). Pollination systems in Iridaceae, another family centred in the GCFR, have been shown to have evolved multiple times (Johnson & Steiner, 2000; Johnson, 2010; Forest *et al.,* 2014). A study on pollination shifts of a particularly florally diverse genus of the Iridaceae, *Lapeirousia* Thunb., indicated that the genus displayed 1.5 shifts per species, which is very high for the region, with an average of 5.4 shifts per species (Forest *et al.,* 2014). The inferred pollination shift in *Conophytum* mirrors that of *Lapeirousia,* with 1.3 shifts per species, suggesting a close relationship between floral adaptation and pollination (Boberg *et al.,* 2014; De Jager & Ellis, 2014) in the genus. Floral adaptation to pollinators has also been proposed as one of the main factors promoting formation of new species (Johnson, 1996; 2010), and therefore the diverse floral morphologies, possibly driven by pollination, may have resulted in the substantially high number of species in the genus, specifically in comparison to the other genera in the *Conophytum*-clade. As the majority of genera in the *Conophytum*-clade (and the family) possess unspecialised and uniform flowers (Hartmann, 1983, 1991), the evolution of unique tubular flowers (Haas, 1976) and subsequent diversification into floral-types as well as phenologies, anthesis, scents and colours in *Conophytum* may have enabled the genus to exploit a range of potential pollinators.

Pollination observations of *Conophytum* have shown that the diurnal flowers are mainly visited by pollen wasps, while the nocturnal flowers were pollinated by moths (Liede *et al.,* 1991; Jürgens & Witt, 2014). The number of observed pollinators does not, however, match the diversity of the floral structures in *Conophytum* (Liede & Hammer, 1990),

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suggesting that there may be additional unknown pollinators, which led to the adaptation of the different floral structures. However, the different floral types A and B may also contribute to the diversity of floral structures, as these different types (A and B), representing fused versus unfused stigmas, have been suggested as a possible adaptation to increase pollination success (Liede & Hammer, 1990). This trait has independently evolved numerous times across the genus (structures A1 & A2, vs. B1 & B2; Fig. 6.7C), although all the species included in clade F had fused stigmas (Fig. 6.7C). In addition, almost all the species of clade C had fused stigmas, with the exception of *C. bilobum* (Fig. 6.7C). As the phylogenetic relationship of this species within the sect. *Conophytum* clade is unresolved, an improved phylogenetic sampling may recover this species as the most early diverging species of this clade.

The flowers of *Conophytum* display a variety of colours, exhibiting almost the full range found in the Aizoaceae, including white, yellow, pink, red-orange and magenta, with colour grades in between (Hammer, 2002). Due to the inherent variation in flower colour across the species, sometimes even within a population (Hammer, 2002), it was not reconstructed on the phylogenetic tree. It is commonly known that flower colour is strongly linked to pollination (e.g. Chittka & Menzel, 1992; Menzel & Shmida, 1993; Peter & Johnson, 2008). The diversity of flower colour in *Conophytum* may therefore be linked to pollinators, but due the limited observation of pollinators, a likely link between flower colour and pollinators in *Conophytum* has not yet been established.

The range of floral and leaf morphologies in *Conophytum* were shown to have evolved numerous times across the genus (Figs. 6.6, 6.7). The multiple evolutions of these characters may have attributed to the large number of species recognised in the genus, with these observed shifts, specifically in floral morphologies, forming in different florally distinct species.

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Chapter 7

Hotbeds of diversity in *Conophytum:* investigating floral and phylogenetic diversity in species-rich *Conophytum* communities

7.1 INTRODUCTION

The speciose genus *Conophytum* N.E.Br., with 106 species, includes a number of range restricted species, 20% of which are point endemics, with the extent of distribution for some species restricted to an area of only 1000 m^2 (Young *et al.,* 2015a). Highly range restricted species are often found within the arid parts of the Greater Cape Floristic Region (GCFR), particularly members of the Aizoaceae which are common the region (Liede *et al.,* 1991; Cowling & Hilton-Taylor, 1994; Cowling & Hilton-Taylor, 1999; Snijman, 2013).

The species of *Conophytum* occupy very small microhabitats due to their small size, consisting of a single highly-reduced pair of leaves (with some exceptions in sect. *Biloba* N.E.Br.) (Hammer, 2002). This enables the species to divide the landscape into very small units (Desmet, *et al.,* 1998; Young *et al.,* 2015a) and exploit all available habitats in a region, often resulting in the formation of complex species communities, with up to 11 species occurring sympatrically (Liede *et al.,* 1991; Young & Desmet, 2016). It is not clear how so many species of the same genus, co-existing in close proximity, are able to maintain their integrity (Liede & Hammer, 1990; Liede *et al.,* 1991). However, other genera in the GCFR, such as *Gladiolus* L., have been reported to form sympatric species complexes (i.e. the *G. carinatus* complex) (Goldblatt & Manning, 1998; Rymer *et al.,* 2010). These species of *Gladiolus*, exist however at a much larger scale than *Conophytum*, with species found together within a Quarter Degree Square (QDS) (as defined by Edwards & Leistner, 1971; Leistner & Morris, 1976) and with fewer co-occurring species (Rymer *et al.,* 2010). A study

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of pollination shifts in *Gladiolus* found that species that occur together display shifts in phenology (flowering season), in response to pollination competition (Rymer *et al.*, 2010).

The majority of *Conophytum* species flower in autumn, while the peak flowering season in the family is from winter to spring (Hartmann, 2001). A few species of *Conophytum*, however, flower in other seasons, displaying a shift in phenology (Chapter 6). A shift in flowering season has been observed in species which are found together in complex *Conophytum* species communities (Liede *et al.*, 1991; Young *et al.*, 2015a). More commonly, *Conophytum* species display a range of anthesis (flowering times) across the genus (Hammer, 2002; Chapter 6). These flowering times include diurnal (10 am–5 pm) and nocturnal (after dusk to just before dawn), as well as afternoon (12 pm to 4 pm) and late afternoon (just before dusk to a few hours thereafter) flowering species (Liede *et al.*, 1991; Hammer, 2002; Chapter 6). This range of anthesis is rare in the Aizoaceae, but is also found in *Mesembryanthemum* subgenus *Phyllobolus* (N.E.Br.) Klak section *Vesperifolia* Haw. (Klak & Bruyns, 2013) with the four species exhibiting differences anthesis, i.e. diurnal, afternoon, late afternoon and nocturnal (Gerbaulet, 1996).

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While the majority of Aizoaceae possess unspecialised radiate flowers (Hartmann, 1983), *Conophytum* has uniquely tubular flowers (Haas, 1976) with a range of floral structures (Liede & Hammer, 1990; Chapter 6). The flowers of *Erepsia* N.E.Br. also include a number of structures and a wide range of pollinators were recorded visiting these different floral structures, suggesting the different structures are strongly linked to different pollinators (Liede, 1990). The species of *Conophytum* also exhibit a wide range of floral scents (Jürgens & Manning, 2004), flower colour as well as variability in the presence or absence of filamentous staminodes (Hammer, 2002).

Floral diversification is commonly thought to be driven by pollination (Grant, 1949; Stebbins, 1970; Rymer *et al.*, 2010) and thus the wide range and variation of floral traits in

Conophytum may be as a response to competition for pollinators between species within a community. Few pollination observational studies have been conducted on *Conophytum* species (Liede *et al.*, 1991; Jürgens & Witt, 2014), with pollen wasps (Vespidae) and moths (Lepidoptera), as well as some members of Coleoptera, Diptera and Hymenoptera recorded as visitors of *Conophytum* flowers. Moths were, as expected, found to be the main visitors and pollinators of the nocturnal flowering *Conophytum* species, while the pollen wasps were recorded as the most common visitors of the diurnal species (Liede *et al.*, 1991; Jürgens & Witt, 2014). Although there is some indication of specialisation in *Conophytum* diurnal flowers to pollination by pollen wasps (Jürgens & Witt, 2014), this specialisation does not explain the great diversity of floral morphology in the genus, specifically among the diurnal species. Phylogenetic analyses of *Conophytum* indicate that differences in phenology and anthesis, as well as floral structure, have evolved several times across the genus (Chapter 6). Although these shifts in floral traits have occurred numerous times, the structure of these floral traits, as well as the variation of floral scent, colour and presence or absence of filamentous staminodes (Hammer, 2002) within the complex *Conophytum* species communities is unknown.

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Conophytum is recovered as sister to *Cheiridopsis* N.E.Br. in phylogenetic analyses of the *Conophytum*-clade (Chapters 5 & 6). These genera share leaf characters, especially the presence of sheathing leaves (Powell *et al.*, submitted b; Chapters 5 & 6). However, the genera differ in floral characters, with specialised tubular flowers (formed by the fusion of the petaloid staminodes) in *Conophytum* (Haas, 1976, Struck, 1995; Hammer, 2002; Chapter 6) and unspecialised radiate flowers in *Cheiridopsis* (Hartmann, 1983; Hartmann, 1991; Powell *et al.*, submitted b; Chapter 5). The capsule type and structure also differs between the genera, as *Cheiridopsis* shares the hard, woody, persistent capsules, which includes internal structures such as covering membranes and closing bodies, with the majority of the

Conophytum-clade (Powell *et al.*, submitted b; Chapter 5), while capsules in *Conophytum* are soft, papery and fragile, easily breaking off the plant, with no internal structures (Hartmann, 2001; Hammer, 2002; Chapters 5 & 6). The distribution of *Conophytum* is much wider than that of *Cheiridopsis*, with a larger number of species-rich hotspots (Young & Desmet, 2016). The distributions of these genera overlap, with a shared centre of diversity around Steinkopf (Young & Desmet, 2016; Powell *et al.*, submitted b; Chapter 5). In contrast to *Conophytum*, the species of *Cheiridopsis* do not form large complex species communities, with the distribution of only a few species overlapping (Hartmann & Dehn, 1987; Powell *et al.*, submitted b; Chapter 5). Despite their sister relationship, *Conophytum* has substantially more species, with ca. 106 species (Hammer, 2002; Young & Desmet, 2016), while *Cheiridopsis* only has 38 species (Powell *et al.*, submitted b; Chapter 5).

In this chapter, it is hypothesised that species within the five selected *Conophytum* communities will display a diverse range of floral traits in response to pollination as a driver of diversification and that the communities will include closely as well as distantly related species. In addition, phylogenetic diversity was measured in *Conophytum* and *Cheiridopsis*, and compared between the genera to provide insights into the dynamics of the speciose and relatively widespread genus *Conophytum*, specifically in comparison to its sister genus *Cheiridopsis*.

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7.2 MATERIALS AND METHODS

7.2.1 SELECTION AND DEMARCATION OF THE SPECIES-RICH *CONOPHYTUM* COMMUNITIES

The species diversity of *Conophytum* was mapped using extensive distribution records (National Herbaria records and records from Young & Desmet (2016)) and five areas of high species diversity (10-20 co-occurring taxa), consisting of defined communities e.g. a mountain or series of inselbergs, were identified for community analyses. The five

communities selected for study, namely Augrabies, Gamsberg, Groot-Graafwater, Smorgenskadu and Umdaus (Fig. 7.1), span the winter-summer rainfall gradient as well as a temperature gradient (Jürgens, 2006) in Namaqualand in the northern parts of the GCFR. Augrabies, Gamsberg, Smorgenskadu and Umdaus, represent the most speciose communities of *Conophytum*, while the Groot-Graafwater community represents the most southern locality where complex *Conophytum* communities occur (Young & Desmet, 2016). Other speciose *Conophytum* communities not included in this study are the areas around Anenous Pass, Harras/Rietkloof and the Koeriesburg (Hammer, 2002). The communities in this study are ordered west to east, along the winter-summer rainfall and temperature gradient, and north to south following a decrease in species richness in *Conophytum* (Young & Desmet, 2016).

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7.2.1.1 Augrabies

The Augrabies community is located just inland of the north-western coast of South Africa, close to the Namibian border. This community (±22 km^2) is defined by the Augrabies Mountains, sometimes referred to as the Fyftienmyl-se-Berge. These mountains host a number of unusual species, including monotypic genera of the Aizoaceae (Hartmann, 2001). Augrabies receives winter-rainfall and intercepts coastal fog that develops in the west and moves eastwards (Jürgens, 1991, 1997; Matimati *et al.*, 2012) throughout the year. A range of habitats and geologies, including quartz and gneiss, can be found in the community.

Nine species of *Conophytum* have been recorded on the Augrabies Mountains, namely *C. bilobum* (Marloth) N.E.Br. subsp. *altum* (L.Bolus) S.A.Hammer, *C. breve* N.E.Br., *C. francoiseae* (S.A.Hammer) S.A.Hammer, *C. hians* N.E.Br., *C. jucundum* (N.E.Br.) N.E.Br. subsp. *marlothii* (N.E.Br.) S.A. Hammer, *C. meyeri* N.E.Br., *C. obscurum* N.E.Br. subsp. *barbatum* (L.Bolus) S.A.Hammer and *C. stephanii* Schwantes.

7.2.1.2 Umdaus

The Umdaus community includes an area of approximately 70 km^2 and is characterised by a number of large quartz patches associated with the Witbakenberg Mountain, north of Steinkopf. The area receives moisture from winter rainfall and the Witbakenberg likely intercepts some of the coastal fog (Jürgens, 1991, 1997; Matimati *et al.*, 2012) that moves across the landscape.

This community is particularly speciose, and is included in the highest area of diversity for the genus (Young & Desmet, 2016) with 11 species (including two subspecies of *C. flavum*) recorded. These species include *C. armianum* S.A.Hammer, *C. bilobum*, *C. devium* G.D.Rowley, *C. ectypum* N.E.Br., *C. flavum* N.E.Br. subsp. *flavum*, *C. flavum* subsp. *novicium* (N.E.Br.) S.A.Hammer, *C. herreanthus* S.A.Hammer, *C. longum* N.E.Br., *C. maughanii* N.E.Br. subsp. *latum* (Tischer) S.A.Hammer, *C. pageae* N.E.Br., *C. phoeniceum* S.A.Hammer and *C. stephanii* Schwantes subsp. *helmutii* (Lavis) S.A.Hammer.

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7.2.1.3 Smorgenskadu

The Smorgenskadu community includes a chain of small inselbergs, east of Springbok on the western edge of the Bushmanland Inselberg Region (BIR), located on the farm Smorgenskadu, as well as on the neighbouring farms Kangnas and Areb, with a total area of approximately 28.5 km^2. The Smorgenskadu community straddles the winter-summer rainfall boundary, with the possibility of receiving both winter and summer rainfall. However, as it is on the boundary of both rainfall regions, in some years the community may not receive rainfall at all. Quartz is the dominant geology in the area, with *Conophytum* species found on quartz outcrops and quartz flats on the top and at the base of the inselbergs. *Conophytum* species are confined to these inselbergs as the surrounding lowland habitat adjacent to the inselbergs is dominated by Nama Karoo grasses and sands.

The Smorgenskadu community includes some point endemic species and a total of nine *Conophytum* species are recorded in the community, namely *C. calculus* (A.Berger) N.E.Br. subsp. *vanzylii* (Lavis) S.A.Hammer, *C. ectypum* N.E.Br. subsp. *ignavum* S.A.Hammer, *C. hermarium* (S.A.Hammer) S.A.Hammer, *C. lithopsoides* L.Bolus, *C. lydiae* (H.Jacobsen) G.D.Rowely, *C. marginatum* Lavis, *C. maughanii*, *C. smorenskaduense* de Boer and *C. vanheerdei* Tisch.

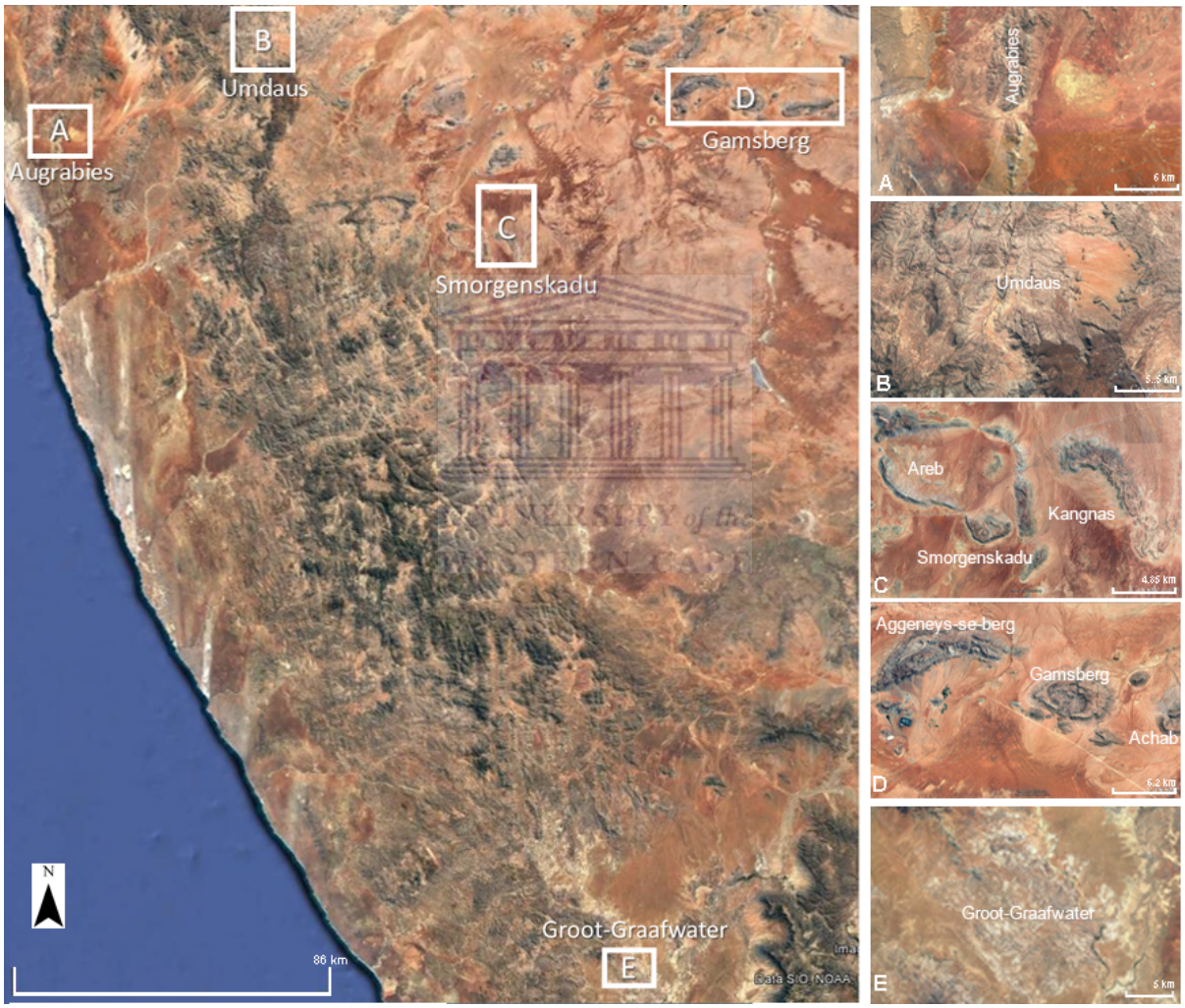

Figure 7.1. Selected sympatric *Conophytum* communities, Augrabies, Umdaus, Smorgenskadu, Gamsberg and Groot-Graafwater, labelled A-E with the approximate areas indicated by the white blocks on the left. The individual communities are illustrated on the right and are labelled accordingly: (A) Augrabies, (B) Umdaus, (C) Smorgenskadu, (D) Gamsberg, (E) Groot-Graafwater.

7.2.1.4 Gamsberg

The Gamsberg community is the most eastern of the selected communities and includes the largest inselberg in the BIR (Gamsberg), as well as the surrounding large inselbergs, Aggeneys-se-berg and Achab. These inselbergs are comprised of metamorphosed granite (gneiss) with extrusions of quartz, which form quartz outcrops or quartz patches, providing suitable habitat for species of *Conophytum.* The Gamsberg community includes a relatively large area (70 km^2), and the region receives mainly summer rainfall. However, the large inselbergs of the Gamsberg community intercept moisture from passing winter-rainfall clouds moving across the landscape from the south-west, due to their high altitude and large size, providing moisture during the winter period to the species occurring on the top and surrounding base of these inselbergs (Desmet, 2000). Although this community spans a large area, the species of *Conophytum* are restricted to the inselbergs, as the surrounding Nama Karoo vegetation does not provide suitable a habitat for the species.

The Gamsberg community includes a number of *Conophytum* species restricted to this community, as well as some point endemic species. The recorded species in the community include *C. achabense* S.A.Hammer, *C. angelicae* N.E.Br., *C. burgeri* L.Bolus, *C. calculus* subsp. *vanzylii*, *C. fulleri* L.Bolus, *C. limpidum* S.A.Hammer, *C. lydiae*, *C. marginatum* subsp. *haramoepense* (L.Bolus) S.A.Hammer, *C. maughanii*, *C. praesectum* N.E.Br. and *C. ratum* S.A.Hammer.

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7.2.1.5 Groot-Graafwater

The Groot-Graafwater community is the most southern locality where complex *Conophytum* species communities occur, with 10 or more taxa included in a QDS (Young & Desmet, 2016). Although the distribution of *Conophytum* extends further south than this community, with up to nine taxa found within a QDS, these species do not form complex communities.

Groot-Graafwater is centred in the Knersvlakte, which is often regarded as a centre of diversity for other Aizoaceae genera (Hartmann, 1991), including the autumn-flowering *Argyroderma* N.E.Br., although this is not the case in *Conophytum*. The Knersvlakte is centred in the arid parts of the winter-rainfall GCFR and is characterised by a network of quartz patches. These quartz patches act as individual units, with population studies of *Argyroderma* showing restricted or no gene flow between quartz patches (Ellis *et al.*, 2007).

Four *Conophytum* species are recorded in the Groot-Graafwater community, including *C. calculus* (A.Berger) N.E.Br., *C. minutum* (Haw.) N.E.Br., *C. subfenestratum* Schwantes and *C. uviforme* (Haw.) N.E.Br.

7.2.2 Floral and Genetic Diversity Analyses

A principal component analysis (PCA) was performed for floral traits (Table 7.1) of species in each of the communities following the methods of Section 2.7.1 of Chapter 2. Pairwise genetic distances of the species within a community (Tables 7.2–7.6) were calculated and presented in a principal coordinate analysis (PCoA) following the methods outlined in Section 2.7.2 of Chapter 2. Phylogenetic diversity (PD) was calculated for *Cheiridopsis* and *Conophytum* following the methods of Section 2.8 of Chapter 2.

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Table 7.1. *Conophytum* species recorded in each of the selected communities and floral traits coded for the species within each community. Phenology (flowering season): AUT=autumn, WIN=winter, SPR=spring, SUM=summer; Anthesis (flowering time): D=diurnal, A=afternoon, LA=late afternoon, N=nocturnal; Structure is coded according to the defined floral structures of Liede & Hammer, (1990) (Fig. 6.2); FS (filamentous staminodes): A=absent, P=present; Scent (floral scent): A=absent, P=present; F.Colour (flower colour): W=white, Y=yellow, P=pink, R=red-orange, M=magenta.

Species	Phenology	Anthesis	Structure	FS	Scent	F. Colour
			Augrabies			
C. bilobum	AUT	D	A2	A	A	Y
C. bolusiae	SPR/SUM	D	B1	A	A	P
C. breve	AUT	N	A2	A	A	Y/R
C. francoiseae	SPR/SUM	D	A2	A	A	P/M
C. hians	AUT	N	B1	A	A	W/P/M
C. jucundum	AUT	D	A2	A	A	P/M
C. meyeri	AUT	D	B1	A	A	Y
C. obscurum	AUT	D	B1	A	A	P/M
C. stephanii	AUT	N	B1	A	A	W/Y/P/M
			Umdaus			
C. armianum	AUT	N	B1	A	P	P
C. bilobum	AUT	D	A2	A	A	Y
C. devium	AUT	D	A2	P	P	W/P
C. ectypum	AUT	D	B1/B2	P	A	W/Y/P
C. flavum	AUT	D	B1	A	A	Y
C. herreanthus	AUT	D	A1	P	P	W/P
C. longum	AUT	D	B1	P	P	W/P
C. maughanii	AUT	N	A2	P	P	W/Y/P
C. pageae	AUT	N	A2	A	P	W/Y/P/R
C. phoeniceum	AUT	N	B1	A	P	P/R
C. stephanii	AUT	N	A2	A	P	R
			Smorgenskadu			
C. calculus	AUT	N	A2	A	P	W/Y/P
C. ectypum	AUT	D	B1/B2	P	A	P
C. hermarium	AUT	D	A1	A	A	Y/P/M
C. lithopsoides	AUT	D	B2	P	A	W/Y/P/R/M
C. lydiae	AUT	D	A1	P	P	P/M
C. marginatum	AUT	D	B1	P	P	P/M
C. maughanii	AUT	N	A2	P	P	W/Y/P
C. smorenskaudense	WIN	A	A2	P	A	W/Y/M
C. vanheerdei	AUT	D	B1	A	A	W/M
			Gamsberg			
C. achabense	AUT	A	B1	A	P	W/P
C. angelicae	AUT	N	A2	A	P	R
C. burgeri	AUT	LA	A2	P	P	P/M
C. calculus	AUT	N	A2	A	P	W/Y/P
C. fulleri	AUT	D	A2	A	P	P/M
C. limpidum	AUT	D	A1	P	P	Q/P
C. lydiae	AUT	D	A1	P	P	P/M
C. marginatum	AUT	D	B1	P	P	M
C. maughanii	AUT	N	A2	P	P	M
C. praesectum	AUT	D	A1	A	P	P/M
C. ratum	AUT	A	A1	A	P	P
			Groot-Graafwater			
C. calculus	AUT	N	A2	A	P	Y/R
C. minutum	AUT	D	B2	P	A	W/P/M
C. subfenestratum	AUT	D	A1	A	P	W/P/M
C. uviforme	AUT	N	A2	A	P	W/Y/P/R

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7.3 RESULTS

7.3.1 AUGRABIES

7.3.1.1 Principal Component Analyses of Floral Traits in the Community

The floral traits, anthesis (flowering time) and phenology (flowering season) separate the species in the Augrabies community into three groups (Fig. 7.2); a group of diurnal autumnal flowering species, including *C. bilobum*, *C. jucundum*, *C. meyeri* and *C. obscurum;* a nocturnal autumnal flowering group, which includes *C. breve*, *C. hians* and *C. stephanii* and a diurnal spring to summer flowering group including *C. bolusiae* and *C. francoiseae*. The first two principal components (Fig. 7.2) were shown to explain most of the variance, with PC1 including 0.692 proportion of variance and PC2 including 0.310 of the variance.

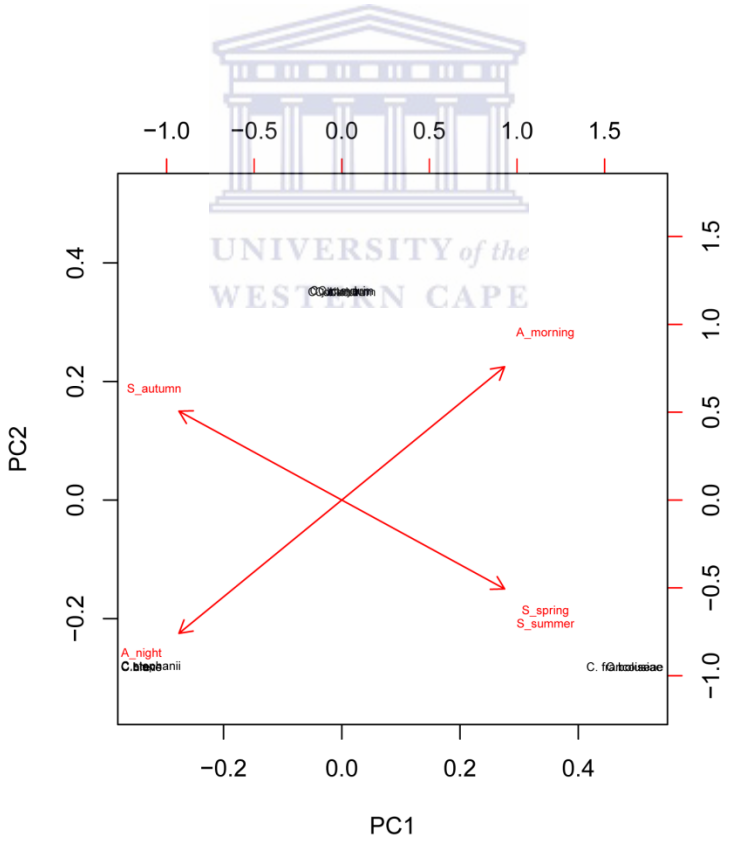

Figure 7.2. Principal component analysis (PCA) of anthesis and phenology of *Conophytum* species in the Augrabies community. Proportion of variance explained by the principal components: PC1=0.692, PC2=0.310.

7.3.1.2 Pairwise Genetic Distance in the community

Table 7.2. Pairwise genetic distance values of *Conophytum* species within the Augrabies community

	C. bilobum	*C. bolusiae*	*C. breve*	*C. hians*	*C. jucundum*	*C. meyeri*	*C. stephanii*
C. bilobum	0	0.007	0.003	0.002	0.003	0.001	0.004
C. bolusiae	0.007	0	0.008	0.006	0.006	0.007	0.009
C. breve	0.003	0.008	0	0.003	0.004	0.004	0.004
C. hians	0.002	0.006	0.003	0	0.003	0.002	0.004
C. jucundum	0.003	0.006	0.004	0.003	0	0.004	0.005
C. meyeri	0.001	0.007	0.004	0.002	0.004	0	0.005
C. stephanii	0.004	0.009	0.004	0.004	0.005	0.005	0

The PCoA of pairwise genetic distance for species (Fig. 7.3) included in the Augrabies communities (Table 7.2), places the majority of species in a loose cluster. In this cluster *C. bilobum, C. jucundum and C. hians* are placed almost equidistant from one another, while *C. meyeri* is placed slightly further from the other species in the group (Fig. 7.3). *Conophytum breve* is positioned the closest to the loose group of species, while *C. bolusiae* is the most distant (Fig. 7.3). *Conophytum stephanii* is also positioned away from the group of species, but is not as isolated as *C. bolusiae* (Fig. 7.3). The proportion of variance is explained mainly by axis 1 and axis 2, with a proportion of variance of 0.656 and 0.164, respectively. Axis 3 to axis 7 only explained 0.004 to 0.088 of the variance. Pairwise genetic distance of the species ranged from 0.001 to 0.009, with an average of 0.005.

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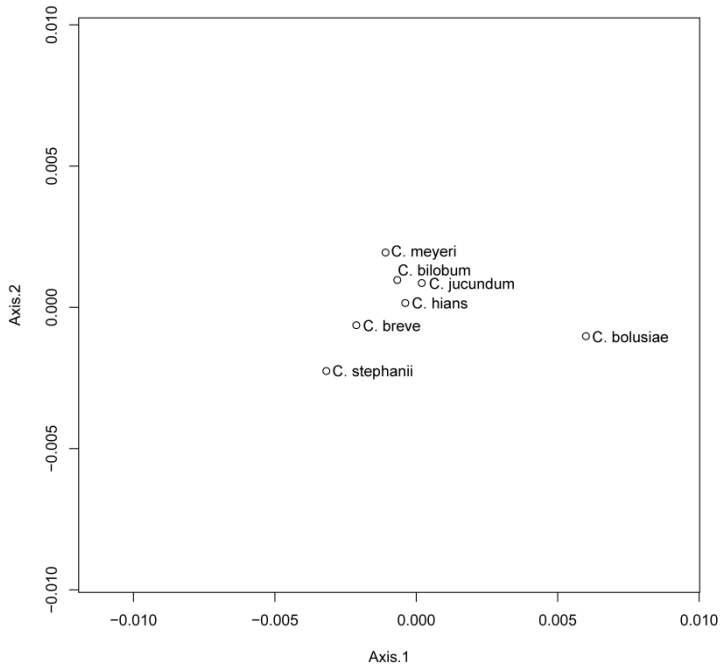

Figure 7.3. Principal coordinates analysis (PCoA) of pairwise genetic distance between *Conophytum* species in the Augrabies community. Proportion of variance explained by the axes: axis 1=0.656, axis 2=0.164.

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7.3.1.3 Floral and Phylogenetic Diversity of Diurnal Species in the Community

Conophytum bolusiae and *C. francoiseae* are temporally separated from the other species in the Augrabies community by differences in phenology, with these species flowering in spring to summer rather than autumn (Fig. 7.4). These species are, however, distinguished florally from one another based on differences in floral structure, with *C. bolusiae* displaying the B2 floral structure while the floral structure in *C. francoiseae* is the A2 type.

The autumnal diurnal species in Augrabies are distinctly separated by floral traits, with *C. meyeri* and *C. obscurum* separated from *C. bilobum* and *C. jucundum* based on the B2 flower structure (Fig. 7.4A). Flower colour separates the species further, with white to yellow flowers in *C. bilobum* and *C. meyeri* and pink to magenta flowers in *C. jucundum* and *C. obscurum* (Fig. 7.4A). The first two principal components explain the variation, with the proportion of variance for PC1=0.800, PC2=0.210.

The sampled species are genetically separated from one another, although *C. bilobum* and *C. meyeri* are positioned somewhat closer to one another than to *C. jucundum* (Fig. 7.4B). The first axis explains the majority of the variance, with a proportion of 0.965.

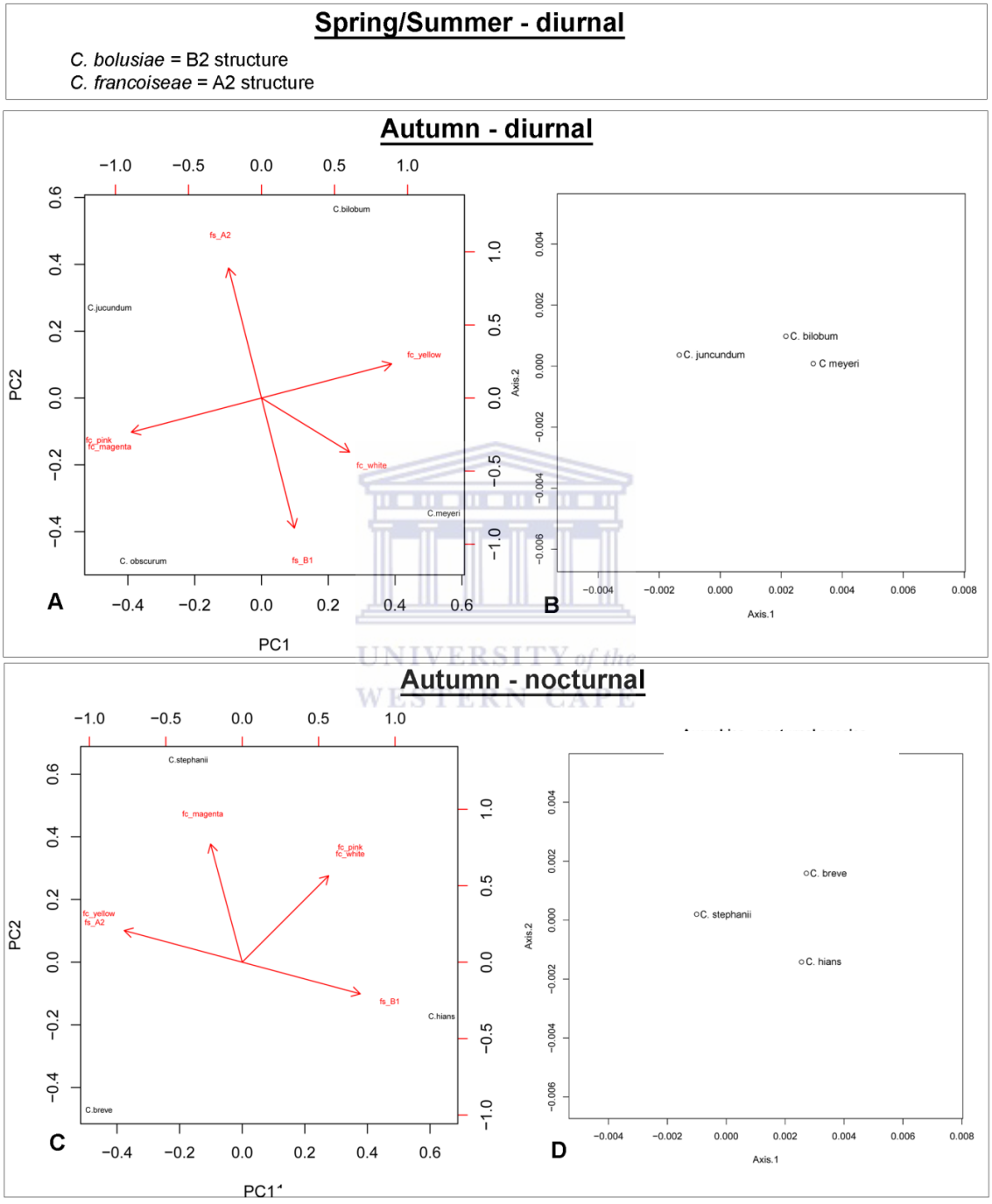

Figure 7.4. Temporal guilds of *Conophytum* species in the Augrabies community. Principal component analysis (PCA) of floral traits and pairwise genetic distance principal coordinates analysis (PCoA) of species in the different temporal guilds, spring to summer flowering, autumn - diurnal flowering (A & B), autumn - nocturnal flowering (C & D) in the Augrabies community; fc=flower colour, fs=flower structure. A: PC1=0.8, PC2=0.21; B: axis 1=0.965; C: PC1 =0.644, PC2=0.356; D: axis 1=0.666, axis 2=0.334.

7.3.1.4 Floral and Phylogenetic Diversity of Nocturnal Species in the Community

The nocturnal species in Augrabies includes *C. breve, C. hians* and *C. stephanii* and are separated in the PCA on the basis of floral traits (Fig. 7.4C). *Conophytum hians* is separated from *C. breve* and *C. stephanii* by the different floral structures (B1), while *C. stephanii* is separated from *C. breve* by the variation in flower colour (Fig. 7.4C), as *C. breve* only displays yellow flowers. The proportion of variance is explained by both PC1 (0.644) and PC2 (0.356).

The species are genetically distinct, placed almost equidistant from one another (Fig. 7.4D). Both the axes explain a proportion of the variance, with the proportion of axis 1=0.666 and axis 2=0.334.

7.3.2 UMDAUS

7.3.2.1 Principal Component Analyses of Floral Traits in the Community

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The PCA including the floral traits anthesis and phenology spilt the species in the Umdaus community into two definitive groups (Fig. 7.5); one including the six diurnal species *C. bilobum, C. devium, C. ectypum, C. flavum, C. herreanthus, C. longum,* and the other including the five nocturnal species *C. armianum, C. maughanii, C. pageae, C. phoeniceum, C. stephanii*. The first principal component (PC1) explained all the variance, with a proportion of 1, and a value of 0 for PC2 (Fig. 7.5).

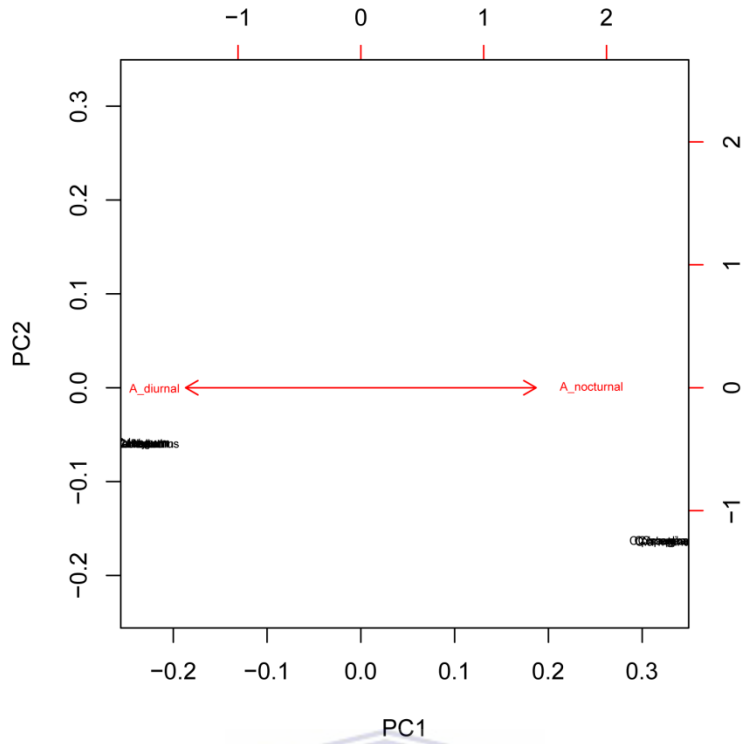

Figure 7.5. Principal component analysis (PCA) of anthesis and phenology of *Conophytum* species in the Umdaus community. Proportion of variance explained by the principal components: PC1=1, PC2=0.

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7.3.2.2 Pairwise Genetic Distance in the community

Table 7.3. Pairwise genetic distance values of *Conophytum* species within the Umdaus community

	C. bilobum	*C. ectypum*	*C. flavum*	*C. herreanthus*	*C. longum*	*C. maughanii*	*C. pageae*	*C. phoeniceum*	*C. stephanii*
C. bilobum	0	0.002	0.006	0.008	0.006	0.003	0.003	0.002	0.004
C. ectypum	0.002	0	0.007	0.008	0.007	0.003	0.003	0.003	0.005
C. flavum	0.006	0.007	0	0.011	0.010	0.006	0.006	0.006	0.006
C. herreanthus	0.008	0.008	0.011	0	0.012	0.008	0.008	0.008	0.010
C. longum	0.006	0.007	0.010	0.012	0	0.007	0.007	0.006	0.009
C .maughanii	0.003	0.003	0.006	0.008	0.007	0	0.003	0.003	0.005
C. pageae	0.003	0.003	0.006	0.008	0.007	0.003	0	0.003	0.005
C. phoeniceum	0.002	0.003	0.006	0.008	0.006	0.003	0.003	0	0.004
C. stephanii	0.004	0.005	0.006	0.010	0.009	0.005	0.005	0.004	0

Species in Umdaus are closely grouped together in the PCoA, based on pairwise genetic distance (Table 7.3), with the exception of four species (Fig. 7.6). *Conophytum stephanii* is placed closest to the larger group, with *C. flavum* positioned further away (Fig. 7.6). *Conophytum herreanthus* is positioned to the right of the group, a distance of approximately 0.007 on axis 1, while *C. longum* is the most isolated from this large group (Fig. 7.6). The remaining species in the Umdaus community form a large group, positioned very close to one another and include *C. bilobum, C. ectypum, C. maughanii, C. pageae* and *C. phoeniceum* (Fig. 7.6). Variance in the PCoA is explained by nine axes, with the largest proportion of variance explained by axis 1 and 2, with proportions of 0.454 and 0.320, respectively. The remaining axes (3-9), explain 0 to 0.01 proportion of the variance. The average pairwise genetic distance of the species is 0.006, while the maximum distance is 0.012 and the minimum distance 0.002.

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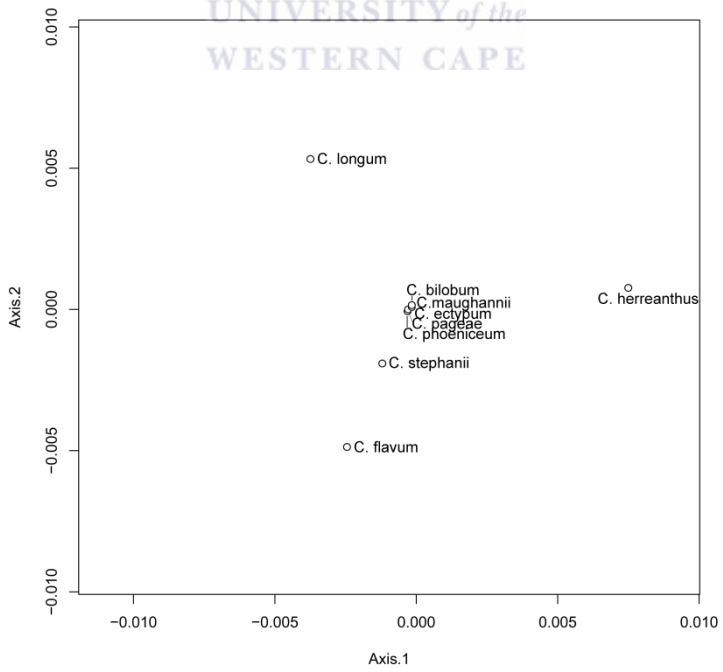

Figure 7.6. Principal coordinates analysis (PCoA) of pairwise genetic distance between *Conophytum* species in the Umdaus community. Proportion of variance explained by the axes: axis 1=0.454, axis 2=0.320.

7.3.2.3 Floral and Phylogenetic Diversity of Diurnal Species in the Community

In Umdaus, there is no variation in phenology, with all species flowering in autumn, with a number of floral traits grouping, as well as separating, the species (Fig. 7.7). *Conophytum bilobum* and *C. devium* are separated from the rest of the species by the A2 floral structure (Fig. 7.7A). *Conophytum herreanthus* is distinguished by the A1 floral structure, while *C. ectypum, C. flavum* and *C. longum* all have a B1 flower structure (Fig. 7.7A). However, *C. herreanthus* shares the presence of filamentous staminodes with *C. ectypum* and *C. longum* (Fig. 7.7A). In addition, *C. herreanthus* shares scented flowers with *C. longum* (Fig. 7.7A). *Conophytum herreanthus, C. devium* and *C. longum* all share the same flower colour (white to pink), while flower colour in the remaining species includes yellow (Fig. 7.7A). The proportion of variance in this community is explained by the first five principal components, with proportions of 0.460, 0.264, 0.167, 0.0814 and 0.028, respectively.

The PCoA of the pairwise genetic distance of these species shows *C. bilobum* and *C. ectypum* close to one another, while the other species in the community are positioned separately from one another (Fig. 7.7B). The proportion of variance explained by the axes ranges from 0 to 0.557.

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7.3.2.4 Floral and Phylogenetic Diversity of Nocturnal Species in the Community

In the PCA, *C. armianum* and *C. phoeniceum* are placed close to one another, based on the shared B1 floral structure (Fig. 7.7C). These two species (*C. armianum* and *C. phoeniceum*) are, however, separated by the red-orange flower colour of *C. phoeniceum,* which is not present in *C. armianum* (Fig. 7.7C). *Conophytum maughanii, C. pageae* and *C. stephanii* all share the A2 floral structure, however, *C. stephanii* is separated by its solely red-orange flower colour, with no variation included (Fig. 7.7C). *Conophytum maughanii* and *C. pageae* are positioned relatively close to one another, based on the shared floral structure (A2) and

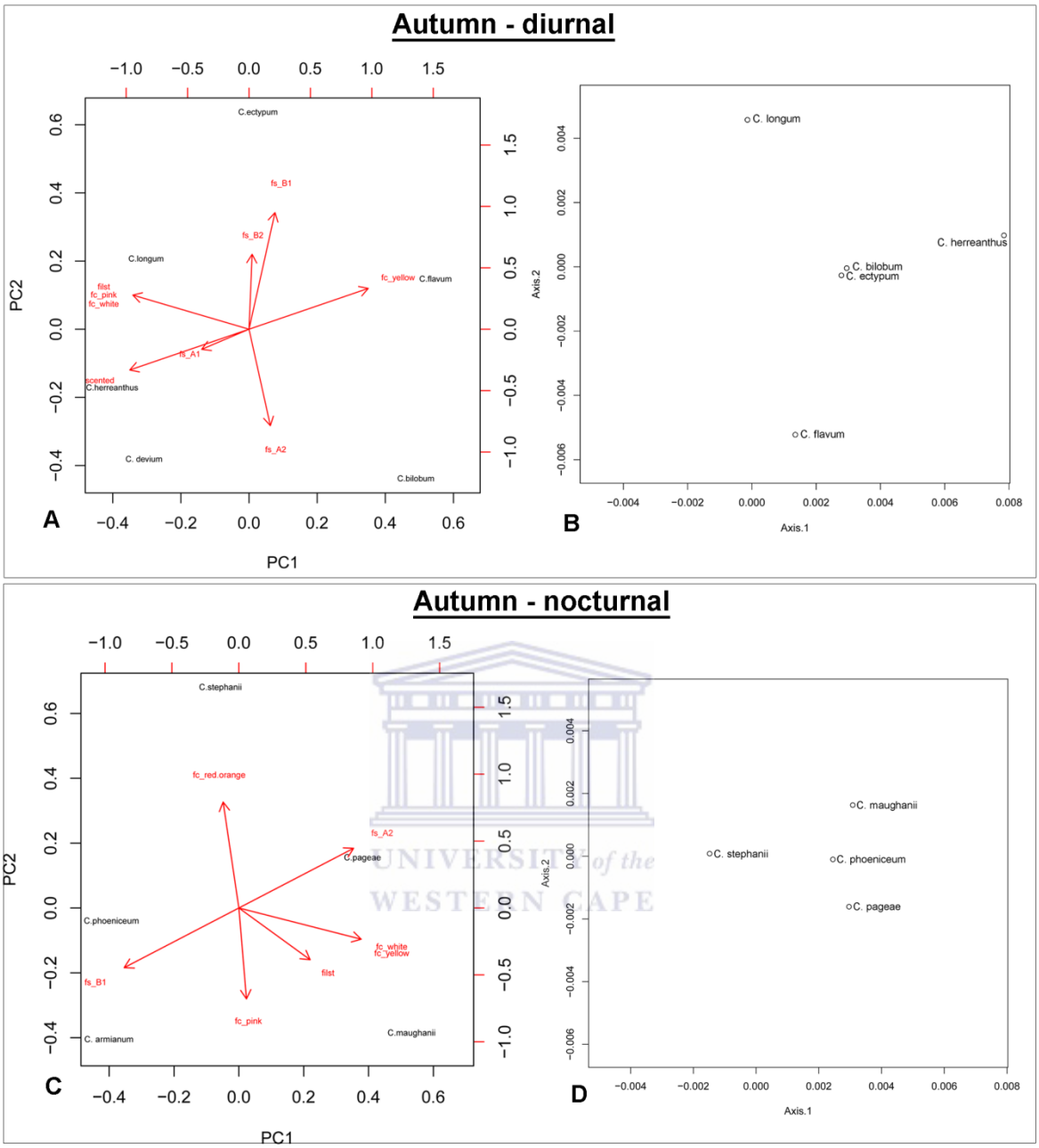

Figure 7.7. Temporal guilds of *Conophytum* species in the Umdaus community. Principal component analysis (PCA) of floral traits and pairwise genetic distance principal coordinates analysis (PCoA) of species in the different temporal guilds, autumn - diurnal flowering (A & B), autumn - nocturnal flowering (C & D) in the Umdaus community; fc=flower colour, fs=flower structure, filst=filamentous staminodes present, scented=flowers scented. A: PC1=0.46, PC2=0.264; B: axis 1=0.557; C: PC1 =0.543, PC2=0.268, PC3=0.111; PC4=0.078; D: axis 1=0.633, axis 2=0.237.

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flower colour, however, *C. maughanii* is separated from *C. pageae* by the presence of filamentous staminodes (Fig. 7.7C). The proportion of variance in the PCA is explained by the first three principal components, including 0.648, 0.244 and 0.109 proportion of the variance, respectively.

The species are genetically separated from one another, however, *C. stephanii* is the most genetically isolated species in the nocturnal Umdaus species guild (Fig. 7.7D). The first three axes explain the variance in the PCoA, with the proportion of variance of axis 1=0.633, axis 2=0.237 and axis 3=0.130.

7.3.3 SMORGENSKADU

7.3.3.1 Principal Component Analyses of Floral Traits in the Community

The species in Smorgenskadu are separated into three groups according to anthesis and phenology in the PCA (Fig. 7.8). *Conophytum smorenskaduense* is separated from all the other species on the basis that it is a winter and afternoon flowering species, while the remaining species are autumnal, separated by differences in anthesis (Fig. 7.8). Although *C. hermarium* is included in the group of autumnal diurnal species, it is slightly separated on account of the species earlier phenology, from summer into autumn (Fig. 7.8). The other diurnal autumnal species include *C. ectypum, C. lithopsoides, C. lydiae, C. marginatum* and *C. vanheerdei*, while the nocturnal autumnal species include *C. calculus* and *C. maughanii* (Fig. 7.8). The first two principal components (Fig. 7.8) explain most of the variance, with a proportion of 0.521 for PC1 and 0.367 for PC2. Principal component three (PC3) includes 0.113 of the proportion of variance.

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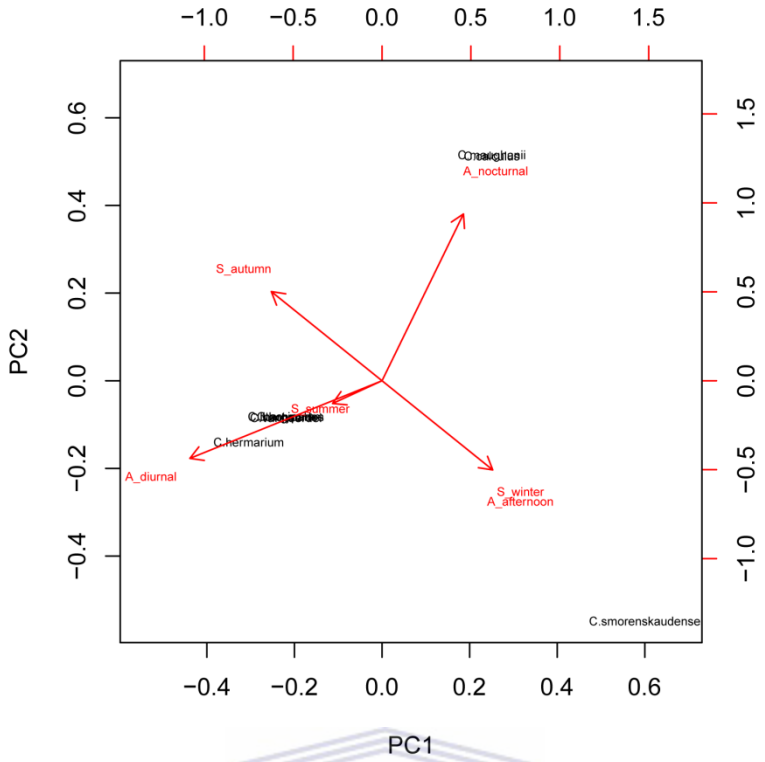

Figure 7.8. Principal component analysis (PCA) of anthesis and phenology of *Conophytum* species in the Smorgenskadu community. Proportion of variance explained by the principal components: PC1=0.521, PC2=0.367, PC3=0.113.

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7.3.3.2 Pairwise Genetic Distance in the community

Table 7.4. Pairwise genetic distance values of *Conophytum* species within the Smorgenskadu community

	C. calculus	*C. ectypum*	*C. hermarium*	*C. lithopsoides*	*C. marginatum*	*C. maughanii*	*C. smorenskaduense*	*C. vanheerdei*
C. calculus	0	0.004	0.007	0.007	0.006	0.004	0.004	0.006
C. ectypum	0.004	0	0.007	0.007	0.005	0.003	0.004	0.006
C. hermarium	0.007	0.007	0	0.010	0.009	0.006	0.006	0.008
C. lithopsoides	0.007	0.007	0.010	0	0.009	0.007	0.007	0.009
C. marginatum	0.006	0.005	0.009	0.009	0	0.005	0.006	0.008
C. maughanii	0.004	0.003	0.006	0.007	0.005	0	0.004	0.006
C. smorenskaduense	0.004	0.004	0.006	0.007	0.006	0.004	0	0.003
C. vanheerdei	0.006	0.006	0.008	0.009	0.008	0.006	0.003	0

The PCoA of pairwise genetic distance in Smorgenskadu (Table 7.4) includes a loose grouping of species, including *C. ectypum*, *C. marginatum*, *C. maughanii* and *C. smorenskaduense* (Fig. 7.9). *Conophytum vanheerdei* and *C. lithopsoides* are placed further away from this grouping, with the position of *C. hermarium* the most isolated (Fig. 7.9). *Conophytum maughanii* and *C. ectypum* are placed very closely together, while the distance of *C. marginatum* from these two species and to *C. smorenskaduense*, is more or less equal (Fig. 7.9). The proportion of variance is explained mainly by the first three axes, with proportions of 0.354, 0.253 and 0.227, respectively, while the proportion of variance for the remaining axes (axis 4–7) ranged from 0.007 to 0.073. The average pairwise genetic distance between the species is 0.006, with a minimum of 0.003 and a maximum distance of 0.009.

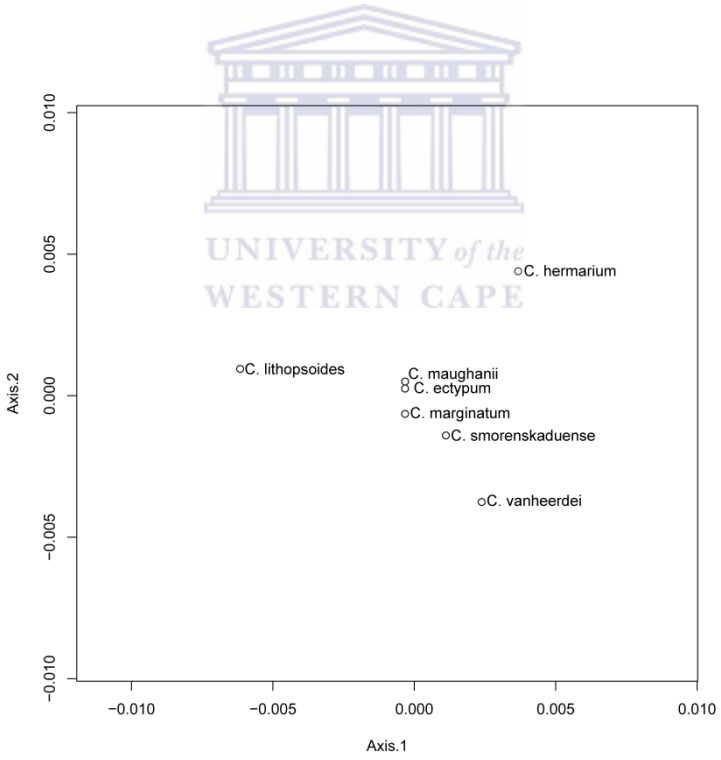

Figure 7.9. Principal coordinates analysis (PCoA) of pairwise genetic distance between *Conophytum* species in the Smorgenskadu community. Proportion of variance explained by the axes: axis 1=0.253, axis 2=0.227.

7.3.3.3 Floral and Phylogenetic Diversity of Diurnal Species in the Community

There is some separation in phenology between the diurnal flowering species in the Smorgenskadu community, with *C. smorenskaduense* and *C. hermarium* flowering in different seasons of the year, and not only in autumn as the majority of species in the community (Fig. 7.10). Although *C. hermarium* flowers in autumn, the start of the flowering season is earlier, into summer (Fig. 7.10). The flowering season of *C. smorenskaduense* is, however, restricted to winter. In addition, this species is the only diurnal species to flower in the afternoon, while the rest of the diurnal species flower throughout the day (Fig. 7.10).

The diurnal autumnal species of the Smorgenskadu community are separated by floral characters in the PCA (Fig. 7.10A). *Conophytum hermarium* is separated from the other diurnal species in the community based on the A2 floral structure, while floral structure (A1) also separates *C. lydiae* from the other species in the community (Fig. 7.10A). Flower structure B2 groups *C. ectypum* and *C. lithopsoides,* however, these species are separated from one another due to the variation of flower colour in *C. lithopsoides,* while in *C. ectypum* the flowers are only pink (Fig. 7.10A). *Conophytum ectypum* and *C. lithopsoides* are also grouped by the presence of filamentous staminodes, which are also present in *C. marginatum* (Fig. 7.10A). *Conophytum marginatum* is also grouped with *C. ectypum* based on the shared B1 flower structure, which is also shared with *C. vanheerdei,* however, *C. marginatum* is the only species in the guild to have scented flowers (Fig. 7.10A). *Conophytum vanheerdei* is separated from *C. ectypum* by flower colour, which is white or magenta in *C. vanheerdei,* and pink in *C. ectypum* (Fig. 7.10A). The variation in floral traits in the diurnal species of Smorgenskadu is included in the first five principal components of the PCA, including the following proportion of variance, PC1=0.315, PC2=0.243, PC3=0.243, PC4=0.146, PC5=0.052.

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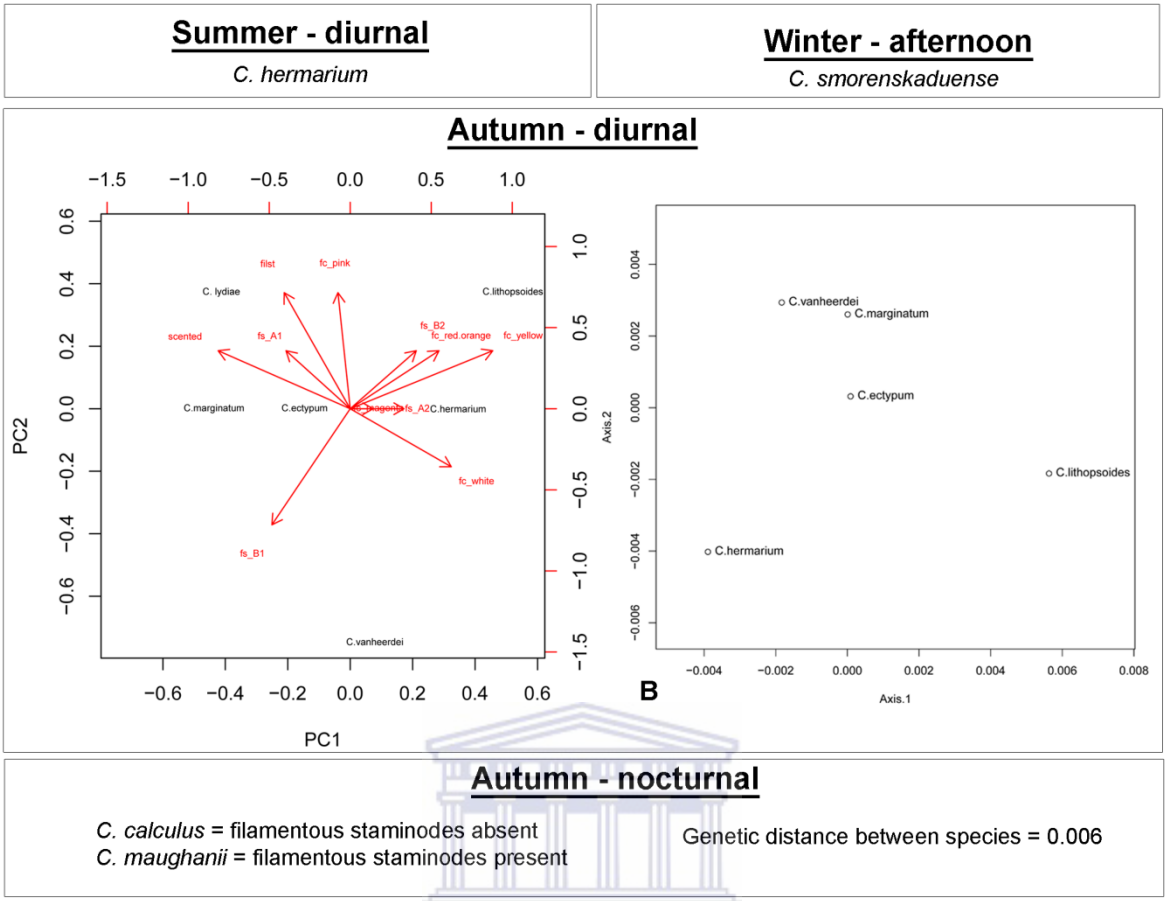

Figure 7.10. Temporal guilds of *Conophytum* species in the Smorgenskadu community. Principal component analysis (PCA) of floral traits and pairwise genetic distance principal coordinates analysis (PCoA) of species in the different temporal guilds, summer - diurnal flowering, winter – afternoon flowering, autumn - diurnal flowering (A & B), autumn - nocturnal flowering in the Smorgenskadu community; fc=flower colour, fs=flower structure, scented=flowers scented. A: PC1=0.315, PC2=0.243; B: axis 1=0.405, axis 2=0.282.

Based on the pairwise genetic distance values of the species, *C. marginatum* and *C. vanheerdei* are shown to be genetically close, with *C. ectypum* placed approximately 0.002 units away on axis 2 (Fig. 7.10B). *Conophytum hermarium* and *C. lithopsoides* are placed separately, on the extremes of axis 1 in the PCoA (Fig. 7.10B). The proportion of variance in the PCoA is explained by four axes, with proportional values of 0.405, 0.282, 0.248 and 0.064, respectively.

7.3.3.4 Floral and Phylogenetic Diversity of Nocturnal Species in the Community

The nocturnal species in Smorgenskadu include only *C. calculus* and *C. maughanii* and as there are only two species, a PCA could not be performed. These species share scented flowers with the A2 floral structure and a variation in flower colour from white to pink, but are separated by the presence of filamentous staminodes in *C. maughanii* (Fig. 7.10). The pairwise genetic distance between the species is 0.006 (Table 7.4).

7.3.4 GAMSBERG

7.3.4.1 Principal Component Analyses of Floral Traits in the Community

The species in the Gamsberg community are only separated according to anthesis, and not phenology, as all the species are autumn-flowering (Fig. 7.11). The PCA groups the species into four groups along three axes, according to anthesis (Fig. 7.11). *Conophytum achabense* and *C. ratum* form a group at the bottom of the PCA, based on the shared anthesis of afternoon-flowering (Fig. 7.11). *Conophytum burgeri* is placed just off centre, towards *C. achabense* and *C. ratum*, as it is a late afternoon-flowering species (Fig. 7.11). A group of diurnal species (*C. fulleri, C. limpidum, C. lydiae, C. marginatum,* and *C. praesectum*) are positioned on the left, while the group of nocturnal species (*C. angelicae, C. calculus* and *C. maughanii*) are placed on the right (Fig. 7.11). The first three principal components (Fig. 7.11) explain the all variance, with the proportion of variance for PC1=0.466, PC2=0.352 and PC3=0.182.

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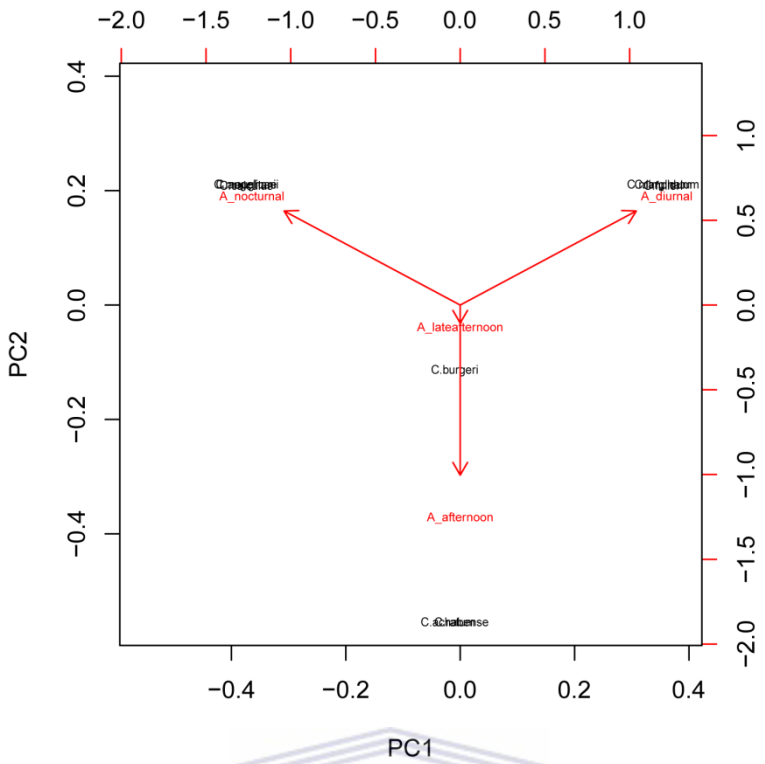

Figure 7.11. Principal component analysis (PCA) of anthesis and phenology of *Conophytum* species in the Gamsberg community. Proportion of variance explained by the principal components: PC1=0.466, PC2=0.352, PC3=0.182.

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7.3.4.2 Pairwise Genetic Distance in the community

Table 7.5. Pairwise genetic distance values of *Conophytum* species within the Gamsberg community

	C. achabense	*C. angelicae*	*C. burgeri*	*C. calculus*	*C. fulleri*	*C. limpidum*	*C. marginatum*	*C. maughanii*	*C. ratum*
C. achabense	0	0.016	0.009	0.009	0.009	0.003	0.010	0.008	0.009
C. angelicae	0.016	0	0.011	0.011	0.011	0.013	0.013	0.011	0.012
C. burgeri	0.009	0.011	0	0.004	0.004	0.007	0.006	0.004	0.005
C. calculus	0.009	0.011	0.004	0	0.004	0.006	0.006	0.004	0.004
C. fulleri	0.009	0.011	0.004	0.004	0	0.006	0.006	0.004	0.005
C. limpidum	0.003	0.013	0.007	0.006	0.006	0	0.008	0.006	0.007
C. marginatum	0.010	0.013	0.006	0.006	0.006	0.008	0	0.005	0.006
C. maughanii	0.008	0.011	0.004	0.004	0.004	0.006	0.005	0	0.003
C. ratum	0.009	0.012	0.005	0.004	0.005	0.007	0.006	0.003	0

The pairwise genetic distance PCoA for Gamsberg presents a large grouping of species (*C. burgeri, C. calculus, C. fulleri, C. maughanii, C. ratum*), with four species separated from this group (Table 7.5; Fig. 7.12). *Conophytum marginatum* is positioned closest to this group, while *C. angelicae* is shown to be the most isolated species (Fig. 7.12). *Conophytum limpidum* and *C. achabense* are placed relatively close to one another, but do not form a group (Fig. 7.12). The first and second axes of the PCoA explain the majority of the variance, with proportions of 0.559 and 0.241, respectively, while the proportion of variance of axes 3 to 8, ranges from 0.002 to 0.0767. The average pairwise genetic distance for these species in the Gamsberg community is 0.007 and ranges from 0.003 to 0.016.

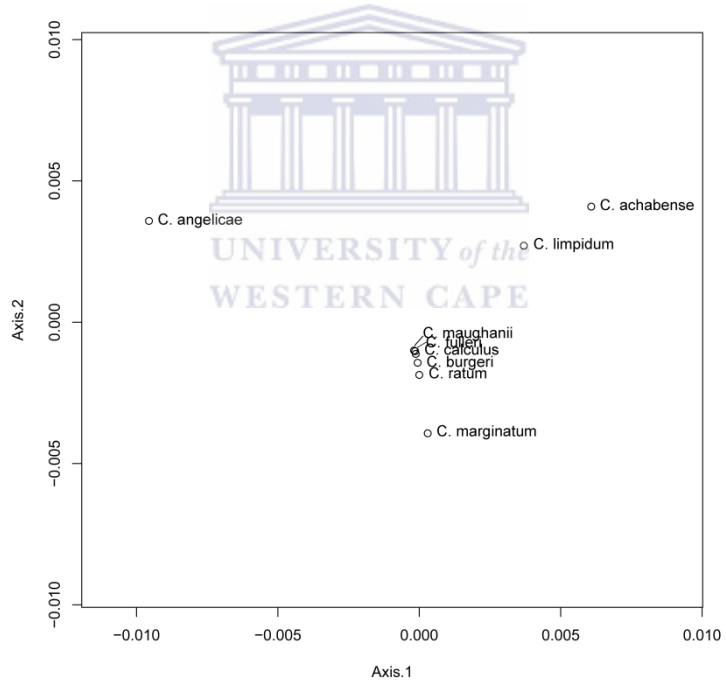

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Figure 7.12. Principal coordinates analysis (PCoA) of pairwise genetic distance between *Conophytum* species in the Gamsberg community. Proportion of variance explained by the axes: axis 1=0.559, axis 2=0.241.

7.3.4.3 Floral and Phylogenetic Diversity of Diurnal Species in the Community

The diurnal species in the Gamsberg community all flower in autumn and thus there are no species that are temporally separated based on a difference in phenology (Fig. 7.13). The species are well-separated by floral traits, with the exception of *C. burgeri* and *C. fulleri,* which are positioned relatively close to one another (Fig. 7.13A). These two species are grouped closely to one another based on the shared A2 floral structure, however, although *C. burgeri* is included in this analysis as a diurnal species, it only flowers in the late afternoon (as indicated by the blue text) (Fig. 7.13A). The A2 floral structure is also shared by *C. lydiae,* but this species is separated from *C. burgeri* and *C. fulleri* by its flower colour, which is never magenta (Fig. 7.13A). Flower structure also groups *C. limpidum, C. praesectum* and *C. ratum* (A1) as well as *C. achabense* and *C. marginatum* (B1) (Fig. 7.13A). However, again, the anthesis of *C. achabense* and *C. ratum* is slightly different to the other diurnal species (indicated by the purple colour), as these species flower only in the afternoon, and not throughout the day as the other diurnal species (Fig. 7.13A). All the diurnal species in the Gamsberg community are further separated by flower colour and presence or absence of filamentous staminodes (Fig. 7.13A). The proportion of variance is explained by the first six principal components, with proportion values of 0.332, 0.237, 0.230, 0.168, 0.026 and 0.007.

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The pairwise genetic distance PCoA for the diurnal Gamsberg species group *C. burgeri, C. fulleri* and *C. ratum* close to one another (Fig. 7.13B). *Conophytum achabense* and *C. limpidum* are also positioned relatively close together, while *C. marginatum* is isolated from the other species (Fig. 7.13B). The proportion of variance in the PCoA is explained mainly by the first two axes, with values of 0.648 and 0.172, while the proportion of variance in the remaining three axes ranges from 0.012 to 0.097.

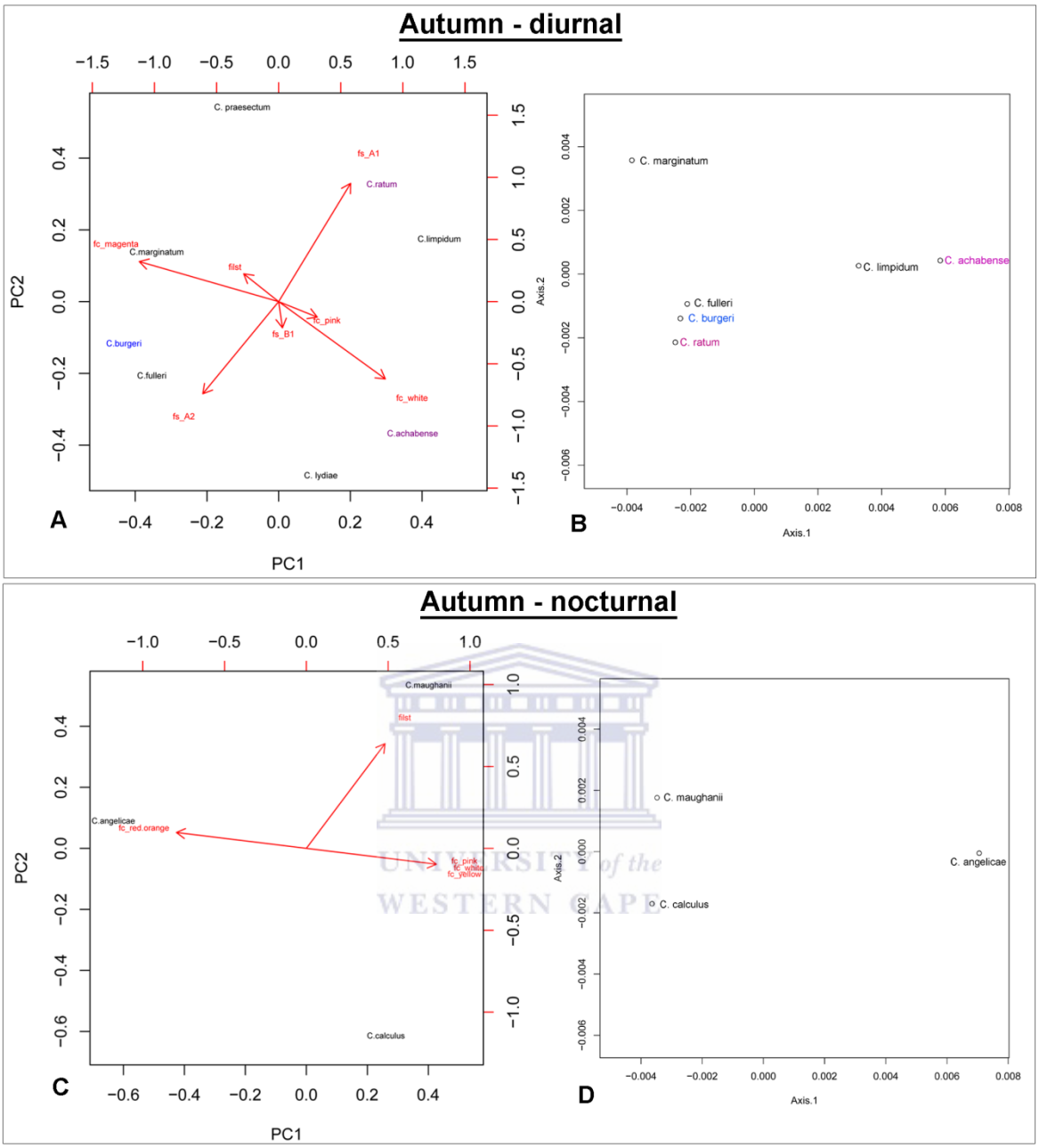

Figure 7.13. Temporal guilds of *Conophytum* species in the Gamsberg community. Principal component analysis (PCA) of floral traits and pairwise genetic distance principal coordinates analysis (PCoA) of species in the different temporal guilds, autumn - diurnal flowering (A & B), with *C. achabense and C. ratum* flowering in the afternoon (indicated in purple) and *C. burgeri* flowering in the late afternoon (indicated in blue) as well as autumn - nocturnal flowering (C & D) in the Gamsberg community; fc=flower colour, fs=flower structure, filst=filamentous staminodes present. A: PC1=0.332, PC2=0.237; B: axis 1=0.648, axis 2=0.172; C: PC1=0.861, PC2=0.139; D: axis 1=0.924, axis 2=0.076.

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7.3.4.4 Floral and Phylogenetic Diversity of Nocturnal Species in the Community

The Gamsberg community includes three nocturnal species, *C. angelicae, C. calculus and C. maughanii* and these three species share scented flowers with the same floral structure (Fig. 7.13C). The species are, however, separated by floral traits, such as the red-orange flowers in *C. angelicae* and the presence of filamentous staminodes in *C. maughanii* (Fig. 7.13C). The variation of floral characters in the PCA is explained by the first two principal components, with the proportion of variance of PC1=0.861 and PC2=0.139.

The species are distinctly separated based on pairwise genetic distance values, as shown in the PCoA (Fig. 7.13D). The proportion of variance is included mainly in the first axis, with a proportion of 0.924, while the second axis includes 0.076 proportion of variance.

7.3.5 GROOT-GRAAFWATER

7.3.5.1 Principal Component Analyses of Floral Traits in the Community

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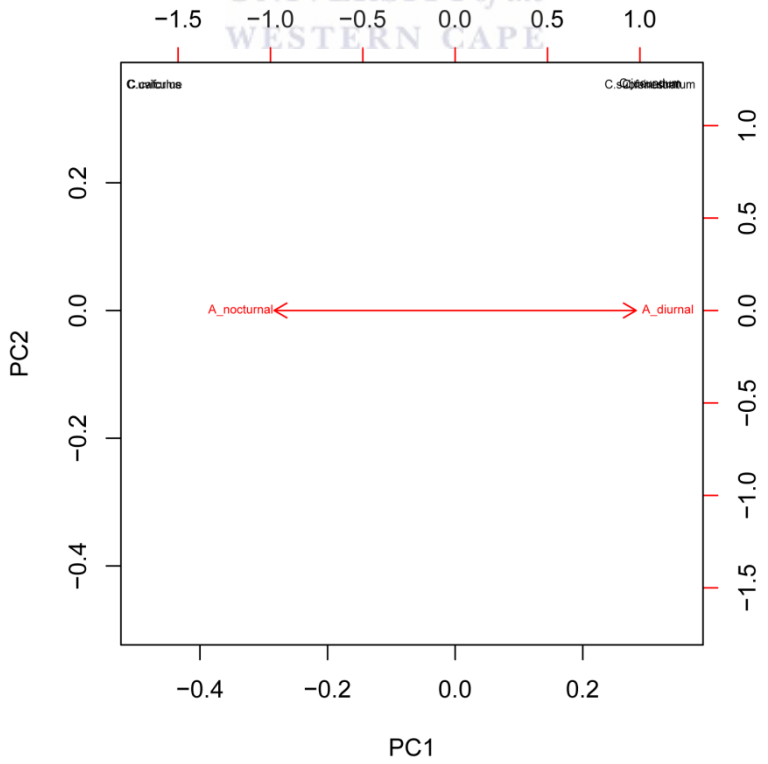

Figure 7.14. Principal component analysis (PCA) of anthesis and phenology of *Conophytum* species in the Groot-Graafwater community. Proportion of variance explained by the principal components: PC1=1, PC2=0.

The PCA for floral traits anthesis and phenology in the Groot-Graafwater separate the species in the community into two groups based on anthesis only, as all the species are autumnal (Fig. 7.14). The diurnal species include *C. minutum* and *C. subfenestratum*, while the nocturnal species in the community are *C. calculus* and *C. uviforme* (Fig. 7.14). The first principal component (Fig. 7.14) explains all the variance, with a proportion of variance of 1.

7.3.5.2 Pairwise Genetic Distance in the community

Table 7.6. Pairwise genetic distance values of *Conophytum* species within the Groot-Graafwater community

	C. calculus	*C. minutum*	*C. subfenestratum*	*C. uviforme*
C. calculus	0	0.008	0.005	0.005
C. minutum	0.008	0	0.009	0.009
C. subfenestratum	0.005	0.009	0	0.005
C. uviforme	0.005	0.009	0.005	0

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The PCoA of pairwise genetic distance for species in the Groot-Graafwater community separates the species in the community, with *C. minutum* most isolated from the other species (Table 7.6; Fig. 7.15). The proportion of variance is mainly explained axis 1, with a proportion of 0.696, while the proportion of variance of axis 2 and 3 is 0.158 and 0.146, respectively. The average pairwise genetic distance between species in this community is 0.005, with a maximum of 0.009 and a minimum of 0.004.

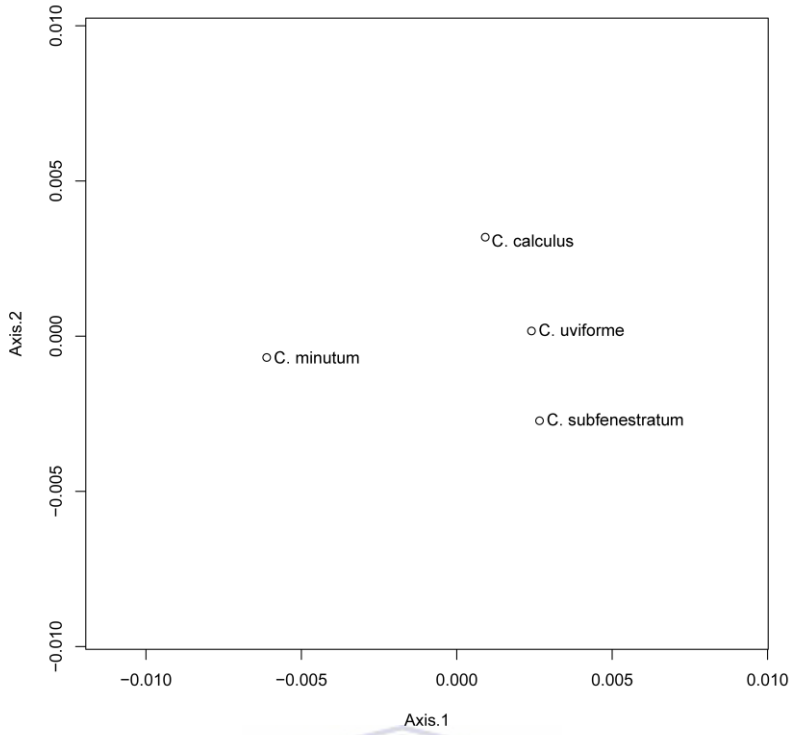

Figure 7.15. Principal coordinates analysis (PCoA) of pairwise genetic distance between *Conophytum* species in the Groot-Graafwater community. Proportion of variance explained by the axes: axis 1=0.696, axis 2=0.158, axis 3=0.146.

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7.3.5.3 Floral and Phylogenetic Diversity of Diurnal Species in the Community

As the Groot-Graafwater community includes two diurnal species which flower in autumn, a PCA was unable to be performed. However, the species are separated by the different floral structures (A1 in *C. subfenestratum* and B2 in *C. minutum* (Fig. 7.16)). In addition, *C. subfenestratum* is scented, while filamentous staminodes are present in *C. minutum* (Fig. 7.16). The pairwise genetic distance between the species is 0.009 (Table 7.6).

7.3.5.4 Floral and Phylogenetic Diversity of Nocturnal Species in the Community

There are only two nocturnal species in the Groot-Graafwater community, *C. calculus* and *C. uviforme.* Although a PCA could not be performed on only these two species, the species share the A2 floral structure and are both scented, but are separated by flower colour, with flower colour in *C. calculus* ranging from golden yellow to reddish orange, while in *C.*

uviforme, the flowers are white to pink, sometimes straw-yellow (Fig. 7.16). The pairwise genetic distance between the species is 0.005 (Table 7.6).

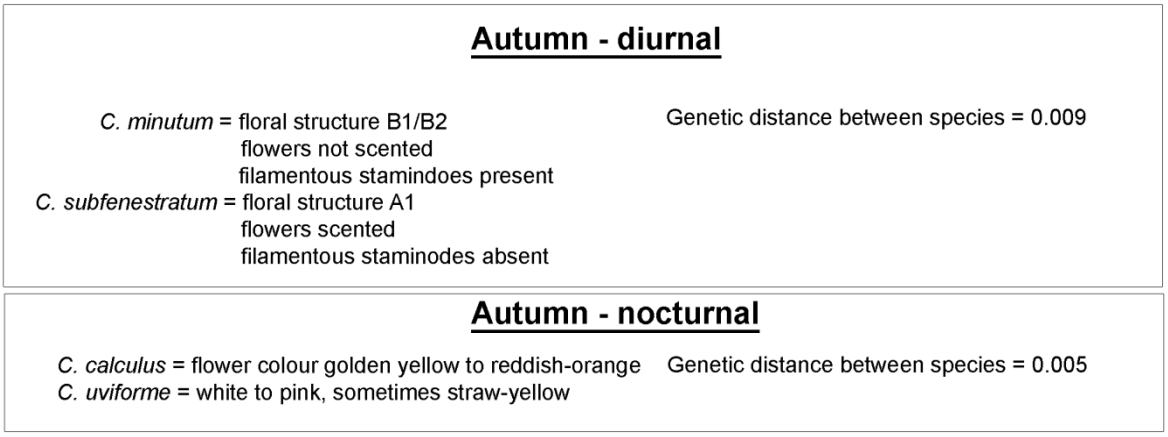

Figure 7.16. Temporal guilds of *Conophytum* species in the Groot-Graafwater community. Autumn - diurnal flowering which includes the species *C. minutum* and *C. subfenestratum* and autumn - nocturnal flowering including *C. calculus* and *C. uviforme*

7.3.6 PHYLOGENETIC DIVERSITY OF *CONOPHYTUM* AND ITS SISTER GENUS *CHEIRIDOPSIS*

Phylogenetic diversity (PD) for *Conophytum* was calculated for 65 QDS with an average PD value of 0.0227, a maximum of 0.0479 in a QDS and a minimum of 0.0069. Species richness was also variable across the QDS, ranging from 3 to 14, with an average of 5.9 species per QDS in *Conophytum* (Table 7.7). *Conophytum* had several QDS with a high PD, spanning the main distribution of the genus (Fig. 7.17A).

Twenty one QDS were used to calculate PD in *Cheiridopsis*, with an average value of 0.0182 per QDS. The maximum PD in a QDS was 0.0464, while the minimum was 0.0046. The average number of species included in a QDS was 5.7, and species richness ranged from 3 to 9 for *Cheiridopsis* (Table 7.8). The PD of *Cheiridopsis* is highest around Steinkopf and the QDS with three or more species are also centred in this region (Fig. 7.17B).

Comparison of PD between shared QDS (where three or more species of both genera occur) in *Cheiridopsis* and *Conophytum,* showed that the PD of *Conophytum* is significantly higher than in *Cheiridopsis* ($t_{(df)}$ = -2.354, P < 0.012), (Table 7.9). The proportional value of PD per QDS was also shown to be significantly greater in *Conophytum* ($t_{(df)}$ = -1.94, P < 0.03) when compared to *Cheiridopsis*, with average proportional values of 0.0039 and 0.0030, respectively. The maximum proportional value of *Conophytum* QDS was 0.0079, while in *Cheiridopsis* it was 0.0073, with an equal minimum proportion value in both genera of 0.0014.

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Table 7.7. Phylogenetic diversity (PD) and species richness (SR) values for *Conophytum* Quarter Degree Squares (QDS) with three or more species.

QDS	PD	SR
2816BB	0.0096	4
2817AA	0.0072	4
2816BD	0.0096	4
2817AC	0.0211	4
2816DB	0.0072	4
2817CA	0.0072	4
2817CB	0.0115	5
2817CC	0.0145	8
2817CD	0.0320	11
2817DC	0.0107	3
2817DD	0.0164	9
2917AA	0.0164	9
2917AB	0.0423	12
2917BA	0.0423	12
2917BB	0.0479	11
2918AA	0.0277	5
2918BB	0.0276	7
2919AB8	0.0175	4
2917AC	0.0069	5
2917AD	0.0101	7
2917BC	0.0473	12
2917BD	0.0277	7
2918AA	0.0469	14
2918AB	0.0187	4
2919AB	0.0272	7
2917AC	0.0341	8
2917AD	0.0393	8
2917BC	0.0393	8
2917BD	0.0467	8
2918AC	0.0192	6
2917CB	0.0147	7
2917DA	0.0452	11
2917DD	0.0439	8
2918CA	0.0327	5
2918CB	0.0078	3
2917CD	0.0132	5
2917DD	0.0329	6
2918CC	0.0360	7
2918CD	0.0182	3
2919CA	0.0182	3
3017AB	0.0186	6
3017BA	0.0108	4
3017BB	0.0263	5
3018AA	0.0292	5
3018AB	0.0100	3
3017BC	0.0168	4
3017BD	0.0420	9
3018AC	0.0368	7
3018AD	0.0292	5
3018BC	0.0158	4
3018BD	0.0169	4
3017DA	0.0139	4
3017DB	0.0131	6
3018CA	0.0238	5
3018CB	0.0094	3
3118AA	0.0124	4
3118AB	0.0124	4
3118BA	0.0137	4
3118AD	0.0150	5
3118BC	0.0145	5
3118BD	0.0307	5
3118CB	0.0119	4
3118DB	0.0242	4
3118DD	0.0236	3
3218BA	0.0073	3
Average	**0.0227**	**5.9**
Maximum	**0.0479**	**14**
Minimum	**0.0069**	**3**

Table 7.8. Phylogenetic diversity (PD) and species richness (SR) values for *Cheiridopsis* with Quarter Degree Squares (QDS) with three or more species.

QDS	PD	SR
2816BB	0.0084	4
2816BD	0.0162	5
2817AC	0.0090	4
2816DA	0.0259	7
2817CA	0.0175	7
2817CC	0.0259	7
2817CD	0.0175	7
2917BA	0.0340	9
2917BB	0.0139	7
2919AB	0.0062	4
2917BC	0.0354	7
2917BD	0.0219	3
2917DA	0.0464	9
2917DB	0.0464	9
2918CA	0.0095	6
2917DC	0.0126	4
2918CD	0.0068	5
3017BB	0.0082	4
3018AB	0.0099	5
3018DA	0.0046	3
3118DA	0.0056	3
Average	**0.0182**	**5.7**
Maximum	**0.0464**	**9**
Minimum	**0.0046**	**3**

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Table 7.9. Comparison of phylogenetic diversity values in shared Quarter Degree Squares with three or more species in *Conophytum* and *Cheiridopsis*. Results of pairwise t-test ($t_{(df)} = -2.354$, $P < 0.012$) indicated a significant difference between shared QDS of genera.

Conophytum		*Cheiridopsis*	
QDS1	0.0096	QDS1	0.0084
QDS3	0.0096	QDS2	0.0162
QDS4	0.0211	QDS3	0.0090
QDS6	0.0072	QDS5	0.0175
QDS8	0.0145	QDS6	0.0259
QDS9	0.0320	QDS7	0.0175
QDS14	0.0423	QDS8	0.0340
QDS15	0.0479	QDS9	0.0139
QDS18	0.0175	QDS10	0.0062
QDS21	0.0473	QDS11	0.0354
QDS22	0.0277	QDS12	0.0219
QDS27	0.0393	QDS13	0.0464
QDS28	0.0393	QDS14	0.0464
QDS29	0.0467	QDS15	0.0095
QDS32	0.0452	QDS16	0.0126
QDS35	0.0078	QDS17	0.0068
QDS38	0.0360	QDS18	0.0082
QDS40	0.0182	QDS19	0.0099
QDS49	0.0292	QDS20	0.0046

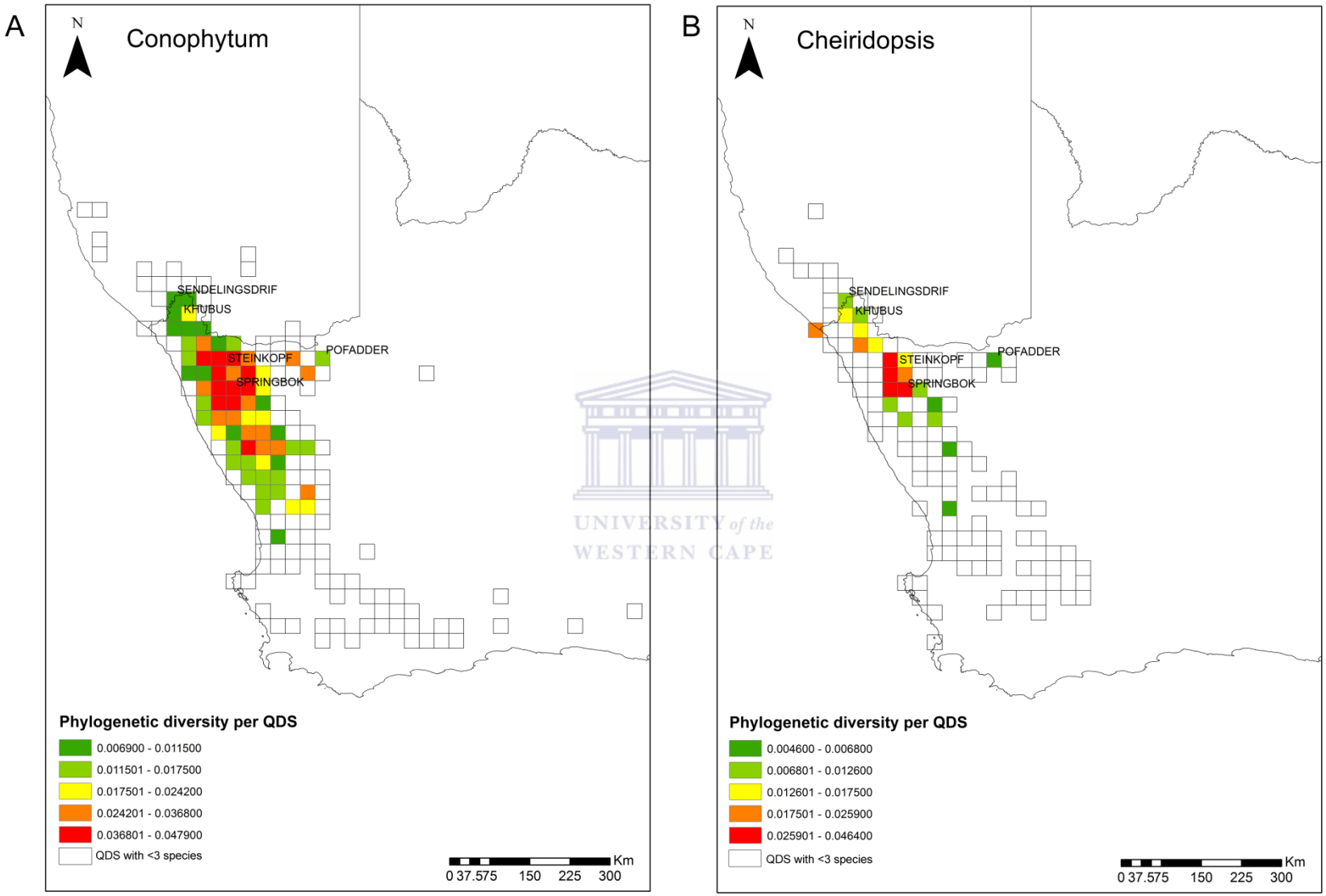

Figure 7.17. Phylogenetic diversity per Quarter Degree Square (QDS) which include three or more species for (A) *Conophytum;* (B) *Cheiridopsis*

7.4 DISCUSSION

7.4.1 FLORAL DIVERSITY IN *CONOPHYTUM* COMMUNITIES

7.4.1.1 Shifts in Phenology and Anthesis

Phenological shifts between co-occurring species, as observed in the Augrabies and Smorgenskadu *Conophytum* communities (Figs. 7.2, 7.5, 7.8, 7.11, 7.14), have also been reported in *Gladiolus* (Rymer *et al.,* 2010), with closely occurring species exhibiting shifts in flowering season, thought to be driven by pollination (Rymer *et al.,* 2010). As the peak flowering season of *Conophytum* is in autumn, earlier than the majority of the Aizoaceae which dominate the GCFR, interspecific competition for pollinators can be considered high, with *Conophytum* providing one of the few floral resources in the region (Jürgens & Witt, 2014). Therefore, the observed shifts in phenology in *Conophytum* are likely to be driven by pollination competition.

The shift in flowering season in the Augrabies and Smorgenskadu communities may be attributed to the position and associated climatic conditions of these communities. The Augrabies community includes the spring to summer flowering *C. bolusiae* and *C. francoiseae* and is positioned just inland of the north-western coast of South Africa. Although this community is located in Namaqualand, where rainfall is low and plants are subject to semi-desert climatic conditions, the Augrabies Mountains intercepts coastal fog that develops on the west coast and moves inland (Desmet & Cowling, 1999; Matimati *et al.,* 2012). The intercepted coastal fog provides moisture and cooler temperatures during the summer and spring months, therefore allowing *C. bolusiae* and *C. francoiseae* to flower in these otherwise hot and arid seasons

The Smorgenskadu community straddles the winter-summer rainfall boundary and the bimodal rainfall provides a longer climatic period suitable for flowering than in other communities. The increased possibility of summer rainfall has allowed the flowering season

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of *C. hermarium* to shift earlier into summer (Hammer, 2002; Chapter 6). A shift in flowering season is also exhibited in *C. smorenskaduense* as the species flowers in winter and is the only species in the genus which strictly flowers in this season (Hammer, 2002; Chapter 6). In addition, the shift in anthesis of *C. smorenskaduense* to the warmer winter afternoon hours (Fig. 7.8) may be attributed to peak pollinator activity during this period.

The autumn flowering *Conophytum* species in the selected communities, which made up the majority of species, were all separated by differences in anthesis, with the main distinction between diurnal and nocturnal flowering (Figs. 7.2, 7.5, 7.8, 7.11, 7.14). In the Gamsberg community, the diurnal species were separated further by anthesis, with *C. achabense* and *C. ratum* flowering only in the afternoon, while flowers of *C. burgeri* only open in the late afternoon (Fig. 7.11). This range in anthesis was previously also found in the Aizoaceae in a section of *Mesembryanthemum* (subgenus *Phyllobolus* section *Vesperifolia*) (Gerbaulet, 1996; Klak & Bruyns, 2013). The distribution of the four species in the section overlapped and the species displayed the full range of anthesis found in *Conophytum,* with diurnal and nocturnal species as well as afternoon and late afternoon flowering species (Gerbaulet, 1996). No conclusions were made suggesting why the four species would have differences in anthesis, however, as the distribution of the species overlap; the switch in anthesis may be driven by pollination. Similarly, the differences in anthesis of sympatric *Conophytum* species may be driven by competition for pollinators, as well as minimising hybridisation between closely related species, as a switch in anthesis between closely related species may act as a barrier to gene flow (Nosil *et al.,* 2008). This is evident in all the selected communities, with a shift in anthesis between closely related species. Gene flow between species that occupy the same temporal niche (e.g. diurnal flowering species in a community) appears to be avoided or minimised by genetic dissimilarity (Figs. 7.4, 7.7, 7.10, 7.13, 7.16).

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Although there is a clear separation in flowering times between diurnal and nocturnal species, the distinction between diurnal and afternoon and late afternoon flowering species is not as well defined. Diurnal flowers are open from around 10 am to 5 pm, while afternoon flowers open around midday (12 pm), closing a few hours thereafter, around 4 pm. There is therefore an overlap in flowering between diurnal and afternoon flowering species, with flowers of both groups of species open during the afternoon hours. Similarly, there is an overlap, although not as pronounced, between diurnal, afternoon and late afternoon species, with late afternoon species opening a few hours before dusk, which may coincide with the last flowering hours of diurnal and afternoon flowering species. In addition, the flowers of some species remain permanently open towards the end of the flowering season (Liede *et al.*, 1991; Hammer, 2002). It is not clear why there is such an overlap in anthesis between these species, as there are no clear barriers to prevent gene flow, however, this overlap may be attributed to competition and maximum allocation of pollination resources. Individuals of *Conophytum* within a population usually flower together resulting in a mass 'bouquet' effect presented by a number of contiguous flowers (Liede *et al.*, 1991). The mass flowering of *Conophytum* species in a community provides ample floral resources, and studies showed that pollinators forage on a mass of flowers at a time before moving onto another mass, rather than mixed foraging (Goulson, 1999; Gegear & Laverty, 2001). Due to the preference of mass foraging of pollinators, perhaps the shift in anthesis of *Conophytum* species is an adaptation towards pollination optimisation, with pollinators foraging on different floral masses of *Conophytum* throughout the day. This may be especially important in the Gamsberg community, as the community is located as the very eastern edge of the winter-rainfall region, where pollinator availability may be limited.

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7.4.1.2 Divergence of floral morphology

The groups of species which are separated by anthesis and phenology form temporal guilds within each of the communities. Species within a temporal guild display a unique combination of floral traits including flower structure, flower colour, absence or presence of floral scent and absence or presence of filamentous staminodes (Figs. 7.4, 7.7, 7.10, 7.13, 7.16). As floral diversification is commonly thought to be driven by pollination (Rymer *et al.*, 2010), with the force of pollination diversification in plants is driven by competition and adaptation to pollinators (Harder & Johnson, 2009; Schiestl & Schluter, 2009; Rymer *et al.*, 2010), these drivers are believed to be one of the main factors driving floral divergence in *Conophytum.* The diverse floral morphology suggests an adaptation and specialisation to different pollinators, thus reducing the competition for pollination resources within the guild (Schiestl & Schluter, 2009; Rymer *et al.*, 2010). The floral diversity of *Conophytum* is not only found within the sympatric communities, but also in the phylogenetic analyses of the genus, with floral traits indicated to have evolved numerous times across the genus (Chapter 6). These traits include phenology, anthesis and floral structure (Chapter 6), with inferred pollination shifts in *Conophytum* (based on anthesis and structure) having taken place every 1.8 species (Chapter 6). A similar high rate of pollination syndromes shifts was found also in *Lapeirousia* Thunb. (Iridaceae) with 1.5 shifts per species (Forest *et al.*, 2014). There is considerable evidence that shifts in pollination syndromes lead to floral evolution (Bradshaw & Schemske, 2003), suggesting that these shifts in floral traits across the phylogeny, driven by pollination, is in turn driving the floral evolution of the genus.

The unique tubular flowers of *Conophytum* and range of floral structures suggests a specialisation towards pollinators (Liede *et al.*, 1991). Although no direct link between the floral structures of *Conophytum* and pollinators of the species has been found, records show that the flowers are visited by a number of pollinators (Liede *et al.*, 1991; Jürgens & Witt,

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2014). Pollen wasps (*Quartina* spp.) were found to be frequent visitors, specifically of the diurnal flowering species, while moths were the main pollinators of the nocturnal species (Liede *et al.*, 1991; Jürgens & Witt, 2014). A number of other insects (Coleoptera, Diptera and Hymenoptera) were also recorded visiting *Conophytum* flowers, however, it was unclear whether the observed insect visitors were pollinators or just coincidental flower visitors (Jürgens & Witt, 2014).

The *Quartina* pollen wasps were observed not only collecting pollen and nectar, but also using the flowers of *Conophytum* as shelter from the elements (sun and wind etc.) and a place for mating (Jürgens & Witt, 2014). As the pollen wasps have long proboscises, the unique tubular flowers of *Conophytum* may represent a specialisation towards these pollinators. Observations by Liede *et al.* (1991) also suggested that the inclusion of filamentous staminodes (which are variably absent or present in the flowers of *Conophytum*) encourages the pollinators to crawl down the tube. Although specialisation of *Conophytum* flowers towards pollen wasps is proposed, the position of the anthers and stigmas at the mouth of the tube does not restrict pollination from generalist pollinators. As *Conophytum* flowers represent a floral resource mostly unavailable in the region during autumn (Jürgens & Witt, 2014), it should be attractive to a range of pollinators, and therefore may be pollinated by specialist as well as generalist pollinators in the region.

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The relationship between the nocturnal *Conophytum* species and their proposed moth pollinators is clear, with the strongly scented tubular flowers inferring a strong adaptation to moth pollination (Jürgens & Witt, 2014). Some of the nocturnal *Conophytum* species are white, which is a common nocturnal moth-pollinated flower colour. However, there are a few nocturnal species that are unusual in their red-orange coloured flowers (Hammer, 2002; Jürgens & Witt, 2014). The flowers of these species are however strongly scented (Jürgens & Witt, 2014).

Flower colour in *Conophytum* is very variable and some species include almost the full range of colours found in the Aizoaceae, including white, yellow, pink, red-orange and magenta. Although flower colour is usually strongly driven by pollination specialisation (Chittka & Menzel, 1992; Menzel & Shmida, 1993; Peter & Johnson, 2008), Liede *et al.* (1991) found that different coloured *Conophytum* flowers in a community were visited by the insects. Flower colour in *Conophytum* also includes an inherent variation, with variation in flower colours across populations, or even in some cases within a population (A.Young, Pers. Comm.).

Despite the overlap in flower colour within species of a community, the results of this present study has revealed that there is a separation of flower colour between species in a community (Figs. 7.4, 7.7, 7.10, 7.13, 7.16). This distinction in flower colour within a community, e.g. in species A flowers are white to yellow, but never magenta, while in species B the flowers are yellow to magenta, is likely to be related to pollination adaptation (Peter & Johnson, 2008).

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Although the pollination syndromes of *Conophytum* indicate high specialisation, the observed pollinators of the diurnal and nocturnal species seem to be relatively uniform across the species within the respective anthesis groups. Therefore further investigation of potential pollinators of the genus is required, perhaps linking the function of the diverse floral traits to specialisation towards a specific pollinator.

7.4.2 PHYLOGENETIC STRUCTURE IN *CONOPHYTUM* COMMUNITIES

7.4.2.1 A Novel Mechanism of Seed Dispersal in *Conophytum*

The selected *Conophytum* communities were comprised of genetically closely related species as well as genetically distant species (Figs. 7.3, 7.6, 7.9, 7.12, 7.15). This suggests that a mechanism of long distance seed dispersal is present, with seeds able to move between non-

contiguous populations across the distribution of the genus. *Conophytum* seeds are released from the capsules during rainfall events, as is typical of the family (Hartmann, 1991). The capsules open when they come into contact with water and the seeds are released from the capsules by various mechanisms, such as wash-out, splash-cup, spring-broad and ejection dispersal (Hartmann, 1991). As the capsules of *Conophytum* are simple, with no internal structures, the seeds were thought to be dispersed by the wash-out mechanism, where capsules open during rainfall events and the collection of water in the capsules results in the seeds washing out the capsule due to the overflow of water (Hartmann, 1991). Although the seed is able to travel considerable distances (up to 30 m) from the parent individual (Hartmann, 1991), this dispersal mechanism does not explain the dispersal of seed across much larger distances, resulting in the differences in phylogenetic structure within the *Conophytum* communities. The capsules of *Conophytum* are small, light and papery, often breaking off the plant (Hartmann, 2001; Hammer, 2002) which potentially allows for the capsules to be dispersed by wind (Van Jaarsveld, 2011). Anemochory or wind dispersal occurs in a few genera in the Aizoaceae, including *Eberlanzia* Schwantes, *Fenestraria* N.E.Br., *Mesembryanthemum* (specifically *M.* sect. *Phyllobolus* (N.E.Br.) Klak) and *Ruschia* Schwantes (Hartmann, 1991), where the whole capsule breaks off the pedicel and is transported as a tumble fruit (Hartmann, 1982). These tumble fruits are, however, woody (Hartmann, 2001), not papery as those of *Conophytum*, and therefore are slightly heavier, suggesting that the capsules of *Conophytum* can be dispersed by wind across further distances. The adaptation to a long distance dispersal mechanism was already indicated in the phylogeny presented in Chapter 6, with a disjunction in the distributions of the recovered sister pair *C. angelicae* and *C. luckhoffii*, as *C. angelicae* is distributed in the southern Namib and Bushmanland, while the distribution of *C. luckhoffii* is confined to the southern Cape between Citrusdal and Clanwilliam (Hammer, 2002). The seeds of the wind dispersed tumble

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fruits are released hygroscopically when capsules come into contact with water during dispersal (Hartmann, 1991). This is also likely to be the case in *Conophytum*, with no internal structures restricting the dispersal of seeds and therefore the potential release of a large number of seeds could result in the establishment of a population in the dispersed habitat (Hartmann, 2001; Hammer, 2002).

Long distance dispersal events have been found to form pocket populations (Mollison, 1977; Nichols & Hewitt, 1994), which may explain the eastern expansion of *Conophytum* into the Bushmanland Inselberg Region (BIR). The pairwise genetic distances show that the Gamsberg community in the BIR has the highest average pairwise genetic distance, as well as the largest genetic distance between species (Fig. 7.12), suggesting that this community includes the most distantly related species which have colonised this region through long distance dispersal events, forming a pocket population. The proposed wind dispersal mechanism in *Conophytum* may also explain the high number of range restricted and point endemic species in the genus, with an adaptation in floral morphology to a newly colonised community and without subsequent contact from closely related species, the newly colonised population possibly forms a new species, as per the allopatric speciation model (Wiens, 2004). This newly formed species is therefore only found within that specific community and is thus regarded as a point endemic.

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7.4.2.2 Contribution of Long Distance Dispersal to Floral Diversity in *Conophytum*

As in many communities, floral diversity in a *Conophytum* community is thought to be shaped by interspecific pollination competition (Schiestl & Schluter, 2009; Rymer *et al.*, 2010). However, the divergence of floral traits in a *Conophytum* community is also related to the long distance dispersal mechanism. Long distance dispersal events introduce new species into a *Conophytum* community and these newly colonised species require a slightly different

floral morphology than the rest of the species in the community or they may be outcompeted (Liede & Hammer, 1990; Liede *et al.,* 1991). Therefore, introduction of distantly related species with different floral morphologies increases the floral diversity within a community. In addition, an adaptation in floral morphology of a newly colonised species in a community may not be in response to pollination competition, but rather an adaptation to the local pollinators of the newly colonised habitat (Harder & Johnson, 2009) or through assimilation by hybridisation (Levin *et al.,* 1996) with local species in the community. Without subsequent contact from other closely related species, these newly adapted species may become genetically isolated, possibly forming a new species as suggested by the allopatric species model (Coyne & Orr, 2004; Hey & Nielsen, 2004). The combination of interspecific pollination competition as well as adaptation to pollinators in the new environment is likely to be driving floral diversity in closely as well as distantly related species in these diverse *Conophytum* communities.

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Capsules of *Conophytum* species may not only be dispersed into new communities, but may also be dispersed back into their source communities, as a dispersal pattern is random (Peres & Baider, 1997; Nathan & Muller-Landau, 2000). In this case, the floral traits of a newly colonised population of species may have adapted to its new community (Harder & Johnson, 2009), possibly forming a new species (Hey & Nielsen, 2004) and so when the capsules of this adapted species are dispersed to the source community, the adapted species does not compete with the species in the source community as a result of the adapted floral morphology (Wiley & Mayden, 1985). This results in different floral morphologies between closely related species found in the same community, as is suggested by the phylogenetic structure of the communities (Figs. 7.3, 7.6, 7.9, 7.12, 7.15) and is possibly indicative of parapatric speciation (Coyne & Orr, 2004).

The occurrence of closely related species with different floral morphologies within a community may also be indicative of sympatric speciation (Coyne & Orr, 2004). This model is highly controversial (Gavrilets, 2003), although some studies provide strong support for this speciation model (Barluenga *et al.*, 2006; Savolainen *et al.*, 2006).

7.4.2.3 Habitat Diversity in *Conophytum* Communities

Adaptation to different habitats may also separate the species in a community, especially as *Conophytum* species occupy very small habitat units due to the small size of the plants (Desmet, *et al.*, 1998; Young *et al.*, 2015a). The species occupy a variety of habitats and are found growing in mostly quartz, but also in granite, calcrete, shale and even in some cases dolerite (Hammer, 2002). A number of species are highly habitat specific with vertical stratification of species usually found within a community (Hammer, 2002; Young *et al.*, 2015a).

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This vertical stratification is particularly noticeable in the Smorgenskadu community, where although species are all found on quartz, the different types of quartz habitats within the community separate the species. For instance, *C. marginatum* is found on the quartz outcrops that cap the top of the inselbergs throughout the community, while *C. maughanii* is found on quartz patch flats which are found on the tops and bases of the inselbergs (Hammer, 2002). *Conophytum lithopsoides* also occurs on the base quartz patch flats, but is not found on the quartz flats on the tops of the inselbergs in the community (Hammer, 2002). *Conophytum hermarium* is endemic to the community and has a very specific habitat only found growing in a large series of quartz blocks (Hammer, 2002). The range of quartz habitats in this community may have attributed to the large number of *Conophytum* species found within it (Young & Desmet, 2016).

However, the large number of species in a community cannot always be attributed to a range of available habitats, as the community which includes the most *Conophytum* species, Umdaus, is characterised by a single habitat type, i.e. quartz patches associated with the Witbakenberg Mountains (Mucina *et al.*, 2006). Similarly, the Knersvlakte which is also comprised of different quartz patches is regarded as a region of high species diversity and endemism in the family (Hartmann, 1991; Schmiedel & Jürgens, 1999), though not particularly so for *Conophytum* (Young & Desmet, 2016). Although these habitat types are broadly categorised here, they include variation in aspect, slope, pebble size etc. creating very specific microhabitats. A large number of *Conophytum* species occupy these highly specific microhabitats, however, due the variability and uniqueness of these microhabitats across the genus, it is difficult at assess these microhabitats at a broad scale.

The results of this study indicate that species diversity in the *Conophytum* communities is mainly explained by the high floral and phylogenetic diversity. This diversity is likely driven by the combination of interspecific competition, floral adaptation and the adaptation of wind dispersed capsules in *Conophytum*. However differences in habitat preferences of the species adds an additional layer of complexity to the communities.

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7.4.3 PHYLOGENETIC DIVERSITY IN *CONOPHYTUM* AND *CHEIRIDOPSIS*

The significantly higher PD of *Conophytum* in comparison to *Cheiridopsis* (Table 7.9; Fig. 7.17) suggests a different in seed dispersal mechanisms (Waser & Elliot, 1991) between the sister genera. This difference in seed dispersal mechanisms is evident from the different in capsule morphologies of the genera. *Conophytum* has soft, papery capsules with no internal structures and often break off the plant, while the capsules of *Cheiridopsis* are hard, woody, with complex internal structures and are persistent (Powell *et al.*, submitted b; Chapter 5). While *Conophytum* is suggested to be wind dispersed, the dispersal of the seed in

Cheiridopsis is facilitated by the internal structures. These internal structures (covering membranes and closing bodies) play an important role in the ejection-type seed dispersal as well as restricting the complete release of all the seeds during a rainfall event (Hartmann, 1991). Although the ejection-type dispersal in *Cheiridopsis* disperses seeds a number of meters away from the parent plant (Hartmann, 1991), the PD results (Tables 7.7–7.9; Fig. 7.17) suggest that the seeds of *Conophytum* are dispersed across much larger distances, with more species from different lineages included in *Conophytum* QDS than in *Cheiridopsis* QDS. This long distance dispersal is further indicated by the higher number of QDS with a high PD in *Conophytum* (Fig. 7.17A) compared to *Cheiridopsis*, where areas of high PD only coincide with the centre of species diversity around Steinkopf (Fig. 7.17B). In addition, although the Steinkopf region was identified as the centre of PD for both genera (Fig. 7.17), the distribution of *Conophytum* is wider than *Cheiridopsis*, providing further evidence of long distance dispersal in *Conophytum.*

Conophytum includes a significantly larger amount of species (106 species) than *Cheiridopsis* (38 species) (Hammer, 2002; Powell *et al.*, submitted b; Chapter 5). Also, *Cheiridopsis* does not form complex species communities as found in *Conophytum*, with only a few species of *Cheiridopsis* found together at a very broad scale (Powell *et al.*, submitted b; Chapter 5). The unusual combination of the long distance dispersal mechanism and specialised flowers of *Conophytum* have enabled the formation of the complex species communities in the genus, while in *Cheiridopsis* the seeds are only dispersed a few meters and the flowers are unspecialised, as is typical of the family (Hartmann, 1983, 2001). The formation of the complex communities may have therefore enabled the explosive radiation of *Conophytum*, specifically in comparison to its sister genus and the floral and genetic separation of species within these communities possibly maintains the integrity of the species within them.

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Chapter 8

Population dynamics of three *Conophytum* species endemic to the Bushmanland Inselberg Region, South Africa

8.1 INTRODUCTION

The Bushmanland Inselberg Region (BIR) is a chain of inselbergs between Springbok and Pofadder, in the Northern Cape Province of South Africa, which straddles the boundaries of the winter and summer rainfall regions. These inselbergs are dominated by Succulent Karoo vegetation with the surrounding lowlands characterised by Nama Karoo vegetation. As a result the inselbergs essentially form islands of Succulent Karoo in the Nama Karoo Biome (Desmet, 2000; Mucina *et al.,* 2006; Young & Desmet, 2016). Although the surrounding Nama Karoo grasses are found growing in the characteristic red sandy soil of the region, the geology of the inselbergs includes metamorphosed granite (gneiss) with intrusions of quartz (McCarthy & Rubridge, 2005). The quartz intrusions are found in the form of quartzite outcrops which cap the summits of the inselbergs, or quartz gravel flats, found on the summit and surrounding inselberg bases (Desmet, 2000). Although the position of the BIR on the boundary of the winter-summer rainfall regions could potentially place it in a position to take advantage of both winter and summer rainfall, rainfall in the region is erratic and low, with a mean annual rainfall of less than 100 mm (Mucina *et al.,* 2006), and therefore there is a possibility that the region might not receive rainfall from either rainfall regime in some years (Desmet, 2000). The BIR also experiences an extreme range of temperatures, with a mean minimum temperature of -3°C and a mean maximum temperature of 38°C (Mucina *et al.,* 2006). Areas of higher altitude provide some refuge from these unfavourable environmental conditions, with cooler temperatures and moisture intercepted from fog that develops in the

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Koa River Valley (just south of Aggeneys) or from low cloud generated by passing cold fronts (Desmet, 2013).

A number of the larger inselbergs have been identified as areas of high endemism for Aizoaceae, the so-called Pofadder centre (Hartmann, 1991), with a number of species found to be endemic to these inselbergs (Desmet, 2000; Mucina *et al.*, 2006). Several of these endemics belong to *Conophytum* N.E.Br., mainly from sections *Ophthalmophyllum* (Dinter & Schwantes) G.D.Rowley and *Chesire-feles* S.A.Hammer, which have soft-bodied leaves and are found in the quartz habitats across the region (Desmet, 2000; Young & Desmet, 2016).

Conophytum ratum S.A.Hammer (Fig. 8.1A) is endemic to the largest inselberg in the BIR, the Gamsberg (Fig. 8.2B), and is found on fine-grain quartz patches, a habitat-type restricted to the Gamsberg (Desmet, 2000). Due to the unique habitat and high number of endemics found on the Gamsberg, this inselberg was identified as the highest priority for conservation of the flora in the BIR (Desmet, 2000), yet as of July 2016 open-cast zinc mining has commenced on this inselberg. Due to this mining activity on the Gamsberg, and the restricted distribution of *C. ratum*, this species is regarded as a flagship for conservation and is listed as vulnerable (VU) on the IUCN Red List (Victor & Desmet, 2005; Raimondo *et al.*, 2009). *Conophytum ratum* often occurs with *C. maughanii* N.E.Br. subsp. *maughanii* (Fig. 8.1A, B; hereafter referred to as *C. maughanii*) and although *C. maughanii* is also endemic to quartz patches in the BIR, it is not as habitat specific as *C. ratum* (Young & Desmet, 2016), and is found across a wider distribution spanning the extent of the BIR. *Conophytum ratum* and *C. maughanii* are sister species in the phylogeny of *Conophytum* (Chapter 6) and are morphologically very similar, with some differences in floral morphology, anthesis and phenology (Hammer, 2002; Chapters 6 & 7).

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Figure 8.1. (A) *Conophytum ratum* in habitat; (B) *Conophytum ratum* (top) and *Conophytum maughanii* (bottom) co-occurring on a quartz patch at the base of Achab; (C) *C. maughanii* in habitat, illustrating the red tannins which are developed later in the growing season; (D) *C. ratum* in its highly specific fine-grain quartz habitat; (E) quartz outcrop habitat of *Conophytum marginatum*; (F) *C. marginatum* growing in crevices of quartz outcrops; (G) *C. marginatum* subsp. *marginatum* with the soft papery capsules, open after contact with fog; (H) *C. marginatum* subsp. *haramoepense*; (I) habitat of the northern-most population of *C. marginatum* subsp. *haramoepense*, in a quartz outcrop surrounded by red dune sand; (J) inselbergs of Succulent Karoo among the Nama-Karoo sands in the Bushmanland Inselberg Region (BIR).

Conophytum marginatum Lavis subsp. *marginatum* (Fig. 8.1G) and *C. marginatum* subsp. *haramoepense* (L.Bolus) S.A.Hammer (Fig. 8.1H) are clump-forming endemics, restricted to quartz outcrops on the summits of the inselbergs across the BIR (Hammer, 2002; Young & Desmet, 2016). On the western inselbergs of the BIR (i.e. Areb – Concordia, Fig. 8.2), *C. marginatum* subsp. *marginatum* is present, while from Areb eastwards to the Gamsberg, it is replaced by *C. marginatum* subsp. *haramoepense* (Fig. 8.2). The two subspecies do not co-occur and have slightly different leaf morphologies. *Conophytum marginatum* subsp. *marginatum* has sharply-keeled leaves with red-lined margins, while the leaves of subsp. *haramoepense* are densely spotted, due to the presence of small windows across the leaf surface, with rounded lobes (Hammer, 2002).

Conophytum has hygrochastic capsules which disperse seeds during rainfall events through a wash-out mechanism (Hartmann, 1991; Hartmann, 2001; Hammer, 2002; Chapter 7). In addition, the capsules of *Conophytum* are soft and papery (Fig. 8.1G) and easily detach from the plant, with the whole capsule potentially dispersed by runoff during a rainfall event, or through wind dispersal as postulated in Chapter 7. As drainage has been shown to directly influence seed dispersal, and in turn the population dynamics in *Argyroderma* N.E.Br (Aizoaceae) (Ellis *et al.,* 2007), it is hypothesised that drainage directly influences the population dynamics of the BIR endemic *Conophytum* species. This hypothesis is potentially already evident in the distribution of *C. maughanii* and *C. ratum,* with populations found on the summit as well as the surrounding base of the inselbergs, suggesting that seed is dispersed down the slope during a rainfall event. The populations of *C. marginatum* are only found on the summits of the inselbergs in the quartz outcrop habitat, with some populations up to 25 km apart. It is unclear how the populations have colonised the often inaccessible habitat of high altitude (above 800 m) quartz outcrops, however the proposed wind dispersed capsules of the genus, as postulated in Chapter 7, may influence population dynamics in this species

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The Amplified Fragment Length Polymorphism (AFLP) population genetics technique has been the method of choice for many studies of plant populations across the globe (Vos *et al.*, 1995; Belaj *et al.*, 2003; Woodhead *et al.*, 2005; Barluenga *et al.*, 2006; Meudt & Clarke, 2007) and relies on largely non-coding DNA that is distributed throughout the genome, allowing for assessment of genome-wide variation (Wong *et al.*, 2001; Shirasawa *et al.*, 2004). The technique is advantageous for studies where there is no *a priori* sequence information, for intraspecific studies with low genetic variability, and for short-term studies where rapid generation of data is required (Mueller & Wolfenbarger, 1999; Sunnucks, 2000; Schlötterer, 2004; Bensch & Åkesson, 2005; Meudt & Clarke, 2007) Previous studies in Aizoaceae have used the AFLP technique for the production of resolved phylogenies to assess species complexes in *Delosperma* N.E.Br. (Buys *et al.*, 2008) and genetic differentiation of species in *Lithops* N.E.Br. (Kellner *et al.*, 2011), as well as the assessment of the population dynamics of *Argyroderma pearsonii* (N.E.Br.) Schwantes in the Knersvlakte (Ellis *et al.*, 2007).

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This study seeks to investigate the interactions between populations of *C. maughanii* and *C. ratum* that co-occur on quartz patches on the summit of the inselbergs and the surrounding quartz patches at the base through use of the AFLP technique. Species delimitation between the sister taxa *C. maughanii* and *C. ratum*, as well as population dynamics of *C. marginatum* will also be assessed. By understanding the interactions and connectivity of these *Conophytum* populations across the inselbergs, inference can be made on the connectivity and interactions of other species across the Bushmanland inselbergs as well as providing insight into conservation of the biodiversity that is found on these inselbergs.

8.2 MATERIALS AND METHODS

8.2.1 POPULATION SAMPLING

Comprehensive distribution data and locality information was obtained from Prof. Andrew Young from Liverpool John Moores University (Young & Desmet, 2016) for the two subspecies of *C. marginatum, C. maughanii* and *C. ratum*. Three extensive field visits (May 2014, April 2015, and June 2015) were undertaken. All known populations of *C. ratum* were visited, with material collected from populations that were relocated at both the summit and base of the inselbergs. One population on the northern slope of the Gamsberg is now extinct due to recent mining activity (Fig. 8.2) and three populations of *C. ratum* along the base of the Namiesberg are apparently lost, as they have not been relocated in the last 10 years (A. Young, Pers. Comm.). A total of 28 individuals of *C. ratum* from five populations were sampled. The sampling of *C. maughanii* includes 42 individual from eight populations, which spans the full distribution of the species in the BIR (Fig. 8.2). The full distribution ranges of both subspecies of *C. marginatum* were sampled, with 59 individuals from 11 populations sampled across the range of inselbergs in the BIR, excluding the Lemoenpoortberge where collection was not permitted by the land owner.

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Leaf material was collected across the full range of each population (on each of the corners and two widely spaced individuals from the centre) and dried on silica gel. Six individuals of each population were usually sampled, however, in more restricted populations, only four or five individuals were sampled so as to not negatively impact the populations.

8.2.2 AFLP POPULATION GENETICS TECHNIQUE

Total DNA extractions, AFLP reactions and analyses of genetic structure, diversity and drainage pattern were carried out following the methods outlined in Section 2.9 of Chapter 2.

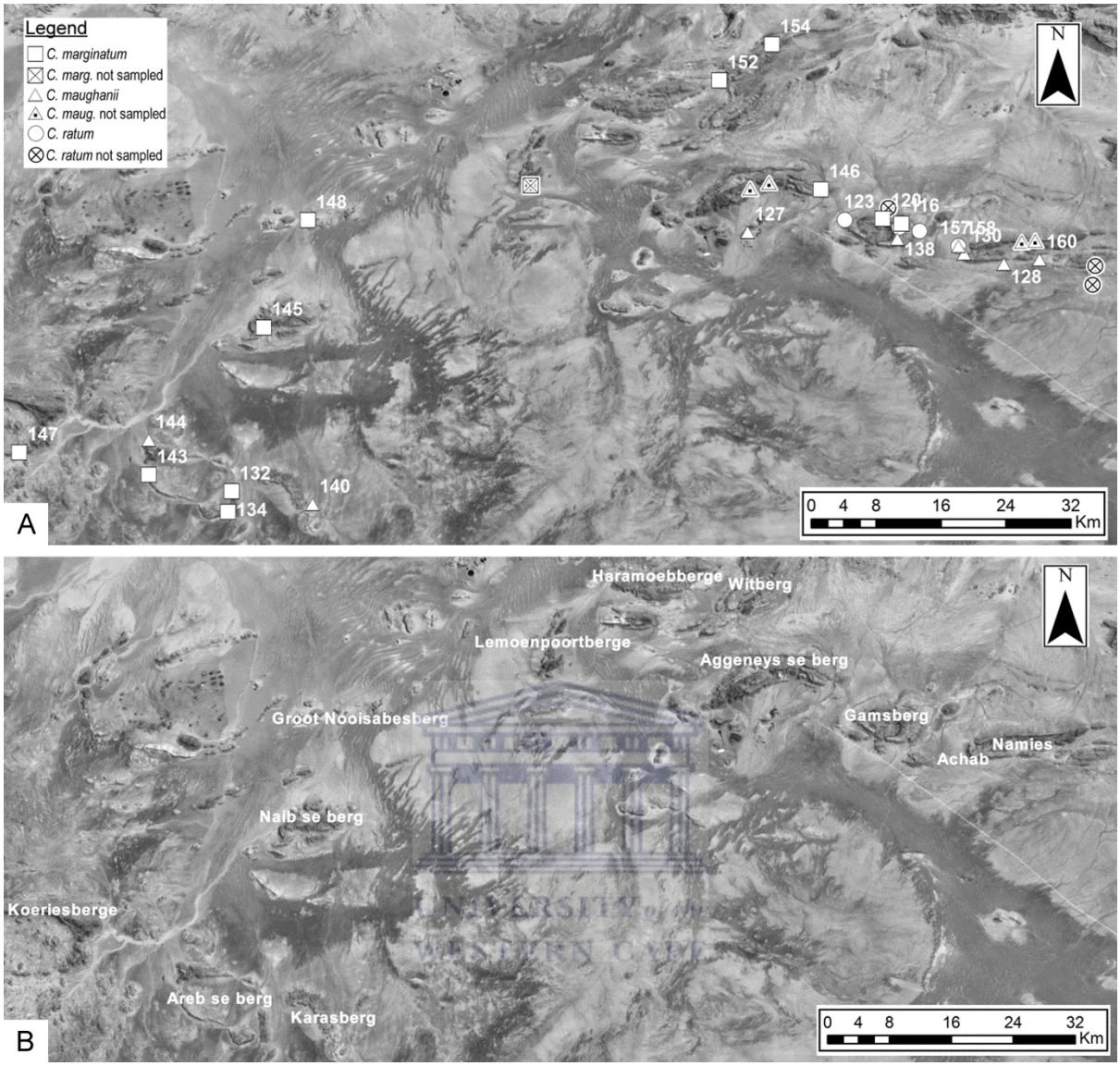

Figure 8.2. (A) Distribution of *Conophytum marginatum*, *C. maughanii* and *C. ratum* populations sampled as well as populations not relocated or lost. (B) The major inselbergs across the BIR.

8.3 RESULTS

8.3.1 *CONOPHYTUM RATUM*

8.3.1.1. Molecular Analysis of Variance (AMOVA)

Table 8.1. Molecular analysis of variance (AMOVA) of *Conophytum ratum* populations, indicating variance among and within populations.

	Df	SS	MS	Est. Var.	%
Among Pops	4	333.350	83.338	9.691	25%
Within Pops	23	670.900	29.170	29.170	75%
Total	27	1004.250		38.861	100%

A total of 289 loci were amplified and scored for the five *Conophytum ratum* populations, of which 96.9% (280 loci) were polymorphic. Molecular analysis of variance (AMOVA) was shown to be much greater within populations (75%) than among populations (25%) (Table 8.1).

The PCoA of the AMOVA results showed that the populations formed two clear groupings on either side of the coordinate 1 axis, with individuals of populations from the summit of the inselbergs, 115 and 119, grouped to the right of the coordinate 1 axis, while individuals of populations from the base, 123, 155, 157 grouped on the left (Fig. 8.3). Two individuals of population 119 and one individual of population 155 were separated from the groupings on the right (Fig. 8.3).

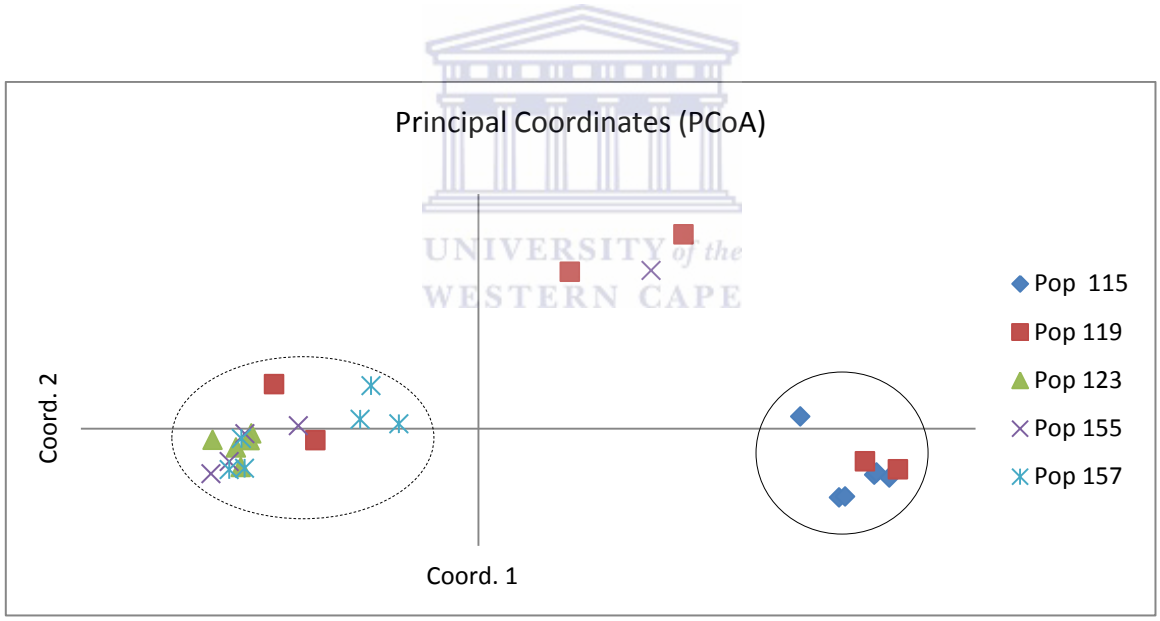

Figure 8.3. PCoA of AMOVA results for individuals in the different populations of *Conophytum ratum*. Individuals of the summit populations in a solid circle on the right (excluding two individuals from population 119 in the top right quadrant) and the base population individuals in a dotted circle (excluding an individual from population 155 in the top right quadrant) on the left. Percentage of variance explained by axis 1: 26.71%, axis 2: 10.04%, axis 3: 4.94%.

8.3.1.2 Genetic Diversity

Table 8.2. Genetic diversity indices for the five populations of *Conophytum ratum* sampled: n-number of individuals; #loc.-number of loci scored; #loc_P-number of polymorphic loci at 5% level; PLP-percentage polymorphic loci at 5% level; Hj-Nei's genetic diversity.

Population	N	#loc.	#loc_P	PLP	Hj	S.E.(Hj)
Pop115	6	289	172	59.5	0.26711	0.01382
Pop119	6	289	190	65.7	0.28684	0.01321
Pop123	5	289	90	31.1	0.14948	0.01347
Pop155	5	289	129	44.6	0.20329	0.01383
Pop157	6	289	112	38.8	0.16753	0.01308

Genetic diversity was higher in populations located on the summit of the Gamsberg (populations 115 and 119), with Nei's genetic diversity (Hj) values of 0.26711 and 0.28684, respectively, compared to the populations at the base of the inselbergs (populations 123, 155 and 157), with Hj values of 0.14948–0.20329 (Table 8.2). The summit populations also had a higher number of polymorphic loci (up to 65.7%) than the base populations (up to 44.6%) (Table 8.2).

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Table 8.3. Pairwise Fst (fixation index) values between the five populations of *Conophytum ratum.*

	Pop115	Pop119	Pop123	Pop155	Pop157
Pop115	0	0.0601	0.3998	0.2966	0.3439
Pop119	0.0601	0	0.2395	0.1317	0.1821
Pop123	0.3998	0.2395	0	0.1389	0.1786
Pop155	0.2966	0.1317	0.1389	0	0.1104
Pop157	0.3439	0.1821	0.1786	0.1104	0

Pairwise Fst (fixation index) values indicated a greater difference in genetic diversity between populations at the summit (populations 115 and 119) and populations at the base (populations 123, 155, 157) with pairwise values between 0.1821–0.3998. Pairwise Fst values

between populations 123, 155 and 157 ranged between 0.1104–0.1786, while the pairwise Fst value between population 115 and 119 was 0.0601 (Table 8.3).

Table 8.4. Genetic diversity indices for the combined summit populations (115 & 119) and the combined base populations (123, 155 & 157) of *Conophytum ratum*: n-number of individuals; #loc.-number of loci scored; #loc_P-number of polymorphic loci at 5% level; PLP-percentage polymorphic loci at 5% level; Hj-Nei's genetic diversity.

Population	N	#loc.	#loc_P	PLP	Hj	S.E.(Hj)
summit	12	289	242	83.7	0.28822	0.01023
base	16	289	186	64.4	0.19442	0.01116

The comparison of genetic diversity indices for the summit and base populations of *C. ratum* showed that the summit populations had a greater genetic diversity (Hj=0.28822) and a higher proportion of polymorphic loci (83.7%) than the base populations (Hj=0.19442, PLP=64.4%) despite the greater total number of individuals in the base populations (Table 8.4).

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8.3.1.3 Genetic Structure

The Bayesian clustering analysis suggested a population structure with two genetic clusters (K=2), based on the highest ΔK value (Table 8.5, Fig. 8.4A). The results of the analysis for K=3 did not provide further cluster resolution and agreed with the results of K=2 (Fig. 8.4A). Most of the populations shared the assigned green cluster, while population 115 was represented by only the red cluster. This red cluster was shared with the summit populations (119 and 155; Fig. 8.4). The mapped drainage lines and transect (Fig. 8.4B, C), suggested movement of propagules from population 115 to 119 and 155, while movement from population 119 to 155 and 157 was suggested.

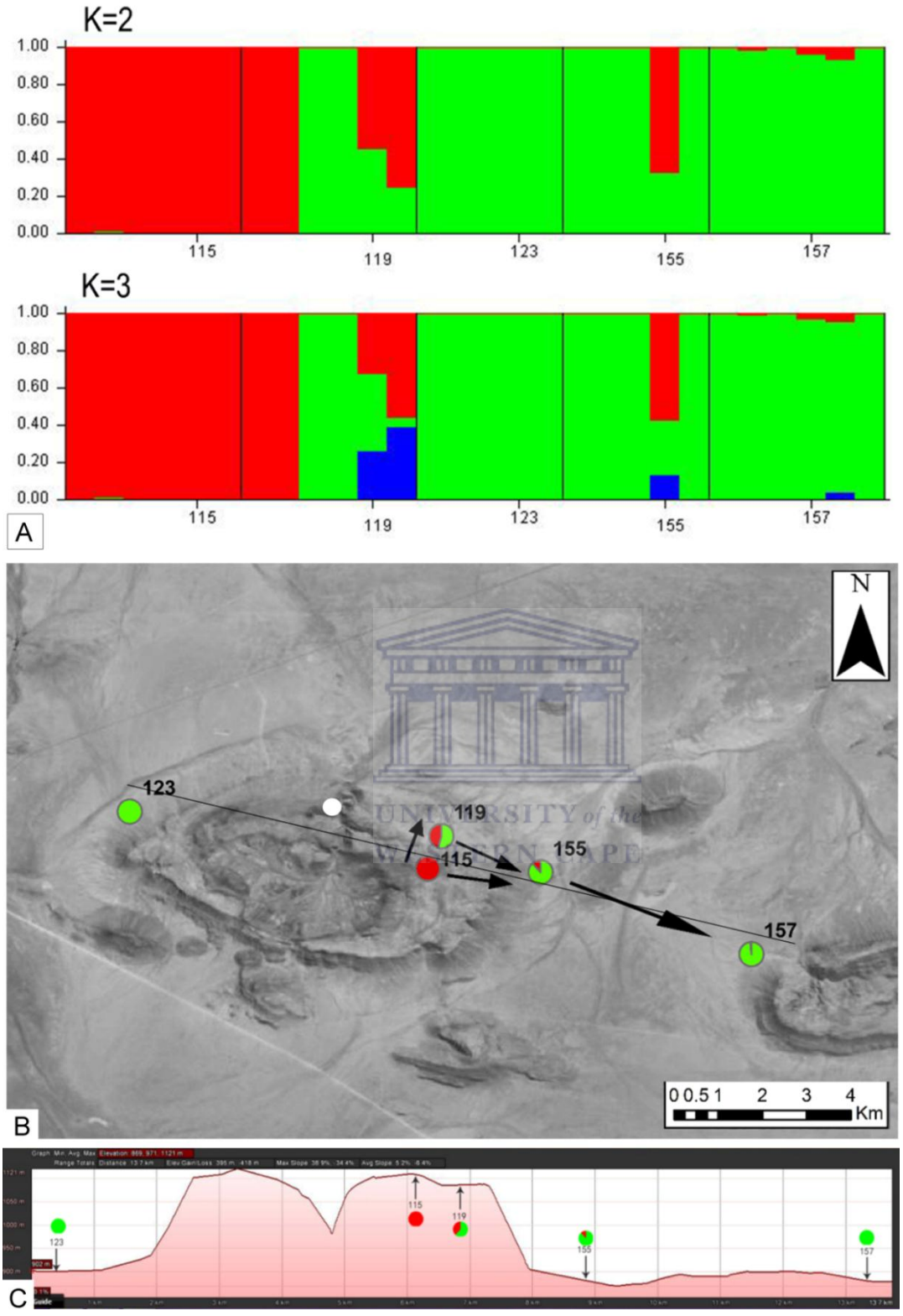

Figure 8.4. (A) Cluster membership of individuals in each of the populations of *Conophytum ratum* at K=2 and K=3. Each cluster is assigned a colour, with solid black lines separating the populations; (B) the mapped geographic distribution of the proportion of genetic clusters in the populations. The arrows indicate the drainage lines and the lost *C. ratum* population is mapped as a white dot. Transect for elevation profile indicated by thin line across the populations; (C) elevation profile across the populations of *C. ratum*, with the genetic structure of each population indicated.

Table 8.5. Values of ΔK for K of 10 iterations for populations of *Conophytum ratum.* K=2 presents the highest ΔK value.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	ΔK
1	10	-3381.16	0.1506	NA	NA	NA
2	10	-2868.3	0.5185	512.86	595.09	1147.615
3	10	-2950.53	2.6132	-82.23	70.64	27.03162
4	10	-2962.12	3.6475	-11.59	11.02	3.021277
5	10	-2962.69	5.0227	-0.57	1.61	0.320544
6	10	-2964.87	4.95	-2.18	10.38	2.096977
7	10	-2956.67	6.0544	8.2	8.7	1.436973
8	10	-2957.17	4.4937	-0.5	6.73	1.497648
9	10	-2950.94	4.51	6.23	1.32	0.29268
10	10	-2946.03	4.7891	4.91	NA	NA

8.3.2 *CONOPHYTUM MAUGHANII*

8.3.2.1 Molecular Analysis of Variance (AMOVA)

Table 8.6. Molecular analysis of variance (AMOVA) of *Conophytum maughanii* populations, indicating variance among and within populations.

	Df	SS	MS	Est. Var.	%
Among Pops	7	317.888	45.413	4.376	16%
Within Pops	34	765.638	22.520	22.520	84%
Total	41	1083.571		26.896	100%

The eight *C. maughanii* populations sampled included 229 loci, of which 99.1% (227 loci) were polymorphic. Molecular analysis of variance (AMOVA) was shown to be much greater within populations (84%) than among populations (16%) (Table 8.6).

The PCoA of the AMOVA results did not show a clear separation between the populations, but rather a spread of individuals from the populations (Fig. 8.5). Individuals of populations 128 and 130 were clustered on either side of the coordinate 2 axis, while

individuals from the remaining populations were spread across the quadrants of the PCoA (Fig. 8.5).

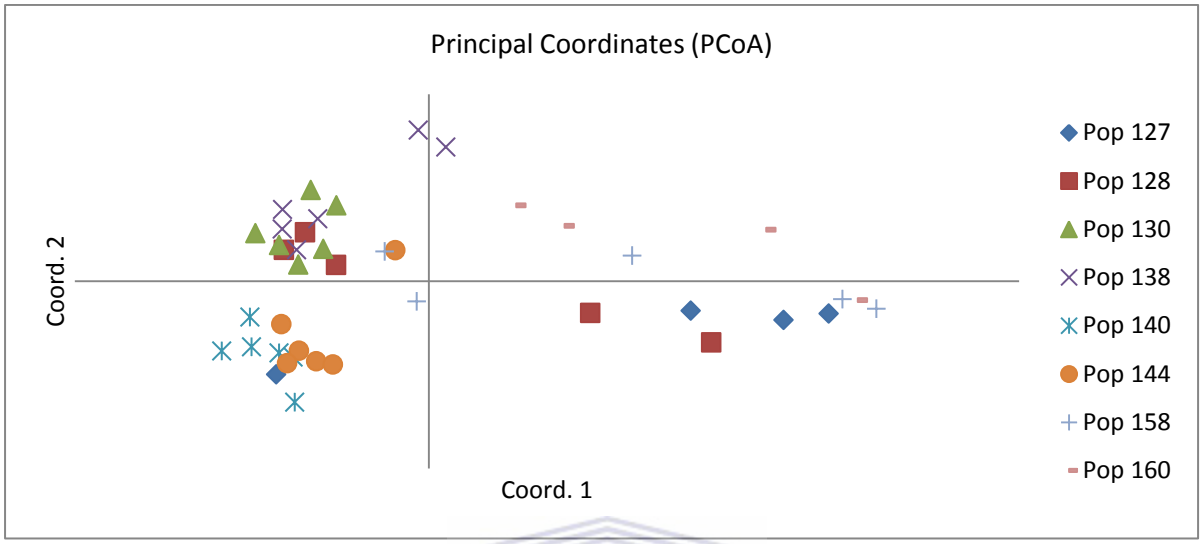

Figure 8.5. PCoA of AMOVA results for individuals in the different populations of *Conophytum maughanii*. Percentage of variance explained by axis 1: 17.93%, axis 2: 6.51%, axis 3: 5.24%.

8.3.2.3 Genetic Diversity

Table 8.7. Genetic diversity indices for the eight populations of *Conophytum maughanii* sampled: n-number of individuals; #loc.-number of loci scored; #loc_P-number of polymorphic loci at 5% level; PLP-percentage polymorphic loci at 5% level; Hj-Nei's genetic diversity.

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)
Pop127	4	229	121	52.8	0.26747	0.01704
Pop128	5	229	112	48.9	0.22889	0.01611
Pop130	6	229	82	35.8	0.16218	0.01504
Pop138	6	229	93	40.6	0.17293	0.0146
Pop140	6	229	75	32.8	0.13925	0.01386
Pop145	6	229	91	39.7	0.16133	0.01387
Pop158	5	229	103	45	0.21045	0.01598
Pop160	4	229	102	44.5	0.22858	0.01718

Genetic diversity was the greatest in population 127, with a value of 0.26747, followed by populations 128 and 160, with Hj=0.22889 and Hj=0.22858, respectively (Table 8.7). The genetic diversity indices ranged from 0.13925–0.21045 for the remaining populations. The number of polymorphic loci was also greatest in population 127 with 121 (52.8%) polymorphic loci.

8.3.2.3 Genetic Structure

Table 8.8. Values of ΔK for K of 10 iterations for populations of *Conophytum maughanii*. K=2 presents the highest ΔK value.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-3634	0.0816	NA	NA	NA
2	10	-3273	0.4082	361	367.53	900.261
3	10	-3279.53	0.4855	-6.53	4.49	9.249053
4	10	-3281.57	0.5208	-2.04	0.31	0.59525
5	10	-3283.3	0.6146	-1.73	0.08	0.130158
6	10	-3285.11	0.5466	-1.81	0.16	0.292716
7	10	-3286.76	1.0124	-1.65	0.44	0.434625
8	10	-3287.97	0.8381	-1.21	1.12	1.336431
9	10	-3290.3	1.1719	-2.33	0.45	0.383994
10	10	-3292.18	0.7729	-1.88	NA	NA

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The Bayesian clustering analysis suggested a population structure with two genetic clusters (K=2), based on the highest ΔK value (Table 8.8, Fig. 8.6A). The results of the analysis for K=3 did not provide further cluster resolution and agreed with the results of K=2 (Fig. 8.6A). All the populations of *C. maughanii* shared the green cluster with only the eastern populations (populations 127, 128, 130, 138, 158, 160) sharing the red cluster.

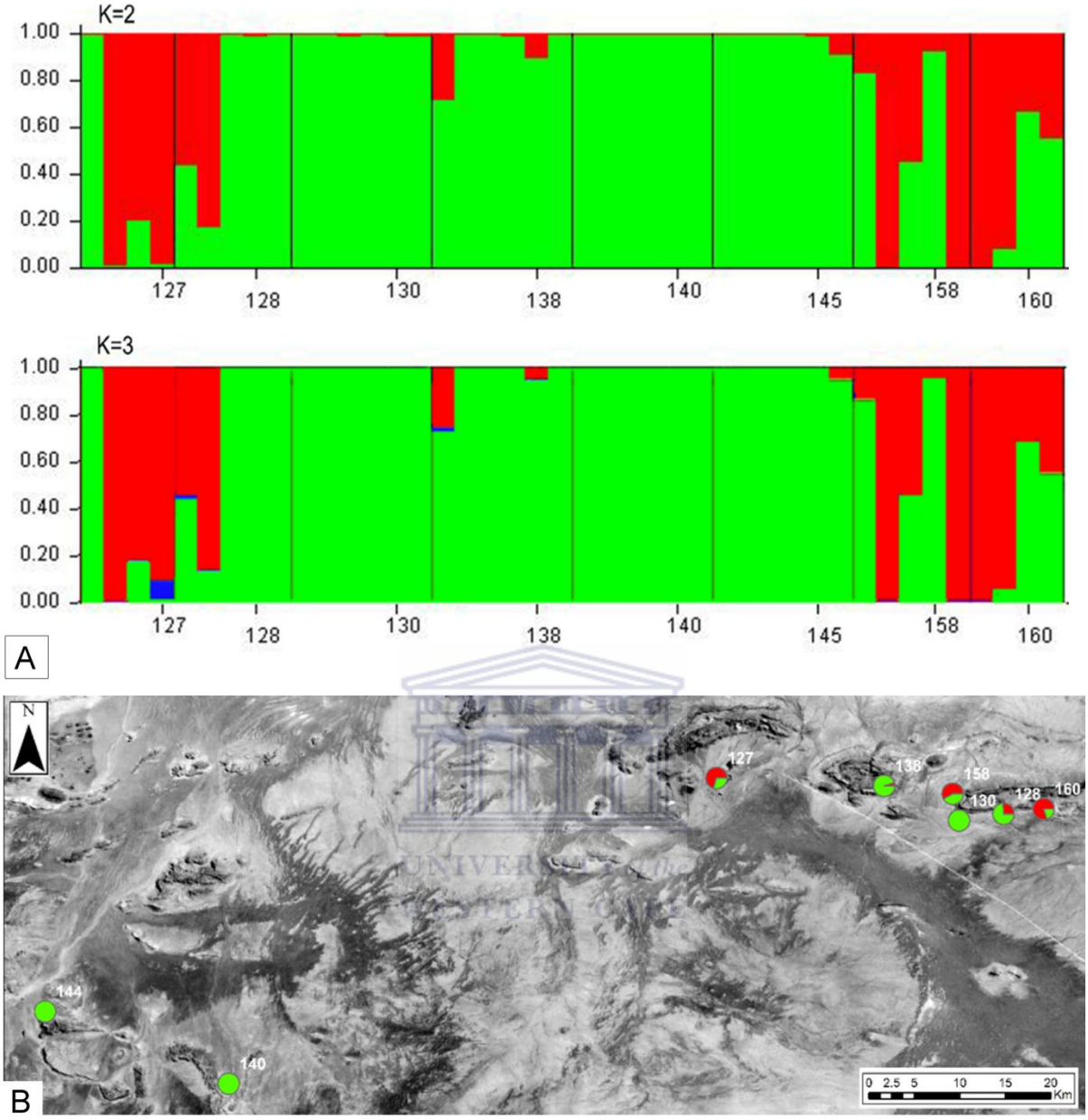

Figure 8.6. (A) Cluster membership of individuals in each of the populations of *Conophytum maughanii* at K=2 and K=3. Each cluster is assigned a colour, with solid black lines separating the populations; (B) the mapped geographic distribution of the proportion of genetic clusters in the populations of *C. maughanii*.

8.3.3 GENETIC STRUCTURE AND SEPARATION OF *C. MAUGHANII* AND *C. RATUM*

8.3.3.1 Molecular Analysis of Variance (AMOVA)

Table 8.9. Molecular analysis of variance (AMOVA) of populations of *Conophytum maughanii* and *C. ratum* and indicating variance among and within the populations of the species.

	Df	SS	MS	Est. Var.	%
Among Pops	1	1372.907	1372.907	39.947	57%
Within Pops	68	2087.821	30.703	29.170	43%
Total	69	3460.729		70.650	100%

Molecular analysis of variance (AMOVA) was shown to be much greater among the populations of *C. maughanii* and *C. ratum* (57%) than within the populations (43%) (Table 8.9).

The PCoA of the AMOVA results show that the individuals from the two species were clearly divided by the coordinate 1 axis, with individuals of *C. maughanii* positioned on the left and individuals of *C. ratum* on the right (Fig. 8.7). The individuals of *C. ratum* were further separated by the coordinate 2 axis, with the individuals from populations found on the summit of the Gamsberg in the bottom right quadrant and individual from populations at the base surrounding the Gamsberg in the top right quadrant (Fig. 8.7).

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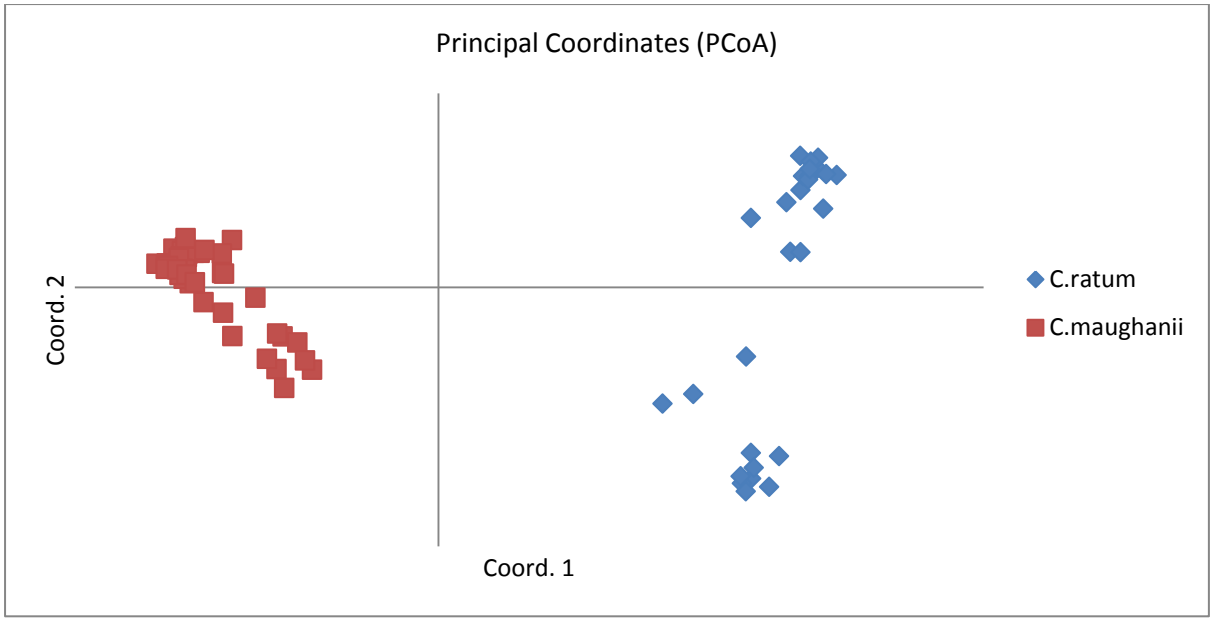

Figure 8.7. PCoA of AMOVA results for individuals of *Conophytum maughanii* and *C. ratum*. Percentage of variance explained by axis 1: 40.49%, axis 2: 8.04%, axis 3: 5.40%.

8.3.3.2 Genetic Structure

The Bayesian clustering analysis suggested a population structure with two genetic clusters (K=2), based on the highest ΔK value for the combined *C. maughanii* and *C. ratum* populations (Table 8.10). The *C. ratum* populations were assigned the red cluster, with some individuals containing a proportion of the green cluster, while *C. maughanii* populations were assigned the green cluster, with some individuals sharing a small proportion of the red cluster (Fig. 8.8).

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Table 8.10. Values of ΔK for K of 10 iterations for the combined populations of *Conophytum maughanii* and *C. ratum*. K=2 presents the highest ΔK value.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	ΔK
1	10	-8461.28	0.1398	NA	NA	NA
2	10	-6943.02	0.8574	1518.26	1018.59	1188.018
3	10	-6443.35	2.2501	499.67	294.15	130.7297
4	10	-6237.83	112.1281	205.52	158.84	1.416595
5	10	-6191.15	1.4215	46.68	51.84	36.46948
6	10	-6196.31	2.1794	-5.16	23.91	10.97079
7	10	-6225.38	83.1664	-29.07	49.2	0.591585
8	10	-6205.25	2.7383	20.13	76.09	27.78723
9	10	-6261.21	110.7276	-55.96	103.58	0.935449
10	10	-6213.59	3.8127	47.62	NA	NA

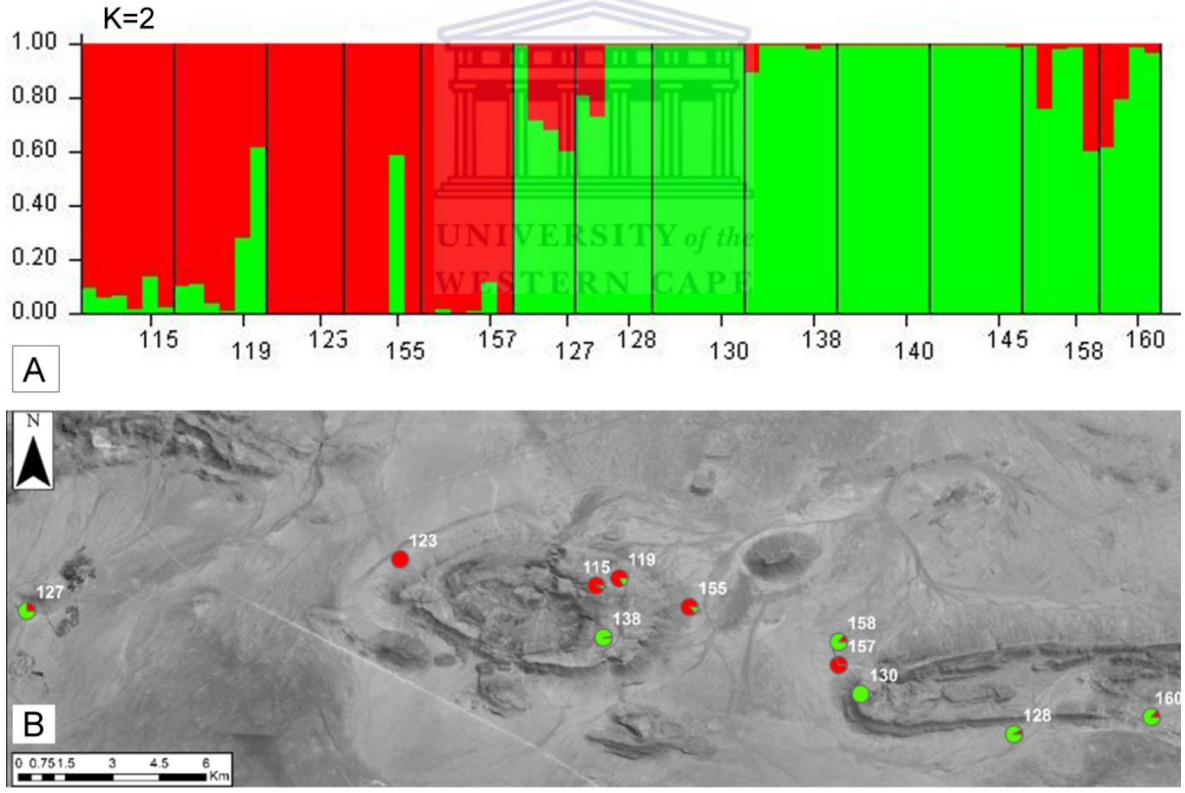

Figure 8.8. (A) Cluster membership of individuals in each of the populations of *Conophytum maughanii* (127–160) and *C. ratum* (115–157) for K=2. Each cluster is assigned a colour, with solid black lines separating the populations; (B) the mapped geographic distribution of the proportion of genetic clusters in the populations of *C. maughanii* (mostly green) and *C. ratum* (mostly red) where the populations both occur.

8.3.4 *CONOPHYTUM MARGINATUM*

8.3.4.1 Molecular Analysis of Variance (AMOVA)

Table 8.11. Molecular analysis of variance (AMOVA) of populations of *Conophytum marginatum*, indicating variance among and within populations.

	df	SS	MS	Est. Var.	%
Among Pops	10	1020.384	102.038	12.133	25%
Within Pops	48	1780.667	37.097	37.097	75%
Total	58	2801.051		49.230	100%

A total of 500 loci were amplified and scored for the eleven *C. marginatum* populations, with 96.2% (481 loci) of the loci polymorphic. Molecular analysis of variance (AMOVA) was shown to be much greater within populations (75%) than among populations (25%) (Table 8.11).

The PCoA of the AMOVA results revealed no clear groupings of populations in *C. marginatum*, although there was some separation between a cluster formed by individuals of population 116, 145 and 150 (Fig. 8.9). Population 120 and 147 showed individuals clustered on the coordinate 1 axis (Fig. 8.9).

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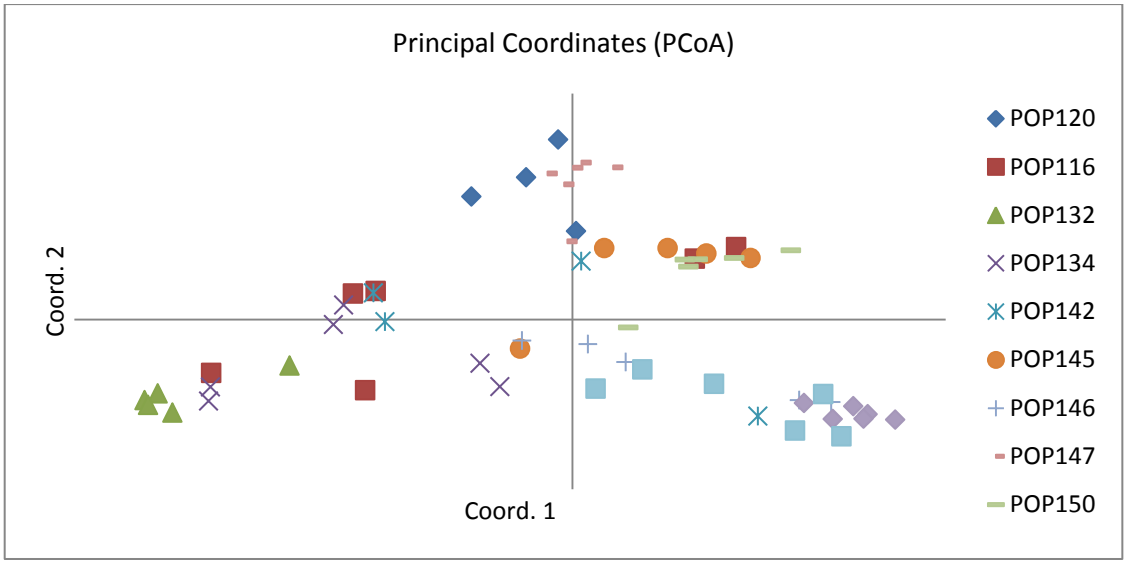

Figure 8.9. PCoA of AMOVA results for individuals in the different populations of *Conophytum marginatum*. Percentage of variance explained by axis 1: 11.32%, axis 2: 8.97%, axis 3: 7.93%.

8.3.4.2 Genetic Diversity

Genetic diversity was greatest in population 142, with an Hj value of 0.19531, followed by populations 116 and 134, with Hj=0.18628 and Hj=0.16580, respectively (Table 8.12). Genetic diversity for the remaining populations ranged from 0.15620–0.1036 (Table 8.12). The number of polymorphic loci was greatest in population 116 with 220 (44%) polymorphic loci.

Table 8.12. Genetic diversity indices for the eleven populations of *Conophytum marginatum* sampled: n-number of individuals; #loc.-number of loci scored; #loc_P-number of polymorphic loci at 5% level; PLP-percentage polymorphic loci at 5% level; Hj-Nei's genetic diversity.

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)
POP116	6	500	220	44	0.18628	0.00993
POP120	4	500	106	21.2	0.11031	0.00964
POP132	5	500	116	23.2	0.10368	0.00867
POP134	6	500	184	36.8	0.16580	0.01012
POP142	4	500	189	37.8	0.19531	0.01139
POP145	5	500	144	28.8	0.12826	0.00928
POP146	5	500	161	32.2	0.15014	0.01005
POP147	6	500	144	28.8	0.12457	0.00917
POP150	6	500	150	30	0.12496	0.00893
POP152	6	500	157	31.4	0.12743	0.00884
POP154	6	500	181	36.2	0.15620	0.00974

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8.3.4.3 Genetic Structure

The Bayesian clustering analysis suggested a population structure with two genetic clusters (K=2), based on the highest ΔK value (Table 8.13, Fig. 8.10A). The results of the population structure of K=3, however, provided an additional grouping with improved resolution (Fig. 8.10A) and as such was used for interpretation of the genetic structure for the *C. marginatum* populations (Fig. 8.10B). There was no clear geographical grouping of populations across the

distribution of the species, with all populations including a green allele cluster (Fig. 8.10B). The red allele cluster was shared by the populations in the west, but is also found in a population in the east (Pop 116) on Gamsberg (Fig. 8.10B). The blue allele cluster was dominant in the populations in the northern extent of the distribution (Pop 146, 152 & 154) (Fig. 8.10B).

Table 8.13. Values of ΔK for K of 10 iterations for populations of *Conophytum marginatum*. K=2 presents the highest ΔK value, with K=3 presenting the second highest ΔK value.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-9631.36	0.1578	NA	NA	NA
2	10	-9081.09	40.5822	550.27	372.91	9.188993
3	10	-8903.73	75.0541	177.36	210.69	2.807175
4	10	-8937.06	149.0256	-33.33	183.88	1.233882
5	10	-8786.51	205.8786	150.55	157.96	0.767248
6	10	-8793.92	159.9023	-7.41	423.27	2.647053
7	10	-9224.6	1534.841	-430.68	796.86	0.519181
8	10	-8858.42	196.1328	366.18	382.55	1.950464
9	10	-8874.79	162.011	-16.37	141.6	0.874015
10	10	-9032.76	398.2056	-157.97	NA	NA

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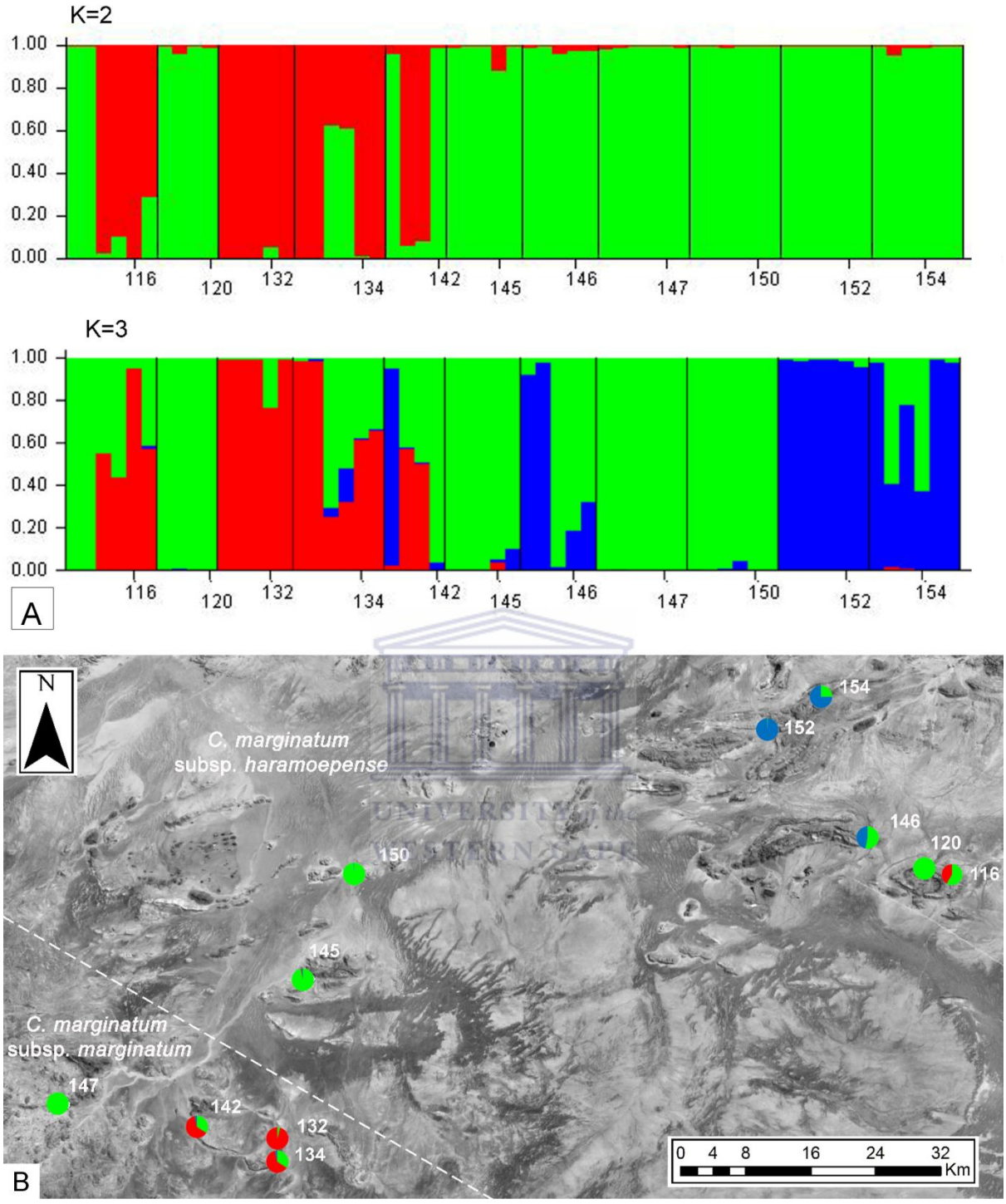

Figure 8.10. (A) Cluster membership of individuals in each of the populations of *Conophytum marginatum* at K=2 and K=3. Each cluster is assigned a colour, with solid black lines separating the populations. (B) The mapped geographic distribution of the proportion of genetic clusters in the populations of *C. marginatum* for K=3. The white dotted line indicates the separation of populations of *C. marginatum*, with subsp. *marginatum* in the west and subsp. *haramoepense* in the east.

8.4 DISCUSSION

8.4.1 *CONOPHYTUM RATUM*

8.4.1.1 Population dynamics and seed dispersal

The AFLP results suggest that the dispersal of seed and population dynamics of *C. ratum* are directly influenced by the drainage pattern. The species is endemic to fine grain quartz patches found on the summit and surrounding base of the Gamsberg (Figs. 8.2; 8.4B) (Young & Desmet, 2016) with the seeds dispersed from the summit populations (115 & 119) to the base populations (123, 155 & 157) along the drainage lines (Fig. 8.4B, C). The pattern of dispersal is illustrated by the genetic structure of the populations (Figs. 8.3, 8.4), with the base populations (123, 155 & 157) sharing the same allele clusters with the summit populations (115 &119) (Fig. 8.4). The relationship between the summit population 119 and population 155 clearly shows the connectivity and direction of gene flow between the summit and base populations, with population 155 located directly at the base of the slope upon which population 119 is situated. These populations are genetically very similar, sharing both the red and green allele clusters, albeit in different proportions (Fig. 8.4B). The difference in proportion of allele clusters between the populations (119 & 155) may be the result of additional genetic material transferred from the summit population 115 to 155, as a drainage line also connects these two populations. The genetic connectivity between populations 119 and 155 is also recovered in the PCoA of the AMOVA results, with three potential hybrid individuals (forming a separate cluster in the PCoA) (Smith & Waterway, 2008) recovered in these populations. The higher genetic diversity and greater number of polymorphic loci in the summit populations than in the base populations (Table 8.4), suggests putative gene flow from the summit to the base of the inselberg (Loveless & Hamrick, 1984). This direction of gene flow is further supported by the large population size of the summit populations (>100 individuals) compared to the base populations (<20 individuals) (Loveless & Hamrick, 1984).

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8.4.1.2 Stable state and transient founder populations

Although the BIR is characterised by low very low, erratic rainfall (<100 mm annual rainfall) and high temperatures (mean maximum of 38°C) (Desmet, 2000; Mucina *et al.*, 2006), the summit populations occupy a more hospitable habitat on the top of the Gamsberg which often intercepts moisture from fog and cloud that moves across the landscape (Matimati, 2012; Desmet, 2013). The base populations however, are susceptible to the more unfavourable lowland environmental conditions, resulting in a higher rate of mortality in these populations and possible subsequent extinction. The populations which could not be relocated at the base of the Namiesberg (Fig. 8.2) were susceptible to the unfavourable lowland conditions and therefore the fact that they have not been found over the last 10 years may suggest that these populations are extinct.

The high genetic diversity and differences in genetic structure (Tables 8.1–8.5; Fig. 8.3–8.5) indicate that the summit populations (155 & 199) are stable populations (Loveless & Hamrick, 1984). These two summit populations are the most genetically distinct (Fig. 8.4B) despite positioned within 500 m of one another (Fig. 8.4). In contrast, the base populations share a very similar genetic structure (Fig. 8.4B). The poor differentiation of genetic structure in the base populations may suggest high rates of mortality (Wade & McCauley, 1988). As a result of the putative gene flow and favourable conditions on the summit of the Gamsberg, the summit populations are predicted to follow a stable state population model, with population size increasing at the rate λ until it reaches a certain number of individuals, reaching a stable state (Fig. 8.11A) (Caswell, 2007). The base populations, however, have potentially high mortality rates due to the unfavourable lowland conditions experienced and as a result there is a continual decrease in population size in these populations (Fig. 8.11B).

The AFLP results (Tables 8.1–8.4; Figs 8.3–8.5) therefore suggest that the stable summit populations sustain the base populations through seed dispersal down the drainage

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lines during rainfall events. These base populations, situated in the unfavourable lowland habitat experience a continual decrease in population size, but are replenished and sustained by seed dispersed from the summit populations. However, after a long period of insufficient rainfall, these base populations could become severely depleted (as is the case in the lost Namies populations) (Fig. 8.2), only to be re-established again from seed from the summit populations during the next rainfall event. The summit populations are therefore crucial to the survival of the species.

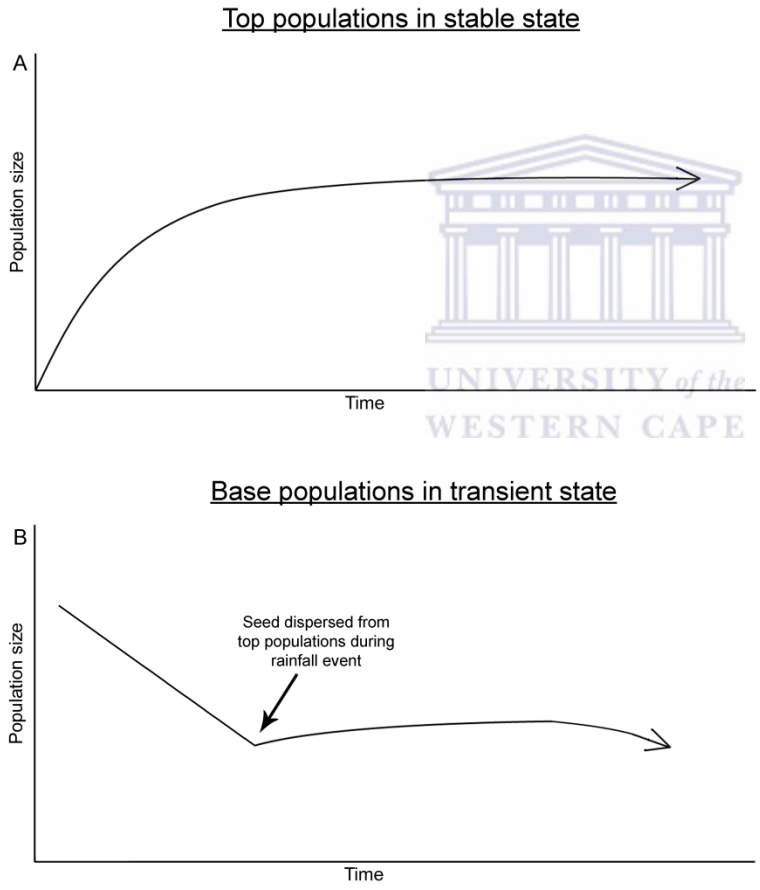

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Figure 8.11. (A) Stable state population model as is found in the summit populations of *Conophytum ratum.* (B) Population model of the base populations of *C. ratum*, indicating a continual steady decline in population size due to unfavourable environmental conditions, with increase in number of individuals after a rainfall event and then subsequent decline after a period of time.

8.4.2 *CONOPHYTUM MAUGHANII*

8.4.2.1 Population dynamics and seed dispersal

As *C. maughanii* occupies the same quartz flat habitat on the summit and surrounding base of the inselbergs as *C. ratum,* the population dynamics of this species is thought to follow the same pattern as *C. ratum,* with seeds released from the hygrochastic capsules during a rainfall event moving down the slope to establish populations at the base of the inselbergs (Figs. 8.2, 8.6). The assessment of population dynamics of *C. maughanii* was limited due to the number of samples that were not relocated. However, it can be assumed that the *C. maughanii* populations at the summit of the inselbergs sustain the base populations through seed dispersal down the slope during rainfall events.

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8.4.2.2 Genetic connectivity across the disjunction in distribution

The disjunction in the distribution of *C. maughanii,* with populations in the west and east, but not in the centre of the BIR, may either be attributed to poor sampling or unavailable habitat. Although the inselbergs have been visited by a number of collectors, the species are often cryptic, hidden by a white sheath among the white quartz stones and therefore could possibly have been missed. However, as *C. maughanii* occupies the quartz patch habitat, found at the base of the inselbergs, the disjunction may more likely be due to the unavailable habitat in the centre of the BIR with only a few very small inselbergs found in this part of the region. The genetic structure results recovered no genetic separation between the western and eastern populations, with all populations sharing the green allele cluster (Fig. 8.6). However, the western populations, as well as population 130 in the east, do not share the red allele cluster with the majority of the eastern populations. The genetic connectivity between the geographically separated east and west populations may be linked to the pollinator of *C. maughanii*. Observational studies on pollinators in *Conophytum* (Liede *et al.,* 1991; Jürgens

& Witt, 2014) did not include *C. maughanii*, however, based on the pollination syndrome of the species, with nocturnal, scented, white flowers, the species is mostly likely moth-pollinated (Chapters 6 & 7). Another explanation for the genetic connectivity between the east and west populations is the proposed mechanism of wind dispersal in *Conophytum* (Chapter 7), with the dispersal of seeds through strong winds across to the east or west populations, bridging the gap between these populations and thus maintaining genetic connectivity between them.

8.4.3 GENETIC SEPARATION OF *C. MAUGHANII* AND *C. RATUM*

Conophytum maughanii and *C. ratum* are often found co-occurring on fine gravel quartz patches around the Gamsberg (Fig. 8.1B). These species were also recovered as sister taxa in the phylogenetic analyses of the genus *Conophytum* (Chapter 6). The AFLP results of the combined populations of *C. maughanii* and *C. ratum* showed that the species are clearly separated from one another, with their own dominant genetic allele structures (Fig. 8.8). Although there is some sharing of the allele clusters in some individuals between the two species (Fig. 8.8), this is negligible, and expected as a result of the species sister relationship (Chapter 6). The AMOVA results also show that the variation between the populations (57%) is greater than the variation within the populations (43%) (Table 8.9) with a clear separation of individuals of the species in the PCoA of the AMOVA results (Fig. 8.7).

Although the two species are found together around the Gamsberg (Desmet, 2000), *C. maughanii* has a wider distribution, occurring across the BIR, while *C. ratum* is restricted to the fine-grain quartz patches of the Gamsberg (Fig. 8.2A). The species also differ in floral morphologies, with *C. maughanii* possessing white, scented flowers opening in the evening in mid- to late autumn (mid-April to May), while the flowers in *C. ratum* are pink and scented, opening in the afternoon in early to mid-autumn (March to mid-April). These

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differences in floral morphologies represent a shift in phenology and anthesis between the species (Hammer, 2002; Chapters 6 & 7).

Although there are many characters that distinguish *C. maughanii* and *C. ratum* from one another (Hammer, 2002; Chapters 6 & 7), these two sister species (*C. maughanii* and *C. ratum*) sometimes co-occur and may be easily confused, especially early in the season when not in flower. The AFLP results (Tables 8.9, 8.10; Figs. 8.7, 8.8) however, indicate that these two sister taxa are distinct reproductively isolated species with no confusion in the sampling strategy used here.

8.4.4 *CONOPHYTUM MARGINATUM*

8.4.4.1 Genetic connectivity across populations

Conophytum marginatum subsp. *haramoepense* and *C. marginatum* subsp. *marginatum* are distributed across the BIR, with subsp. *marginatum* found in the west and subsp. *haramoepense* found in the east (Fig. 8.2). Both subspecies occupy the same habitat, growing in crevices of large quartz outcrops on the summits of the inselbergs, above 800 m in altitude. As the populations are only found at the summits of inselbergs, with no suitable habitat between inselbergs, the populations are geographically isolated from one another, with the inselbergs acting as islands across the BIR. Due to the geographic isolation of the populations, little genetic connectivity between the populations was expected, however, the AFLP results indicate the contrary (Table 8.11; Figs. 8.9, 8.10).

The genetic structure results, inferring two genetic allele clusters (K=2), showed nearly no difference in genetic structure (Fig. 8.10B), while the results inferring three genetic allele clusters (K=3), which was analysed for improved resolution, only revealed an additional grouping of the northern populations (145, 152 & 154) (Fig. 8.10B). Although this additional grouping corresponds to the geographic distribution, there was no further indication of

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directionality of gene flow across the populations. The genetic similarity of the populations across the inselbergs was further supported by the AMOVA results, with variance within populations much greater than variance between populations (Table 8.11) and the PCoA recovering no clear groupings or separation of individuals across the populations (Fig. 8.9).

The genetic connectivity of the populations was further demonstrated in the two subspecies, with the populations of *C. marginatum* subsp. *haramoepense* in the east and *C. marginatum* subsp. *marginatum* in the west showing no clear genetic distinction (Fig. 8.10). Although these subspecies are distinguished by their leaf morphology (Hammer, 2002), the population genetic results indicate that these subspecies are not as distinct as previously thought and perhaps a reassessment of their taxonomic ranking is required.

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8.4.4.2 Population dynamics and seed dispersal

The geographically isolated populations of *C. marginatum* might be regarded as refugial populations, with the genetic connectivity between the populations attributed to an expanded historical distribution for the species. However, *C. marginatum* is restricted to quartz outcrops which caps the BIR inselbergs and are not present further down the slopes or on the plains. As a result it seems unlikely that the species could have had an expanded historical distribution as the species currently occupies the entire available habitat in the region (Young & Desmet, 2016).

The genetic connectivity between the geographically isolated populations of *C. marginatum* may be explained by the pollinator of the species. Pollination observations of *Conophytum* was conducted by Jürgens & Witt (2014) who observed that flowers of *C. marginatum* were regularly visited by pollen wasps (*Quartinia* sp.) and that they were likely to be the main pollinators of this species. Pollen wasps, however, are thought not to travel more than 100 m between populations (Jürgens & Witt, 2014) and so it is unlikely that this

potential pollinator could visit all the populations across the distribution range of approximately 95 km. Also the fact that the proportion of genetic allele clusters included in each of the *C. marginatum* populations is different indicates that the pollination cannot solely explain the genetic connectivity between the populations of this species, as populations that are visited by the same pollinators usually have a homologous genetic structure (Eguiarte *et al.,* 1992).

Finally, the genetic connectivity of the *C. marginatum* populations may be linked to the proposed wind dispersal of the soft and papery capsules of *Conophytum,* as postulated in Chapter 7. As successful seed dispersal into a suitable habitat is considered a random event (Peres & Baider, 1997; Nathan & Muller-Landau, 2000), populations of wind dispersed species exhibit differences in genetic structure, as found in *C. marginatum.* The proposed wind dispersed capsules also provide an explanation for how the populations are able to occupy the often inaccessible habitat of the summits of the inselbergs.

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8.4.5 Population Dynamics and Different Dispersal Mechanisms of Endemic *Conophytum* Species across the Bushmanland Inselberg Region

Two different mechanism of dispersal are prevalent in the investigated *Conophytum* species. The population dynamics of *C. maughanii* and *C. ratum* are directly influenced by drainage, while in *C. marginatum* the wind dispersed capsules resulted in low genetic differentiation between the populations. Although the drainage pattern was shown to directly influence the population dynamics of *Argyroderma* (Ellis *et al.,* 2007), populations separated by drainage lines, were genetically different (Ellis *et al.,* 2007). In contrast in *C. maughanii* and *C. ratum,* there was little genetic separation between populations across drainage lines (Figs. 8.4, 8.6). The influence of drainage patterns is not relevant in *C. marginatum* as there is no suitable available habitat lower down along the drainage lines. Rather, the wind dispersal of the

capsules is more evident in the population genetic results of *C. marginatum* (Fig. 8.10). The capsules of *C. maughanii* and *C. ratum* may also be dispersed by wind, as they share the same capsule type with *C. marginatum*, which may explain how populations are able to colonise the summit of the inselbergs, specifically in the case of *C. maughanii*, which has a relatively wide distribution (Fig. 8.6); however, the influence of the drainage pattern on the population dynamics of *C. ratum* and *C. maughanii* is more evident in these species.

The results of the AFLP analyses of the selected endemic *Conophytum* species provides for the first time an understanding of the population dynamics of species in the genus, as well as the dynamics of species in the BIR. However, it is important to note that the sampling size of the study is relatively small due to the limited distribution and few populations of the studied species, and therefore the interpretation of some of the results may be exaggerated due to the small sample size. Nevertheless, this study provides essential data to understand and assess the dynamics of species in the BIR.

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8.4.6 Conservation Recommendations for the Selected Endemic *Conophytum* Species and the Bushmanland Inselberg Region

Conophytum ratum is already regarded as a flagship species for conservation, as it is listed as vulnerable (VU) on the IUCN Red List (Victor & Desmet, 2005; Raimondo *et al.*, 2009). The results of this study provide further insight into the conservation of this species, with the populations on the summit of the Gamsberg sustaining and replenishing the base populations. The summit populations are therefore high conservation priorities as the removal of these populations may result in the extinction of this species. As the two summit populations are stable state populations, they have different genetic structures, and with regards to conservation of genetic diversity, population 119 is the highest priority (Table 8.2). However, conservation of both these populations is essential as they include the total genetic diversity

of the species, as well as providing the pool of genetic material for all the populations of the species.

Following the hypothesis of seed dispersal from the summit populations to the base populations, population 123 is currently in danger, as the genetic structure results indicate this population only includes the green allele cluster, which is different from the other base populations. Population 123 is directly at the base of the slope where the recently removed population (removed due to mining activity), was located (Fig. 8.4B). The removal of this population may be detrimental to the survival of population 123, as based on the drainage lines and elevation profile, the results show that this population was possibly sustaining the base population 123 (Fig. 8.4).

The *C. maughanii* population (population 127) at the base of Aggeneys-se-berg (Figs. 8.2, 8.6) contained the highest genetic diversity (Table 8.7) and although this population is currently included in a small conservation area just outside the mining town of Aggeneys, restriction to the area has been lax and the area has been recently disturbed by vehicles driving on the quartz patches (R. Powell, Pers. Obs.). Adequate conservation of this population should be prioritised by the mining company (Black Mountain Mining (Pty) Ltd.), with restricted access to this conservation area enforced.

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Although the populations of *C. marginatum* were recovered as genetically similar, the conservation of each of the populations is important, as there was no clear directionality of gene flow (Fig. 8.10B), and therefore it is not clear what the impact of the removal of one population on the dynamics of the species would be. As little is known about the population dynamics of species in the BIR, with this study providing insights for the first time, the impact of removal of species or the inselberg itself (e.g. through mining) is uncertain. However, as the inselbergs act as islands of Succulent Karoo, with a number of endemic or highly specific species on each of the inselbergs, each inselberg and the connectivity between

them can be assumed to be important and therefore the conservation of all the inselbergs in the region is essential.

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Chapter 9

General Conclusions

This thesis presented a multidisciplinary study assessing taxa of the *Conophytum*-clade (Aizoaceae) across a range of taxonomic levels (generic, subgeneric, species and population) and has led to new insights into the classification, evolution and ecology of species in this group of succulents.

9.1 Generic Circumscription in the *Conophytum*-clade

The number of recognised genera in the *Conophytum*-clade decreased from ten to seven following assessment of generic circumscriptions in the clade using molecular, morphological, anatomical and palynological data.

The polyphyly of *Octopoma* N.E.Br. was confirmed, with five species, together with three species of the polyphyletic *Arenifera* A.G.J.Herre, placed in the *Conophytum*-clade (Powell *et al.*, 2016; Chapter 3). These *Arenifera* and *Octopoma* species were placed sister to *Schlechteranthus* N.E.Br., and *Schlechteranthus* was expanded to include these species based on shared leaf, floral and capsule characters (Powell *et al.*, 2016; Chapter 3). The species of the expanded *Schlechteranthus* formed two morphological groupings, one including the species of *Schlechteranthus* s.s. and the other including species previously classified in *Arenifera* and *Octopoma.* These two groupings were subsequently recognised as the subgenera *Schlechteranthus* and *Microphyllus* R.F.Powell, distinguished by differences in capsule, leaf and anatomical characters. A taxonomic revision of the newly erected subgenus *Microphyllus* was presented as part of this thesis (Powell *et al.*, submitted a; Chapter 4).

An expanded phylogenetic sampling of *Cheiridopsis* N.E.Br., *Ihlenfeldtia* H.E.K.Hartmann and *Odontophorus* N.E.Br., recovered the species of *Ihlenfeldtia* and *Odontophorus* embedded in *Cheiridopsis.* The species of *Ihlenfeldtia* were placed together

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with species of *Cheiridopsis* subgenus *Aequifoliae* H.E.K.Hartmann, while the species of *Odontophorus* were recovered within the clade including species of *Cheiridopsis* subgenus *Odontophorus* H.E.K.Hartmann. *Cheiridopsis* was subsequently expanded to include species of *Ihlenfeldtia* and *Odontophorus* and these species were accommodated in the relevant subgenera based on shared capsule morphology (Powell *et al.*, submitted b; Chapter 5). Although leaf anatomical characters, specifically papillae type, was useful in grouping species of *Cheiridopsis* subgenus *Cheiridopsis,* anatomical characters did not support the phylogenetic groupings in the other subgenera (Powell *et al.*, submitted b; Chapter 5).

Leaf anatomical characters, as well as palynological characters, were also useful in the assessment of the generic statuses of the monotypic genera *Enarganthe* N.E.Br., *Jensenobotrya* A.G.J.Herre, *Namaquanthus* L.Bolus, and *Ruschianthus* L.Bolus. These monotypic genera were found to have a glabrous leaf surface, with no epidermal extensions. In contrast, the other genera of the *Conophytum*-clade all had some form of epidermal extensions (papillae or trichomes) (Powell *et al.*, submitted b; Chapter 5). In addition, the pollen structures (exine and colpi) of these genera were distinct, with each genus (*Ruschianthus* not sampled) exhibiting a unique pollen structure. The remainder of the genera in the clade had uniform tricolpate pollen with poorly defined colpi (Powell *et al.*, submitted b; Chapter 5). The anatomical and palynological characters, as well as the unique combination of morphological characters, provided an argument to maintain the generic statuses of these monotypic genera (Powell *et al.*, submitted b; Chapter 5).

Despite the large number of species and diverse morphological characters of *Conophytum* N.E.Br., the genus was recovered as monophyletic in expanded phylogenetic sampling. This agrees with the results of Klak *et al.* (2013), although only three species of *Conophytum* were sampled in the tribal phylogeny. The expanded phylogenetic sampling of the *Conophytum*-clade recovered a sister relationship between *Cheiridopsis* and *Conophytum*

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for the first time, and these sister genera share the synapomorphic character of sheathing leaves (Powell *et al.,* submitted b; Chapter 5).

The increased sampling greatly improved phylogenetic signal, with improved resolution and novel phylogenetic relationships recovered within the *Conophytum*-clade. However, due to the few phylogenetic informative characters, phylogenetic relationships were discovered to be somewhat unstable. Therefore a complete sampling was required to suitably assess relationships in the clade, with as little missing data as possible. Although previous phylogenetic analyses of the Aizoaceae (Klak *et al.,* 2003a; Thiede, 2004), have relied on nuclear data, there are a number of problems associated with these data (Klak *et al.,* 2013). Therefore recent phylogenetic analyses, specifically in the Ruschieae (Klak *et al.,* 2013; Powell *et al.,* 2016) are based only on plastid regions. Sequences for between six and nine plastid gene regions are required to resolve and obtain some level of support for clades within the Ruschieae. However, recent studies (Downie & Jansen, 2015) have used the novel Next Generation Sequencing (NGS) technique to identify possible variable markers. The application of this technique in the Aizoaceae may lead to the discovery of new variable markers, which could greatly contribute to the production of well-resolved phylogenies for the family.

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9.2 Diversity of the Speciose Genus *Conophytum*

As *Conophytum* includes diverse leaf and floral morphologies, the genus is popular among succulent collectors. However, the species only comprise of a single reduced leaf pair (some exceptions in sect. *Biloba* N.E.Br.) and are therefore often difficult to identify. Identification of species is also hindered by the poor representation of *Conophytum* in national herbarium collections, despite the large collections included in the personal greenhouse of succulent collectors and growers. However, through collaboration with these succulent collectors, the

production of the digital DELTA key for *Conophytum* provides comprehensive distribution maps for the species. These distribution maps greatly aid the identification of species, as many species in *Conophytum* are point endemics. In addition, the digital key provides images of diagnostic characters, as well as of the species in habitat or greenhouse collections. These images further aid the identification of species. Not only is the key useful identification tool for taxonomists, but also for conservation agencies in the identification of the species and assessment of species distributions. The coded morphological matrix produced during the development of the key was also used to assess the leaf and floral diversity of species in the genus.

Leaf and floral traits were assessed across the species through ancestral trait reconstruction on the phylogeny. The selected reconstructed morphological characters were shown to have evolved multiple times across the genus (Chapter 6). This was particularly interesting with regards to the floral traits, as the reconstruction showed multiple switches in floral morphologies across the genus. The unique floral morphology of *Conophytum*, with fused petaloid staminodes that form a tubular flower (Haas, 1976), seemed to have enabled the species to develop a diverse range of specialised flowers, with a variety of structures (Liede & Hammer, 1990), scents (Jürgens & Manning, 2004) and colours (Hammer, 2002). In contrast, the flowers of the remaining genera of the *Conophytum*-clade were of the typical unspecialised radiate type of the Aizoaceae (Hartmann, 1983, 1991). The floral traits in *Conophytum* were also assessed within the complex species communities, where floral morphology was shown to be diverse within a community (Chapter 7). This diversity was suggested to be as a result of competition and adaptation to pollination resources (Harder & Johnson, 2009; Schiestl & Schluter, 2009; Rymer *et al.,* 2010).

Although there have been few pollination studies done on *Conophytum* (Liede *et al.,* 1991; Jürgens & Witt, 2014), the diversity of the observed pollinators of *Conophytum* does

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not match the floral diversity of the genus. Two main pollinators of *Conophytum* have been observed, i.e. pollen wasps visiting the diurnal species and moths pollinating the nocturnal species (Liede *et al.,* 1991; Jürgens & Witt, 2014). However, a number of other flower visitors (Coleoptera, Diptera and Hymenoptera) have been recorded visiting flowers of *Conophytum.* Although it is not certain whether these other recorded visitors of *Conophytum* flowers are pollinators or coincidental visitors, the floral diversity suggests an adaptation to an array of pollinators. The possibility of additional unknown pollinators of *Conophytum* is also highlighted by the AFLP analyses. The populations of *C. marginatum* Lavis across the inselbergs of the Bushmanland Inselberg Region (BIR) indicate genetic connectivity, which is possibly related to pollination (Chapter 8). Jürgens & Witt (2014) found that flowers of *C. marginatum* are pollinated by pollen wasps, however, these pollen wasps are not thought to travel far distances (<100 m). Therefore it is uncertain whether these pollinators are able to visits the populations of *C. marginatum* approximately 25 km apart. In contrast, the two top populations of *C. ratum* are just a few meters apart, yet have different genetic structures (Chapter 8), suggesting that they are not visited by the same set of pollinators (Eguiarte *et al.,* 1992). The highly specialised flowers of *Conophytum* suggest the potential for a number of specialised pollinators. Further work on pollination is required in *Conophytum*, and the genus lends itself as an ideal group to study specialisation, as well as possibly co-evolution of floral and pollination syndromes.

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9.3 PHYLOGENETIC DIVERSITY AND DISPERSAL MECHANISMS IN *CONOPHYTUM*

Phylogenetic diversity in *Conophytum* was significantly higher than in its sister genus *Cheiridopsis,* despite the overlap in distributions of the genera and shared centre of diversity (Chapter 7). The phylogenetic diversity of *Conophytum* was also indicated in the species complex communities, with the inclusion of closely related, as well as distantly related

species (Chapter 7). The inclusion of distantly related species in the communities suggested a long distance seed dispersal mechanism in *Conophytum.* The soft, papery capsules of *Conophytum,* which easily break off the plant, were suggested to be an adaptation to wind dispersal (Chapter 7). This proposed wind dispersal of *Conophytum* species is postulated for the first time in this thesis, as previously, seeds of the species were thought to solely disperse through a wash-out mechanism (Hartmann, 1991). It is here suggested that this adaptation has enabled the species of *Conophytum* to exploit a variety of habitats, across the Greater Cape Floristic Region, resulting in the high number of species and widespread distribution of the genus.

9.4 Population Dynamics of *Conophytum* and Conservation Implications for the Genus and Family

The proposed mechanism of wind dispersal in *Conophytum* was evident in the assessment of population dynamics of *Conophytum* in the BIR, specifically in the results of *C. marginatum.* Populations of *C. marginatum* were initially suspected to be genetically distinct, as these populations are restricted to the tops of the inselbergs in the BIR, which act as islands of Succulent Karoo, surrounded by Nama Karoo vegetation (Desmet, 2000; Young & Desmet, 2016). However, the opposite was found, with populations across the inselbergs shown to be genetically similar. The indicated genetic connectivity between the populations was likely attributed to the wind dispersed capsules of the genus (Chapter 8).

However, the release of seed and establishment of populations, specifically in the species found at the tops and surrounding bases of the inselbergs (*C. maughanii* N.E.Br., *C. ratum* S.A.Hammer) were shown to be directly influenced by drainage. This finding highlighted the importance of the top populations of *C. ratum*, restricted to the Gamsberg, which sustain the base populations (Chapter 8). These top populations are of high

conservation priority, as mining of the Gamsberg has already commenced. Both the AFLP studies conducted on species of the Aizoaceae (this study, as well as Ellis *et al.* (2007)), found that population structure and genetic diversity was directly influenced by the drainage pattern. This relationship between population structure and drainage pattern is linked to the hygrochastic capsules of the species. As all members of the Aizoaceae include hygrochastic capsules, drainage may play an important role in the genetic structure of all populations in the family. This is important with regards to conservation of the family, as conservation of a single population of a species is not sufficient. It is important to assess all the populations of a species within in a region, specifically in relation to the drainage pattern, to best select populations to prioritise for conservation.

The AFLP study also revealed a possible variation in genome size of Aizoaceae. The genome size scan of *C. marginatum*, which was required to ensure the AFLP primers for the correct genome size were used, was substantially larger (10.69 pg (2C)) than the previously recorded measurement (0.85 pg (2C)) for Aizoaceae (Plant DNA C-values Database; Bennet & Leitch, 2012 (available at http://data.kew.org/cvalues/)). However, the database included only a single measurement for a species in subfamily Mesembryanthemoideae. Previous AFLP studies in Aizoaceae included both the small and regular genome size primers (Ellis *et al.*, 2007; Buys *et al.*, 2008 Kellner *et al.*, 2011), also suggesting variation across genera, although genome size was not explicitly measured in these studies. Additional measurements of genome size across the major phylogenetic lineages in the Aizoaceae may therefore reveal interesting patterns of variation across the family.

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APPENDIX A

APPENDIX A. Voucher information and GenBank numbers for sequence data.

APPENDIX A1. Voucher information and GenBank (https://www.ncbi.nlm.nih.gov/genbank/) reference numbers for new accessions from which cpDNA sequence data were obtained for *Arenifera*, *Leipoldtia* and *Octopoma* presented in Chapter 3 (published in Powell *et al.*, 2016; Appendix E1). The information is listed as follows: *taxon: atpB–rbcL*, *psbJ–petA*, *matK*, *rpl16*, *rps16*, *trnD–trnT*, *trnL–F*, *trnQ–rps16*, *trnS–trnG;* voucher information.

Arenifera A.G.J.Herre: *A. pillansii* (L.Bolus) A.G.J.Herre: KT248239, KT248248, KT248260, KT248267, KT248278, KT248290, KT248302, KT248314, KT248326; *Bruyns 9136* (BOL). *A. pungens* H.E.K.Hartmann: KT248240, KT248249, KT281447, —, KT248279, KT248291, KT248303, KT248315, KT248327; *Powell 28* (NBG). *A.* sp.nov.: —, KT248251, KT281448, KT248269, KT248281, KT248293, KT248305, KT248317, KT248329; *Powell 41* (NBG). *A. spinescens* (L.Bolus) H.E.K.Hartmann: KT248241, KT248250, KT248261, KT248268, KT248280, KT248292, KT248304, KT248316, KT248328; *Klak 2424* (BOL). *A. stylosa* (L.Bolus). H.E.K.Hartmann: KT248242, KT248252, KT248262, KT248270, KT248282, KT248294, KT248306, KT248318, KT248330; *Powell 75* (NBG). ***Leipoldtia*** L.Bolus: *L. gigantea* Klak: KT248243, KT248253, KT248263, KT248271, KT248283, KT248295, KT248307, KT248319, KT248331; *Klak 1275* (BOL). *L. sp.* (undescribed): KT248244, KT248254, KT248264, KT248272, KT248284, KT248296, KT248308, KT248320, KT248332; *Klak 2406* (BOL). ***Octopoma*** N.E.Br.: *O. abruptum* (L.Bolus) L.Bolus: KT248246, KT248258, KT281451, KT248276, KT248288, KT248300, KT248312, KT248324, KT248335; *Powell 5* (NBG). *O. connatum* (L.Bolus) L.Bolus: —, KT248255, —, KT281449, KT248273, KT248285, KT248297, KT248309, KT248321; *Powell 10* (NBG). *O. inclusum* N.E.Br.: —, KT248256, KT281450, KT248274, KT248286, KT248298, KT248310, KT248322, KT248333; *Powell 35* (NBG). *O. nanum* (L.Bolus) Klak: KT248245, KT248257, KT248265, KT248275, KT248287, KT248299, KT248311, KT248323, KT248334; *Klak 2426* (BOL). *O. tetrasepalum* (L.Bolus) H.E.K.Hartmann: KT248247, KT248259, KT248266, KT248277, KT248289, KT248301, KT248313, KT248325, KT248336; *Klak 2411* (BOL).

APPENDIX A2. Voucher information and GenBank (https://www.ncbi.nlm.nih.gov/genbank/) reference numbers for new accessions from which cpDNA sequence data were obtained for *Cheiridopsis*, *Conophytum*, *Ihlenfeldtia* and *Odontophorus* presented in Chapters 5 and 6. The information is listed as follows: *taxon –matK*, *rpl16*, *rps16*, *trnL–F*, *trnQ–rps16*, *trnS–trnG;* voucher information.

Cheiridopsis N.E.Br.: *C. acuminata* L.Bolus: KY631845, —, KY635099, KY635179, KY635259, KY635339; *Powell 68* (NBG). *C. alba-oculata* Klak & Helme: KY631846, —, —, KY635180, KY635260, KY635340; *Klak 2308* (BOL). *C. aspera* L.Bolus: KY631847, KY635021, KY635100, KY635181, KY635261, KY635341; *Powell 14* (NBG). *C. brownii* Schick & Tisch.: KY631848, KY635022, KY635101, KY635182, KY635262, KY635342; *Powell 61* (NBG). *C. caroli-schmidtii* N.E.Br.: KY631849, —, KY635102, KY635183, KY635263, KY635343; *Powell 105* (NBG). *C. denticulata* N.E.Br.: KY631850, KY635023, KY635103, KY635184, KY635264, KY635344; *Powell 9* (NBG). *C. derenbergiana* Schwantes: KY631851, KY635024, KY635104, KY635185, KY635265, KY635345; *Powell 79* (NBG). *C. gamoepensis* S.A.Hammer: KY631852, KY635025, KY635105, KY635186, KY635266, KY635346; *Powell 89* (NBG). *C. glomerata* S.A.Hammer: KY631853, KY635026, KY635106, KY635187, KY635267, KY635347; *Powell 96* (NBG). *C. herrei* L.Bolus: KY631854, KY635027, KY635107, KY635188, KY635268, KY635348; *Powell 100* (NBG). *C. meyeri* N.E.Br.: KY631855, KY635028, KY635108, KY635189, KY635269, KY635349; *Powell 16* (NBG). *C. minor* (L.Bolus) H.E.K.Hartmann: KY631856, KY635029, KY635109, KY635190, KY635270, KY635350; *Powell 94* (NBG). *C. namaquensis* (Sond.) H.E.K.Hartmann: KY631857, KY635030, KY635110, KY635191, KY635271, KY635351; *Powell 11* (NBG). *C. peculiaris* N.E.Br.: KY631858, KY635031, KY635111, KY635192, KY635272, KY635352; *Powell 15* (NBG). *C. pillansii* L.Bolus: KY631859, KY635032, KY635112, KY635193, KY635273, KY635353; *Powell 45* (NBG). *C. pilosula* L.Bolus: KY631860, KY635033, KY635113, KY635194, KY635274, KY635354; *Powell 69* (NBG). *C. ponderosa* S.A.Hammer: KY631861, KY635034, KY635114, KY635195, KY635275, KY635355; *Powell 103* (NBG). *C. purpurea* L.Bolus: KY631862, KY635035, KY635115, KY635196, KY635276, KY635356; *Powell 85* (NBG). *C. robusta* N.E.Br.: KY631863, KY635036, KY635116, KY635197, KY635277, KY635357; *Powell 66* (NBG). *C. rostrata* N.E.Br.: KY631864, KY635037, KY635117, KY635198, KY635278, KY635358; *Powell 88* (NBG). *C. speciosa* L.Bolus: KY631865, KY635038, KY635118, KY635199, KY635279, KY635359; *Powell 107* (NBG). *C. umdausensis* L.Bolus: KY631866, KY635039,

KY635119, KY635200, KY635280, KY635360; *Powell 102* (NBG). *C. verrucosa* L.Bolus: KY631867, KY635040, KY635120, KY635201, KY635281, KY635361; *Powell 99* (NBG). ***Conophytum*** N.E.Br.: *C. achabense* S.A.Hammer: KY631789, KY634965 , KY635043 , KY635123, KY635204, KY635284; *Powell 131* (NBG). *C. angelicae* N.E.Br.: KY631790, KY634966, KY635044, KY635124, KY635205, KY635285; *Bruyns 9479* (NBG). *C. auriflorum* Tisch.: KY631791, KY634967, KY635045, KY635125, KY635206, KY635286; *Mitchell 555* (KBG). *C. bilobum* (Marloth) N.E.Br.: KY631792, KY634968, KY635046, KY635126, KY635207, KY635287; *Powell 19* (NBG). *C. bolusiae* Schwantes: KY631793, KY634969, KY635047, KY635127, KY635208, KY635288; *Mitchell 578* (KBG). *C. breve* N.E.Br.: KY631794, KY634970, KY635048, KY635128, KY635209, KY635289; *Rawé 1162* (NBG). *C. burgeri* L.Bolus: KY631795, KY634971, KY635049, KY635129, KY635210, KY635290; *Donor s.n.* (KBG). *C. caroli* Lavis: KY631796, KY634972, KY635050, KY635130, KY635211, KY635291; *Sauer 88/1004* (NBG). *C. chauviniae* (Schwantes)S.A.Hammer: KY631797, KY634973, KY635051, KY635131, KY635212, KY635292; *Mitchell 1086* (KBG). *C. comptonii* N.E.Br.: KY631798, KY634974, KY635052, KY635132, KY635213, KY635293; *Harrower 5009* (NBG). *C. concavum* L.Bolus: KY631799, KY634975, KY635053, KY635133, KY635214, KY635294; *Lavranos s.n.* (KBG). *C. concordans* G.D.Rowley: KY631800, KY634976, KY635054, KY635134, KY635215, KY635295; *Harrower s.n.* (NBG). *C. depressum* Lavis: KY631801, KY634977, KY635055, KY635135, KY635216, KY635296; *Powell 53* (NBG). *C. ectypum* N.E.Br.: KY631802, KY634978, KY635056, KY635136, KY635217, KY635297; *Meyer 99A* (NBG). *C. ernstii* S.A.Hammer: KY631803, KY634979, KY635057, KY635137, KY635218, KY635298; *Van Jaarsveld 8512* (NBG). *C. ficiforme* N.E.Br.: KY631804, KY634980, KY635058, KY635138, KY635219, KY635299; *Powell 3* (NBG). *C. flavum* N.E.Br.: KY631805, KY634981, KY635059, KY635139, KY635220, KY635300; *Powell 57* (NBG). *C. friedrichiae* Schwantes: KY631806, KY634982, KY635060, KY635140, KY635221, KY635301; *KBG 312/89* (KBG). *C. frutescens* Schwantes: KY631807, KY634983, KY635061, KY635141, KY635222, KY635302; *Hammer 679* (NBG). *C. fulleri* L.Bolus: KY631808, KY634984, KY635062, KY635142, KY635223, KY635303; *Mitchell 966* (KBG). *C. globosum* N.E.Br.: KY631809, KY634985, KY635063, KY635143, KY635224, KY635304; *Mitchell 1200* (KBG). *C. hermarium* (S.A.Hammer)S.A.Hammer: KY631810, KY634986, KY635064, KY635144, —, —; *Powell 32*(NBG). *C. herreanthus* S.A.Hammer: KY631811, KY634987, KY635065, KY635145, KY635225, KY635305; *KBG 103/91* (KBG). *C. hians* N.E.Br.: KY631812, KY634988, KY635066, KY635146, KY635226,

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KY635306; *Harrower 3246* (NBG). *C. joubertii* Lavis: KY631813, KY634989, KY63506, KY635147, KY635227, KY635307; *Barnhill & Hammer 2395* (NBG). *C. jucundum* (N.E.Br.)N.E.Br.: KY631814, KY634990, KY635068, KY635148, KY635228, KY635308; *Wisura 1589* (NBG). *C. khamiesbergense* (L.Bolus)Schwantes: KY631815, KY634991, KY635069, KY635149, KY635229, KY635309; *Klak 2402* (BOL). *C. klinghardtense* Rawé: KY631816, KY634992, KY635070, KY635150, KY635230, KY635310; *Williamson 4614* (KBG). *C. limpidum* S.A.Hammer: KY631817, KY634993, KY635071, KY635151, KY635231, KY635311; *Hammer 385* (NBG). *C. lithopsoides* L.Bolus: KY631818, KY634994, KY635072, KY635152, KY635232, KY635312; *Powell 29* (NBG). *C. longum* N.E.Br.: KY631819, KY634995, KY635073, KY635153, KY635233, KY635313; *Mitchell 738* (KBG). *C. luckhoffii* Lavis: KY631820, KY634996, KY635074, KY635154, KY635234, KY635314; *Harrower 4211* (NBG). *C. marginatum* Lavis: KY631821, KY634997, KY635075, KY635155, KY635235, KY635315; *Powell 20* (NBG). *C. meyeri* N.E.Br.: KY631822, KY634998, KY635076, KY635156, KY635236, KY635316; *Powell 16* (NBG). *C. minutum* N.E.Br.: KY631823, KY634999, KY635077, KY635157, KY635237, KY635317; *Harrower 102* (NBG). *C. obcordellum* N.E.Br.: KY631824, KY635000, KY635078, KY635158, KY635238, KY635318; *Magee 1058* (NBG); *C. pageae* N.E.Br.: KY631825, KY635001, KY635079, KY635159, KY635239, KY635319; *Magee 1059* (NBG). *C. pellucidum* Schwantes: KY631826, KY635002, KY635080, KY635160, KY635240, KY635320; *Harrower 3628* (NBG). *C. phoeniceum* S.A.Hammer: KY631827, KY635003, KY635081, KY635161, KY635241, KY635321; *Hammer 1212* (NBG). *C. piluliforme* (N.E.Br.)N.E.Br.: KY631828, KY635004, KY635082, KY635162, KY635242, KY635322; *Bayer s.n.* (KBG). *C. quaesitum* N.E.Br.: KY631829, KY635005, KY635083, KY635163, KY635243, KY635323; *Hammer 909* (NBG). *C. ratum* S.A.Hammer: KY631830, KY635006, KY635084, KY635164, KY635244, KY635324; *Powell 24* (NBG). *C. regale* Lavis: KY631831, KY635007, KY635085, KY635165, KY635245, KY635325; *Bayer 1081* (KBG). *C. roodiae* N.E.Br.: KY631832, KY635008, KY635086, KY635166, KY635246, KY635326; *Powell 51* (NBG). *C. saxetanum* N.E.Br.: KY631833, KY635009, KY635087, KY635167, KY635247, KY635327; *Wisura 1600* (NBG). *C. smorenskaduense* de Boer: KY631834, KY635010, KY635088, KY635168, KY635248, KY635328; *Mitchell 672* (KBG). *C. stephanii* Schwantes: KY631835, KY635011, KY635089, KY635169, KY635249, KY635329; *Mitchell 999* (KBG). *C. subfenestratum* Schwantes: KY631836, KY635012, KY635090, KY635170, KY635250, KY635330; *Bruyns s.n.* (KBG). *C. swanepoelianum* Rawé: KY631837, KY635013, KY635091, KY635171, KY635251,

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KY635331; *Harrower 4993* (NBG). *C. tantillum* N.E.Br.: KY631838, KY635014, KY635092, KY635172, KY635252, KY635332; *Harrower 3684* (NBG). *C. truncatum* N.E.Br.: KY631839, KY635015, KY635093, KY635173, KY635253, KY635333; *Mitchell s.n.* (KBG). *C. uviforme* (Haw.) N.E.Br.: KY631840, KY635016, KY635094, KY635174, KY635254, KY635334; *Littlewood s.n.* (KBG). *C. vanheerdei* Tisch.: KY631841, KY635017, KY635095, KY635175, KY635255, KY635335; *Powell 141* (NBG). *C. velutinum* Schwantes: KY631842, KY635018, KY635096, KY635176, KY635256, KY635336; *KGB 57/86* (KBG). *C. violaciflorum* Schick & Tisch.: KY631843, KY635019, KY635097, KY635177, KY635257, KY635337; *Mitchell 170C* (KBG). *C. wettsteinii* N.E.Br.: KY631844, KY635020, KY635098, KY635178, KY635258, KY635338; *Powell 50* (NBG). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. vanzylii* (L.Bolus) H.E.K.Hartmann: KY631868, KY635041, KY635121, KY635202, KY635282, KY635362; *KGB 222/98* (KBG). ***Odontophorus*** N.E.Br: *O. angustifolius* L.Bolus: KY631869, KY635042, KY635122, KY635203, KY635283, KY635363; *EVJ 106/87* (NBG).

APPENDIX A3. Voucher information for accessions sent to Canadian Centre for DNA Barcoding (Guelph, Canada) for matK to contribute to the Barcode of Life project (iBOL, 2015. Sequences available at http://www.boldsystems.org/ (Ratnasingham & Herberg, 2007). GenBank accession numbers for the sequences are provided in Appendix A3, while the remaining sequences will be submitted to GenBank upon publication

Arenifera A.G.J.Herre: *A. pillansii* (L.Bolus) A.G.J.Herre, *Bruyns 9136* (BOL). ***Cheiridopsis*** N.E.Br.: *C. aspera* L.Bolus, *Powell 14* (NBG); *C. denticulata* N.E.Br., *Powell 9* (NBG); *C. meyeri* N.E.Br., *Powell 16* (NBG); *C. namaquensis* (Sond.) H.E.K.Hartmann, *Powell 11* (NBG); *C. peculiaris* N.E.Br., *Powell 15* (NBG); *C. pillansii* L.Bolus, *Powell 45* (NBG); *C. robusta* N.E.Br., *Powell 66* (NBG). ***Conophytum*** N.E.Br.: *C. angelicae* N.E.Br., *Bruyns 9479* (NBG); *C. auriflorum* Tisch., *Mitchell 555* (KBG); *C. bilobum* (Marloth) N.E.Br., *Powell 19* (NBG); *C. bolusiae* Schwantes, *Mitchell 578* (KBG); *C. burgeri* L.Bolus, *Donor s.n.* (KBG); *C. caroli* Lavis, *Sauer 88/1004* (NBG); *C. chauviniae* (Schwantes)S.A.Hammer, *Mitchell 1086* (KBG); *C. comptonii* N.E.Br., *Harrower 5009* (NBG); *C. concavum* L.Bolus, *Lavranos s.n.* (KBG); *C. concordans* G.D.Rowley, *Harrower s.n.* (NBG); *C. depressum* Lavis, *Powell 53* (NBG); *C. ectypum* N.E.Br., *Meyer 99A* (NBG); *C. ernstii* S.A.Hammer, *Van Jaarsveld 8512* (NBG); *C. ficiforme* N.E.Br., *Powell 3* (NBG); *C. flavum* N.E.Br., *Powell 57* (NBG); *C. friedrichiae* Schwantes, *KBG 312/89* (KBG); *C. frutescens* Schwantes,

Hammer 679 (NBG); *C. fulleri* L.Bolus, *Mitchell 966* (KBG); *C. globosum* N.E.Br., *Mitchell 1200* (KBG); *C. hermarium* (S.A.Hammer)S.A.Hammer, *Powell 32*(NBG); *C. herreanthus* S.A.Hammer, *KBG 103/91* (KBG); *C. hians* N.E.Br., *Harrower 3246* (NBG); *C. jucundum* (N.E.Br.)N.E.Br., *Wisura 1589* (NBG); *C. klinghardtense* Rawé, *Williamson 4614* (KBG); *C. limpidum* S.A.Hammer, *Hammer 385* (NBG);*C. lithopsoides* L.Bolus, *Powell 29* (NBG); *C. longum* N.E.Br., *Mitchell 738* (KBG); *C. luckhoffii* Lavis, *Harrower 4211* (NBG); *C. marginatum* Lavis, *Powell 20* (NBG); *C. meyeri* N.E.Br., *Powell 16* (NBG); *C. minutum* N.E.Br., *Harrower 102* (NBG); *C. obcordellum* N.E.Br., *Magee 1058* (NBG); *C. pageae* N.E.Br., *Magee 1059* (NBG); *C. pellucidum* Schwantes, *Harrower 3628* (NBG); *C. phoeniceum* S.A.Hammer, *Hammer 1212* (NBG); *C. piluliforme* (N.E.Br.)N.E.Br., *Bayer s.n.* (KBG); *C. quaesitum* N.E.Br., *Hammer 909* (NBG); *C. ratum* S.A.Hammer, *Powell 24* (NBG); *C. regale* Lavis, *Bayer 1081* (KBG); *C. roodiae* N.E.Br., *Powell 51* (NBG); *C. saxetanum* N.E.Br., *Wisura 1600* (NBG); *C. smorenskaduense* de Boer, *Mitchell 672* (KBG); *C. stephanii* Schwantes, *Mitchell 999* (KBG); *C. subfenestratum* Schwantes, *Bruyns s.n.* (KBG); *C. tantillum* N.E.Br., *Harrower 3684* (NBG); *C. truncatum* N.E.Br., *Mitchell s.n.* (KBG); *C. uviforme* (Haw.) N.E.Br., *Littlewood s.n.* (KBG); *C. velutinum* Schwantes, *KGB 57/86* (KBG); *C. violaciflorum* Schick & Tisch., *Mitchell 170C* (KBG); *C. wettsteinii* N.E.Br., *Powell 50* (NBG). ***Enarganthe*** N.E.Br: *E. octonaria* (L.Bolus) N.E.Br, *Powell 45* (NBG). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. excavata* (L.Bolus) H.E.K.Hartmann, *Powell 55* (NBG). ***Schlechteranthus*** Schwantes (including species previously reconginsed in *Arenifera* and *Octopoma*): Schwantes: *S. abruptus* (A.Berger) R.F.Powell, *Powell 5* (NBG); *S. connatus* (L.Bolus) R.F.Powell, *Powell 10* (NBG); *S. inclusus* (N.E.Br.) R.F.Powell, *Powell 35* (NBG); *S. maximillianii* Schwantes, *Powell 48* (NBG); *S. pungens* (H.E.K.Hartmann) R.F.Powell, *Powell 28* (NBG); *S. parvus* R.F.Powell & Klak (unpublished), *Powell 41* (NBG); *S. subglobosus* (L.Bolus) R.F.Powell, *Powell 33* (NBG).

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APPENDIX A4. Voucher information and GenBank reference numbers (https://www.ncbi.nlm.nih.gov/genbank/) for sequences published in Klak *et al.* (2013) which were used in combination with new accessions produced in the thesis. The information is listed as follows: *taxon – atpB, psbJ, matK, rpl16, rps16, trnD, trnL, trnQ, trnS;* voucher information.

Conophytum N.E.Br: *C. bruynsii* S.A.Hammer: KF132665; KF132179; AJ532795; AJ558090; KF131886; KF133146; *Bruyns 6784* (BOL). *C. calculus* (A.Berger) N.E.Br.: KF132666; KF132180; KF132451; KF132793; KF131887; KF133147; *Klak 1641* (BOL). *C. maughanii* N.E.Br.: KF132667; KF132181; KF132452; KF132794; KF131888; KF133148; *Bruyns 8913* (BOL). ***Cheiridopsis*** N.E.Br.: *C. pearsonii* N.E.Br., KF132664; KF132178; KF132450; KF132792; KF131885; KF133145; *Bruyns 9504* (BOL). ***Enarganthe*** N.E.Br: *E. octonaria* (L.Bolus) N.E.Br: KF132684; KF132198; AJ532805; AJ439023; JN896433; JN896380; *Klak 491* (BOL). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. excavata* (L.Bolus) H.E.K.Hartmann: KF132701; KF132214; KF132477; KF132818; KF131921; KF133182; *Wisura 1926* (BOL). ***Jacobsenia*** L.Bolus & Schwantes: *J. vaginata* (L.Bolus) Ihlenf.: KF132703; KF132215; KF132479; KF132820; KF131923; KF133184; *Wisura 882* (BOL). ***Jensenobotrya*** A.G.J.Herre: *J. lossowiana* A.G.J.Herre: KF132704; KF132216; KF132480; KF132821; KF131924; KF133185; *Van Jaarsveld 21145* (BOL). ***Knersia*** H.E.K.Hartmann & Liede (= *Drosanthemum diversifolium* in Klak *et al.*, (2013)): *K. diversifolia* (L.Bolus) H.E.K.Hartmann & Liede: KF132633; KF132149; KF132435; KF132778; KF131856; KF133116; *Klak 1743* (BOL). ***Namaquanthus*** L.Bolus *N. vanheerdei* L.Bolus: KF132724; –; AJ532810; AJ439049; KF131944; KF133205; *Klak 251* (BOL). ***Odontophorus*** N.E.Br: *O. marlothii* N.E.Br.: KF132731; KF132243; AJ532823; AJ558101; KF131951; KF133212; *Klak 862* (BOL). ***Ruschianthus*** L.Bolus *R. falcatus* L.Bolus: KF132758; KF132266; KF132518; KF132857; KF131978; KF133240; *Rawe s.n.*(BOL). ***Schlechteranthus*** Schwantes: *S. hallii* L.Bolus: KF132617; KF132417; KF132760; KF132268; KF132520; KF132128; KF132859; KF131980; KF133242; *Klak 259* (BOL). *S. steenbokensis* (H.E.K.Hartmann) Klak: AJ532671; KF132397; KF132740; KF132251; AJ532817; KF132108; AJ558097; KF131960; KF133221; *Bruyns 8267* (BOL). *S. subglobosus* (L.Bolus) R.F.Powell (=*Octopoma subglobosa* (L.Bolus) L.Bolus in Klak *et al.*, (2013)): KF132592; KF132387; KF132730; KF132242; KF132496; KF132098; KF132837; KF131950; KF133211; *Bruyns 8240* (BOL).

APPENDIX B

APPENDIX B. Voucher information for leaf material studied in transverse section. Voucher information is listed as taxon, voucher information.

Arenifera A.G.J.Herre: *A. pillansii* (L.Bolus) A.G.J.Herre, *Bruyns 9136* (BOL). ***Cheiridopsis*** N.E.Br.: *C. acuminata* L.Bolus, *Powell 68* (NBG);*C. aspera* L.Bolus, *Powell 14* (NBG); *C. denticulata* N.E.Br., *Powell 9* (NBG); *C. caroli-schmidtii* N.E.Br., *Powell 105* (NBG); *C. gamoepensis* S.A.Hammer, *Powell 89* (NBG); *C. glomerata* S.A.Hammer, *Powell 96* (NBG); *C. imitans* L.Bolus, *Powell 91* (NBG); *C. herrei* L.Bolus, *Powell 100* (NBG); *C. meyeri* N.E.Br., *Powell 16* (NBG); *C. minor* (L.Bolus) H.E.K.Hartmann, *Powell 94* (NBG); *C. namaquensis* (Sond.) H.E.K.Hartmann, *Powell 11* (NBG); *C. peculiaris* N.E.Br., *Powell 15* (NBG); *C. pillansii* L.Bolus, *Powell 45* (NBG); *C. pilosula* L.Bolus, *Powell 69* (NBG); *C. ponderosa* S.A.Hammer, *Powell 103* (NBG); *C. purpurea* L.Bolus, *Powell 85* (NBG); *C. robusta* N.E.Br., *Powell 66* (NBG); *C. rostrata* N.E.Br., *Powell 88* (NBG); *C. schlechteri* Tisch., *Powell 17* (NBG); *C. speciosa* L.Bolus, *Powell 107* (NBG); *C. verrucosa* L.Bolus, *Powell 99* (NBG); *C. umdausensis* L.Bolus, *Powell 102* (NBG). ***Enarganthe*** N.E.Br: *E. octonaria* (L.Bolus) N.E.Br, *Powell 45* (NBG). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. excavata* (L.Bolus) H.E.K.Hartmann, *Powell 55* (NBG); *I. vanzylii* (L.Bolus) H.E.K.Hartmann, *KBG 222/98* (KBG). ***Jensenobotrya*** A.G.J.Herre: *J. lossowiana* A.G.J.Herre, *SUG 12618* (NBG). ***Namaquanthus*** L.Bolus *N. vanheerdei* L.Bolus, *EVJ 2475* (NBG). ***Octopoma*** N.E.Br.: *O. nanum* (L.Bolus) Klak, *Klak 2426* (BOL); *O. octojuge* N.E.Br., *Bohnen 8924* (NBG); *O. quadrisepalum* (L.Bolus) H.E.K.Hartmann, *Gwynn-Evans 155.2* (NBG). ***Odontophorus*** N.E.Br: *O. angustifolius* L.Bolus, *EVJ 106/87* (NBG). ***Schlechteranthus*** Schwantes (including species previously reconginsed in *Arenifera* and *Octopoma*): *S. abruptus* (A.Berger) R.F.Powell, *Powell 5* (NBG); *S. albiflorus* (L.Bolus) Klak, *van Jaarsveld 4236* (NBG); *S. connatus* (L.Bolus) R.F.Powell, *Powell 10* (NBG); *S. hallii* L.Bolus, *Powell 71* (NBG); *S. inclusus* (N.E.Br.) R.F.Powell, *Powell 35* (NBG); *S. maximillianii* Schwantes, *Powell 48* (NBG); *S. paruvs* R.F.Powell & Klak (unpublished), *Powell 41* (NBG); *S. pungens* (H.E.K.Hartmann) R.F.Powell, *Powell 28* (NBG); *S. spinescens* (L.Bolus) R.F.Powell, *Klak 2424* (BOL); *S. stylosus* (L.Bolus) R.F.Powell, *Powell 75* (NBG); *S. subglobosus* (L.Bolus) R.F.Powell, *Powell 33* (NBG); *S. tetrasepalus* (L.Bolus) R.F.Powell, *Klak 2411* (BOL).

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APPENDIX C

APPENDIX C. Voucher information for pollen material studied under the light microscope. Voucher information is listed as taxon, voucher information.

Conophytum N.E.Br.: *C. frutescens* Schwantes, *Wisura 862* (NBG); *C. hians* N.E.Br., *Wilkins s.n.* (NBG); *C. uviforme* (Haw.) N.E.Br., *Powell 39* (NBG). ***Cheiridopsis*** N.E.Br.: *C. denticulata* N.E.Br.,,*van Heerde s.n.* (BOL); *C. derenbergiana* Schwantes, *Wisura 2053* (NBG); *C. namaquensis* (Sond.) H.E.K.Hartmann, *van Heerde 69* (BOL); *C. pillansii* L.Bolus, *Wisura 1617* (NBG); *C. pilosula* L.Bolus, *van Jaarsveld 2548* (NBG). ***Enarganthe*** N.E.Br: *E. octonaria* (L.Bolus) N.E.Br., *van Jaarsveld 4206* (NBG). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. excavata* (L.Bolus) H.E.K.Hartmann, *van Heerde 30* (NBG). ***Namaquanthus*** L.Bolus *N. vanheerdei* L.Bolus, *van Jaarsveld 2475* (NBG); ***Odontophorus*** N.E.Br: *O. marlothii* N.E.Br., *Hall NBG 624/57* (NBG); *O. nanus* L.Bolus, *Smithers s.n.* (BOL). ***Schlechteranthus*** Schwantes (including species previously reconginsed in *Arenifera* and *Octopoma*): *S. albiflorus* (L.Bolus) Klak, *Van Berkel 462* (NBG); *Schlechteranthus connatus* (L.Bolus) R.F.Powell, *Helme 3226* (BOL); *S. hallii* L.Bolus, *Burgoyne 7340* (NBG); *S. spinescens* (L.Bolus) R.F.Powell, *Desmet & Ellis 1345* (NBG).

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APPENDIX D

APPENDIX D. Coded morphological characters and character states.

APPENDIX D1. Morphological characters and character states coded in the morphological cladistics analysis for species of *Schlechteranthus* subgenus *Microphyllus.*

1–locule number: 10–12 locules=0; 7–9 locules=1. **2**–closing bodies: large, blocking ¾ of locule=0; small, blocking $^1/_3$ of locule=1. **3**–inflorescence: solitary=0; cymose=1. **4**–bracteole: present=0; absent=1. **5–**peduncles becoming spinescent: absent=0; present=1. **6**–spines derived from aborted buds: absent=0; present=1. **7–**primary axis conspicuously lengthened, at least 1½ longer than the length of the capsule: absent=0; present=1. **8**–spines arranged in compound cyme on secondary axis: absent=0; present=1.

APPENDIX D2. Leaf, floral and capsule morphological characters and character states coded and mapped onto the Bayesian inference tree of the expanded *Conophytum*. Characters were coded using information available in the literature, including Liede & Hammer, (1990), Hartmann, (2001), Hammer, (2002), Opel (2005b), Powell *et al.* (2016; Chapter 3), Powell *et al.* (submitted; Chapter 5) as well as from field observations.

1–papillae type: 0=no epidermal extensions, 1=blunt papillae, 2=trichomes. **2–**windowed leaves: 0=absent, 1=present. **3–**soft-bodied leaves: 0=absent, 1=present. **4–**flowering season (phenology): 1=autumn, 2=winter, 3=spring, 4=summer. **5–**flowering time (anthesis): 1=diurnal, 2=afternoon, 3=late afternoon, 4=nocturnal. **6–**floral structure (Liede & Hammer, 1990): 0=radiate flower, 1=A1, 2=A2, 3=B1, 4=B2. **7–**locule number: 1=<5 locules, 2=5–6 locules, 3=7–9 locules, 4=>9 locules.

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APPENDIX E

APPENDIX E. Publications and products.

APPENDIX E1. **Powell RF, Boatwright JS, Klak C, Magee AR. 2016.** Phylogenetic placement and generic re-circumscription of the multilocular genera *Arenifera, Octopoma* and *Schlechteranthus* (Aizoaceae; Ruschieae): Evidence from anatomical, morphological and plastid DNA data. *Taxon* **65:** 249–261.

APPENDIX E2. **Powell RF, Boatwright JS, Klak C, Magee AR.** Inclusion of *Ihlenfeldtia* and *Odontophorus* in *Cheiridopsis* (Ruschioideae; Aizoaceae) with insights into generic and subgeneric circumscriptions within the *Conophytum*-clade. *Botanical Journal of the Linnean Society.* **Accepted**.

APPENDIX E3. **Powell RF, Klak C, Boatwright JS, Magee AR.** A taxonomic revision of *Schlechteranthus* subgenus *Microphyllus* (Ruschieae; Aizoaceae). *Systematic Botany.* **Submitted**.

APPENDIX E4. **Powell RF, Hammer S, Rodgerson C, Young A. 2015.** *Conophytum*: an interactive DELTA Key. Available at: http://rpowell2.wixsite.com/conophytum-delta-key.

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APPENDIX E1. **Powell RF, Boatwright JS, Klak C, Magee AR. 2016.** Phylogenetic placement and generic re-circumscription of the multilocular genera *Arenifera, Octopoma* and *Schlechteranthus* (Aizoaceae; Ruschieae): Evidence from anatomical, morphological and plastid DNA data. *Taxon* **65:** 249–261.

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Phylogenetic placement and generic re-circumscriptions of the multilocular genera *Arenifera*, *Octopoma* and *Schlechteranthus* (Aizoaceae: Ruschieae): Evidence from anatomical, morphological and plastid DNA data

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Abstract Ruschieae is the largest tribe in the highly speciose subfamily Ruschioideae (Aizoaceae). A generic-level phylogeny for the tribe was recently produced, providing new insights into relationships between the taxa. *Octopoma* and *Arenifera* are woody shrubs with multilocular capsules and are distributed across the Succulent Karoo. *Octopoma* was shown to be polyphyletic in the tribal phylogeny, but comprehensive sampling is required to confirm its polyphyly. *Arenifera* has not previously been sampled and therefore its phylogenetic placement in the tribe is uncertain. In this study, phylogenetic sampling for nine plastid regions (*atpB-rbcL*, *matK*, *psbJ-petA*, *rpl16*, *rps16*, *trnD-trnT*, *trnL-F*, *trnQ*^{UUG}*-rps16*, *trnS-trnG*) was expanded to include all species of *Octopoma* and *Arenifera*, to assess phylogenetic placement and relationships of these genera. Three phylogenetic analyses were carried out, maximum parsimony, maximum likelihood and Bayesian inference. Leaf anatomical sections were studied to further inform generic circumscriptions. The phylogenies showed *Octopoma* to be polyphyletic, with the type, *O. octojuge*, and the related *O. nanum*, resolved as sister to *Zeuktophyllum* and *Smicrostigma*, while the other species were placed in the *Conophytum*-clade. *Arenifera* was also shown to be polyphyletic, with the type, *A. pillansii*, placed in the xeromorphic-clade, and the remainder of the species recovered among the *Octopoma* species in the *Conophytum*-clade (forming the *Octopoma subglobosum–Arenifera spinescens* subclade). Generic affinities of the *O. subglobosum–A. spinescens* subclade were assessed in relation to the sister taxon *Schlechteranthus*. The leaf anatomy was found to be informative within the study group. Bladder cells were observed in *Arenifera pillansii*, a hypodermis in Little Karoo *Octopoma* (*O. octojuge*, *O. nanum*, *O. quadrisepalum*) and epidermal cells forming blunt papillae in *Schlechteranthus* and the *O. subglobosum–A. spinescens* subclade. Upon assessment of the anatomical, morphological and phylogenetic data, *Schlechteranthus* is here expanded to include the species in the *O. subglobosum–A. spinescens* subclade. Eight new combinations are made in *Schlechteranthus*. As a result, *Arenifera* is again monotypic and the circumscription of *Octopoma* is refined to include three species restricted to the Little Karoo. Two subgenera within *Schlechteranthus* s.l. (subg. *Schlechteranthus*, subg. *Microphyllus*) are erected to accommodate differences in leaf size, capsule size, closing body size and locule number.

Keywords anatomy; *Conophytum*-clade; Greater Cape Floristic Region; polyphyly; Succulent Karoo; *Schlechteranthus* subg. *Microphyllus*

Supplementary Material The Electronic Supplement (Figs. S1, S2) is available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax; DNA sequence alignment is available from TreeBASE, study no. 19102

■ INTRODUCTION

Ruschioideae is the largest and most diverse subfamily in the Aizoaceae, with approximately 1600 species (Hartmann, 2001). The subfamily has its centre of diversity in the arid parts of the Greater Cape Floristic Region (Jürgens, 1991) of southern Africa, with ca. 36% of the species endemic to the region (Manning & Goldblatt, 2012; Snijman, 2013). Within the Ruschioideae, four tribes are recognised (Apatesieae, Dorotheantheae, Drosanthemeae, Ruschieae) based on floral nectaries, capsule morphology, leaf characters and molecular data (Chesselet & al., 2002, 2004; Klak & al., 2003a, 2013).

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Early classifications of Aizoaceae were based on leaf characters (Haworth, 1795), floral nectary structure (Rappa & Camarrone, 1953) and capsule morphology (Schwantes, 1926; Brown, 1930). Ihlenfeldt (1960) examined these classifications and argued that the classifications based on leaf and floral nectary structure were conflicting and unnatural and suggested capsule morphology to be more taxonomically informative for predicting a natural classification system for the family. As a result, the current subfamilial classifications are based on capsule morphology, in combination with leaf and floral characters.

Anatomical characters, specifically the leaf epidermal and stomatal structures, have also been noted to be of taxonomic value (Reule, 1937). Leaf anatomy has been used for a range of classifications, from subfamilial to generic, and also for distinguishing species (Ihlenfeldt & Bittrich, 1985; Klak & Linder, 1998; Landrum, 2001; Opel, 2005). Landrum (2001) investigated novel wide-band tracheids, which were found in 89 genera in the Aizoaceae, specifically in the subfamily Ruschioideae, providing a useful character to distinguish this subfamily from other subfamilies of Aizoaceae. Examinations of leaf epidermal cells, floral and capsule morphology of *Psilocaulon* N.E.Br. led Ihlenfeldt & Bittrich (1985) to re-establish the genus *Brownanthus* Schwantes and erect a new genus *Pseudobrownanthus* Ihlenf. & Bittrich. Some 30 years later, Klak & Linder (1998) in their studies of *Psilocaulon* found that leaf epidermal idioblasts were informative for identification of species. Leaf anatomical characters have also been helpful in grouping species of large genera (Opel, 2005), with a number of anatomical characters such as the presence of a hypodermis, crystals, papillae and bladder cells differentiating morphological clades in *Conophytum* N.E.Br.

More recently, phylogenetic analyses based on DNA sequence data have provided further insight into relationships between taxa in the family (Klak & al., 2003b) and in the subfamilies Sesuvioideae (Hassan & al., 2005; Bohley & al., 2015), Mesembryanthemoideae (Klak & al., 2006, 2007, 2014) and Ruschioideae (Klak & al., 2003a, 2013). Klak & al. (2013) produced the first generic-level phylogeny for the large and taxonomically problematic tribe Ruschieae. Although extensive morphological studies had previously been conducted on the tribe (Hartmann, 2001), the phylogeny uncovered novel relationships between taxa (Klak & al., 2013). The phylogenetic results supported Ihlenfeldt's (1960) classification and subsequent classifications (Hartmann, 2001) in many cases. However, very large genera (e.g., *Ruschia* Schwantes) and a few other genera in the subfamily where shown to be polyphyletic (Klak & al., 2013).

Octopoma N.E.Br. is a genus of woody shrubs with multilocular capsules, comprised of nine species distributed across the Succulent Karoo (Hartmann, 2001). The genus was last revised by Hartmann (1996), where it was suggested that the circumscription of the genus may be unnatural. The possible polyphyly of the genus was highlighted by Klak & al. (2013), however, only two of the nine species were sampled. In this analysis the type of the genus, *Octopoma octojuge* N.E.Br., was placed sister to *Zeuktophyllum* N.E.Br. and *Smicrostigma* N.E.Br., while *Octopoma subglobosum* (L.Bolus) L.Bolus was recovered as sister to the multilocular genera *Schlechteranthus* Schwantes and *Polymita* N.E.Br. within the *Conophytum*-clade (recognised by Klak & al. (2013) and currently including 11 genera, viz. *Cheiridopsis* N.E.Br, *Conophytum*, *Enarganthe* N.E.Br., *Ihlenfeldtia* H.E.K.Hartmann, *Jensenobotrya* A.G.J.Herre, *Namaquanthus* L.Bolus, *Octopoma* p.p., *Odontophorus* N.E.Br, *Polymita*, *Ruschianthus* L.Bolus, and *Schlechteranthus*). *Polymita*, however, has recently been included within *Schlechteranthus* by Klak & Bruyns (2016), reducing the number of genera within the *Conophytum* clade to ten.

Arenifera A.G.J.Herre is a shrubby genus with multilocular capsules, and includes four species occurring in the Succulent Karoo (Hartmann, 1996, 2001). The phylogenetic position and relationships of *Arenifera* remain uncertain as the genus has not been sampled in any phylogenetic studies. An affinity with *Psammophora* Dinter & Schwantes has been postulated (Bolus, 1927; Herre, 1948; Hartmann, 1996), based on the distinctly sticky leaves. However, *Psammophora* does not share multilocular capsules with *Arenifera* (Hartmann, 2001), and therefore the placement of *Arenifera* in the subfamily is unclear.

In this study, the polyphyly of *Octopoma* is assessed by expanding the current phylogenetic analyses to include eight of the nine species in the genus. The phylogenetic placement and relationships of *Arenifera* are also determined, with all of the five species (including a new species) sampled. Generic circumscriptions of the sampled taxa and their relationships to sister genera were assessed. In addition, leaf anatomical and morphological characters of the relevant taxa were investigated to provide additional evidence to further inform generic circumscriptions and relationships in *Arenifera*, *Octopoma* and *Schlechteranthus.*

■ MATERIALS AND METHODS

Taxon sampling. — Field visits were conducted to collect and study the species of *Arenifera*, *Octopoma* and *Schlechteranthus* in situ. Twelve (*Arenifera pungens* H.E.K.Hartmann, *Arenifera* sp. nov., *A. spinescens* L.Bolus, *A. stylosa* (L.Bolus) H.E.K.Hartmann, *Octopoma abruptum* N.E.Br., *O. connatum* (L.Bolus) L.Bolus, *O. inclusum* N.E.Br., *O. nanum* (L.Bolus) Klak, *O. tetrasepalum* (L.Bolus) H.E.K.Hartmann, *Schlechteranthus albiflorus* (L.Bolus) Klak, *S. hallii* Bolus, *S. maximiliani* Schwantes) of the seventeen species in these three genera were located in the field and collected for the phylogenetic, anatomical and morphological study.

We also examined 125 herbarium specimens, which included all *Arenifera*, *Octopoma* and *Schlechteranthus* specimens held at BOL, NBG and SAM, as well as the other genera within the *Conophytum*-clade for comparison. Further investigation of *Octopoma abruptum*, known only from the type locality, showed that it was not distinct from the sympatric *O. rupigenum* (L.Bolus) L.Bolus and is therefore considered conspecific with this species (Powell & al., in prep.).

All five species of *Arenifera* (including an undescribed species) and seven of the eight species of *Octopoma*

(*O. quadrisepalum* (L.Bolus) H.E.K.Hartmann could not be relocated) were sampled in the phylogeny. In addition, two anomalous species of the morphologically similar genus *Leipoldtia* L.Bolus were included: the large-flowered *L. gigantea* Klak, and a potential new species with 8 to 10 rather than the 10 to 16 locules usually found in the genus. In order to assess the phylogenetic relationships of *Octopoma* and *Arenifera*, the Ruschieae dataset of Klak & al. (2013) was expanded with 106 new sequences, for nine chloroplast gene regions. This dataset (Klak & al., 2013) included all genera in the subfamily, excluding *Arenifera*, *Circandra* N.E.Br. and the insufficiently known *Calamophyllum* Schwantes. As in Klak & al. (2013), the trees were rooted with *Cleretum papulosum* (L.f.) N.E.Br. and *Conicosia pugioniformis* N.E.Br. These taxa were selected as outgroups as, together with Drosanthemeae, they represent the tribes most closely related to Ruschieae, Dorotheantheae and Apatesieae (Hartmann, 1996; Klak & al., 2003b, 2013). Nuclear regions were excluded as they have been shown to be problematic (Klak & al., 2013), indicating gene duplication or multiple copies. Voucher information and GenBank accession numbers for sequences produced in this study are provided in Appendices 1 and 2. Voucher information and accession numbers for the remainder of the taxa can be found in Klak & al. (2013).

For anatomical study, fresh leaf material of five species of *Arenifera* (*A. pillansii* (L.Bolus) A.G.J.Herre, *A. pungens*, *Arenifera* sp. nov., *A. spinescens*, *A. stylosa*), six species of *Octopoma* (*O. abruptum*, *O. connatum*, *O. inclusum*, *O. nanum*, *O. subglobosum*, *O. tetrasepalum*) and three species of *Schlechteranthus* (*S. albiflorus*, *S. hallii*, *S. maximiliani*) was used. Fresh leaf material was not available for two species of *Octopoma* (*O. octojuge*, *O. quadrisepalum*), and for these samples, leaf material was collected from herbarium (NBG) specimens. These taxa were selected as they are representative of the taxa added to the phylogenies and *Schlechteranthus* was added as it was shown to be sister to *Octopoma* (Klak & al., 2013).

DNA sequence data. — Total DNA was extracted from silica-dried leaf material (0.2 mg) using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.) according to the manufacturer's instructions. Nine chloroplast gene regions were amplified and sequenced. A portion of the *trnQ*^{UUG}*-rps16* intergenic spacer was amplified using the primers trnQ^{UUG} and rps16x1 (Shaw & al., 2007). The *trnS-trnG* intergenic region was amplified using the primers trnS and trnG (Hamilton, 1999). The *trnL-F* region (consisting of the adjacent *trnL* intron and *trnL-F* intergenic spacer) was amplified using the extron primers c and f (Taberlet & al., 1991). The *rps16* region was amplified using the primers rps16F and rps16R2 (Oxelman & al., 1997). The *rpl16* intron was amplified using primers rpl16 71F (Jordan & al., 1996) and rpl16 1516R (Kelchner & Clark, 1997). The intergenic spacer between the *atpB* and *rbcL* genes was amplified using primers 2 and 5 (Manen & al., 1994). The intergenic spacer *psbJ-petA* was amplified using primers psbJ and petA (Shaw & al., 2007) and part of the intergenic spacer *trnD-trnT* was amplified using primers trnE (Shaw & al., 2005) and trnD (Demesure & al., 1995). A portion of the *matK* gene was amplified for four species (*Arenifera spinescens*, *A. stylosa*, *Octopoma nanum*, *O. tetrasepalum*) using DNA barcoding primers 3F-Kim and 1R-Kim (Cuenoud & al., 2002). The remaining seven species (*Arenifera pillansii*, *A. pungens*, *Arenifera* sp. nov., *Octopoma abruptum*, *O. connatum*, *O. inclusum*, *O. subglobosum*) were sent to the Canadian Centre for DNA Barcoding (Guelph, Canada) for *matK* barcode sequencing to contribute towards the International Barcode of Life project, available on BOLD systems (Ratnasingham & Herbert, 2007; iBOL, 2014).

Polymerase chain reactions (PCRs) were performed in 25 µl reactions containing 22.5 µl Thermo Scientific 1.1× ReddyMix PCR Master Mix (Thermo Fischer Scientific, Waltham, Massachusetts, U.S.A.), 0.8 µl Bovine Serum Albumin, 0.6 µl sterile distilled water, 0.3 µl of each primer and 0.5 µl of DNA template. The PCR reactions were carried out using the following thermal conditions: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 8 min. For samples that did not amplify successfully, the protocol was adjusted to include a temperature ramp following Shaw & al. (2005). Successfully amplified samples were cleaned using the ExoSAP protocol of Werle & al. (1994) using 5 units of Exonuclease I and 0.5 units of Shrimp Alkaline Phosphatase. Automated sequencing was carried out by Macrogen (Seoul, Korea). Electropherograms obtained from the sequences were manually checked and inconsistencies in the sequences were edited where necessary using MEGA v.6 (Higgins & al., 1994).

Phylogenetic analyses. — New sequences were automatically aligned into the existing Ruschieae matrices of Klak & al. (2013), using the Clustal W function in MEGA v.6 (Higgins & al., 1994; Tamura & al., 2013). This alignment was checked manually and adjusted accordingly where required, with gaps positioned so as to minimize nucleotide mismatches. Hypervariable regions for *trnQ*^{UUG}*-rps16* (79 bp) and *trnS-trnG* (48 bp) were excluded from the analysis, and gaps were coded using simple indel coding (Simmons & Ochoterena, 2000). The combined chloroplast dataset included a total of 8259 characters (including coded indels) and were analysed using maximum parsimony (MP), Bayesian inference (BI) and maximum likelihood (ML), excluding the coding indels in the latter analysis (8236 characters).

The MP algorithm was implemented in PAUP* v.4.0b4 (Swofford, 2000). Character transformations were unordered and equally weighted (Fitch, 1971). A heuristic search with 1000 random sequence additions, tree bisection-reconnection (TBR) branch-swapping, and the MULPARS option selected, was performed. All character transformations were treated with equal likelihood and a maximum of 10 trees were saved in each replicate to minimise swapping on local minima. Trees of the shortest length were saved and used as starting trees for a second round of TBR swapping with no limit on the number of trees saved, to ensure the shortest trees were recovered in the analysis. Node support was evaluated using the jackknife function in PAUP, with a full search, 1000 replicates and a limit of 1000 trees per replicate (Farris & al., 1996). Only jackknife support (JK) values greater than or equal to 50% were retained, and the following scale was used to evaluate

support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Maximum likelihood analyses were performed using RAxML v.8.1.11 (Stamatakis, 2006) on the combined chloroplast data (excluding the coded indels). The analyses were run on the CIPRES Portal, v.3.3 (Miller & al., 2010), using the default settings. A maximum likelihood tree with bootstrap node support (BS) is presented in Electr. Suppl.: Fig. S2, using the following scale to evaluate support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Bayesian inference (BI) was performed on the combined chloroplast dataset (including the coded indels), using MrBayes v.3.2.3 (Ronquist & Huelsenbeck, 2003). The analyses were run on the CIPRES Portal, v.3.3 (Miller & al., 2010). Parameters were set in a Bayes block and the data were partitioned into 10 partitions (9 gene regions and 1 coded indel partition). All parameters were unlinked (statfreq, revmat, shape, pinvar) between partitions. Following Klak & al. (2013), the most complex model (GTR+G+I) was implemented for the gene regions partitions (Huelsenbeck & Rannala, 2004). The standard coding model in MrBayes was used for the coded indel partition (Ronquist & al., 2011). Two simultaneous runs were completed for 10^7 generations with a sampling frequency of 100. The standard deviation between the split frequencies stabilised below 0.01, providing evidence that a sufficient number of generations had been completed. Using Tracer v.1.5 (Rambaut & Drummond, 2009), suboptimal trees were discarded as the "burn-in" phase. The remaining 75,001 trees were used to construct a 50% majority-rule consensus tree with posterior probabilities (PP). Only support values greater than or equal to 0.5 were retained, and the following scale used: 0.50–0.94, weak; and 0.95–1.00, strong.

Anatomical data. — Fresh leaf material was fixed in formalin-aceto-alcohol (FAA). The FAA was prepared using 90 ml of 70% ethanol, 5 ml of 40% formalin and 5 ml of glacial acetic acid (De Neergaard & al., 2001). Leaf material obtained from herbarium specimens was re-hydrated and then also fixed in FAA. The material was embedded in paraffin wax (Rudall, 1995), and 12–15 µm transverse sections from the central third of the leaf were cut using a Reichert-Jung autocut microtome (Model 2040). The sections were then double-stained with alcian blue and safranin. Permanent slides were made using Entellan and viewed with a Zeiss compound microscope and photographed using an Olympus SC30 camera.

■ RESULTS

Phylogenetic analyses. — Following Klak & al. (2013), the chloroplast matrices for the nine gene regions were not analysed separately due to low sequence divergence, but rather only in combination. The combined chloroplast matrix for the nine gene regions consisted of 8236 unambiguously aligned positions and 23 binary scored indels, resulting in 1247 variable characters and 570 parsimony-informative characters. In the maximum parsimony analysis, 129 trees were retained with a tree length of 2652 steps (consistency index [CI] = 0.78; retention index [RI] = 0.68). The topologies recovered in the MP, ML and BI analyses were all consistent with those presented in Klak & al. (2013) as well as with one another, albeit with expected differences in resolution and support values. The Bayesian inference phylogeny was the most resolved with well-supported clades (Fig. 1). Maximum parsimony showed moderate support for the main clades, but was otherwise poorly resolved (Electr. Suppl.: Fig. S1). The phylogeny produced in the maximum likelihood analysis identified the majority of the clades, however, support for these clades was low, but increased within subclades (Electr. Suppl.: Fig. S2).

Octopoma was recovered as polyphyletic in all three analyses (Fig. 1; Electr. Suppl.: Figs. S1, S2). The type of the genus (*O. octojuge*), together with the closely related *O. nanum*, were recovered together (Little Karoo *Octopoma*, Fig. 1) in a clade with *Zeuktophyllum* and *Smicrostigma* (PP = 0.88, Fig. 1). In contrast, the remaining species of *Octopoma* (*O. abruptum*, *O. connatum*, *O. inclusum*, *O. subglobosum*, *O. tetrasepalum*) were placed in the *Conophytum*-clade (PP = 0.99, BS = 69).

Arenifera was also recovered as polyphyletic (Fig. 1; Electr. Suppl.: Figs. S1, S2), with the type, *A. pillansii* (Fig. 1, *Arenifera* s.str.), placed in the xeromorphic clade (PP = 0.89, sensu Klak & al., 2013), while the remaining four non-sticky *Arenifera* species (*A. pungens*, *Arenifera* sp. nov., *A. spinescens*, *A. stylosa*) were recovered among the Namaqualand species of *Octopoma* in the *Conophytum*-clade (PP = 0.99, BS = 69).

Within the *Conophytum*-clade the non-sticky *Arenifera* and Namaqualand *Octopoma* species formed a subclade *O. subglobosum–A.spinescens* (PP = 1.0, JK = 61, BS = 92), sister (PP = 0.97, BS = 57) to the monophyletic *Schlechteranthus* (PP = 1.0, JK = 92, BS = 99). Together these three groups (non-sticky *Arenifera*, Namaqualand *Octopoma* and *Schlechteranthus*), formed a subclade strongly supported in the Bayesian analysis (PP = 0.97, BS = 57, Fig. 1).

Both of the new accessions of *Leipoldtia* were recovered together with the previously included species of *Leipoldtia* (PP = 1.0, BS = 67, Fig. 1).

Leaf anatomy. — Leaf anatomical characters are summarised in Table 1. The epidermis was conspicuously smooth in *Arenifera pillansii* and three species of *Octopoma* (*O. nanum*, *O. octojuge*, *O. quadrisepalum*; Fig. 2A, D). The outer wall of the epidermal cells formed blunt papillae concentrated around the stomata in the remaining species studied (*Arenifera pungens*, *Arenifera* sp. nov., *A. spinescens*, *A. stylosa*, *Octopoma abruptum*, *O. connatum*, *O. inclusum*, *O. subglobosum*, *O. tetrasepalum*, *Schlechteranthus albiflorus*, *S. hallii*, *S. maximiliani*; Fig. 2B, E). In *Arenifera pungens*, *Arenifera* sp. nov., *A. spinescens*, *A. stylosa*, *Octopoma abruptum*, *O. connatum*, *O. inclusum*, *O. subglobosum* and *O. tetrasepalum* the papillae extended away from the stomata, decreasing in density (Table 1). The epidermal cells of *Schlechteranthus* (*S. albiflorus*, *S. hallii*, *S. maximiliani*) were anticlinally elongated (Fig. 2B). The epidermal cells in *Octopoma octojuge*, *O. nanum*, *O. quadrisepalum* were slightly paraclinally elongated (Fig. 2A), while the epidermal cells of the remaining species were isodiametric (Fig. 2D–F). Prominent bladder cells, that almost obscure the epidermis, were only found in *Arenifera pillansii*

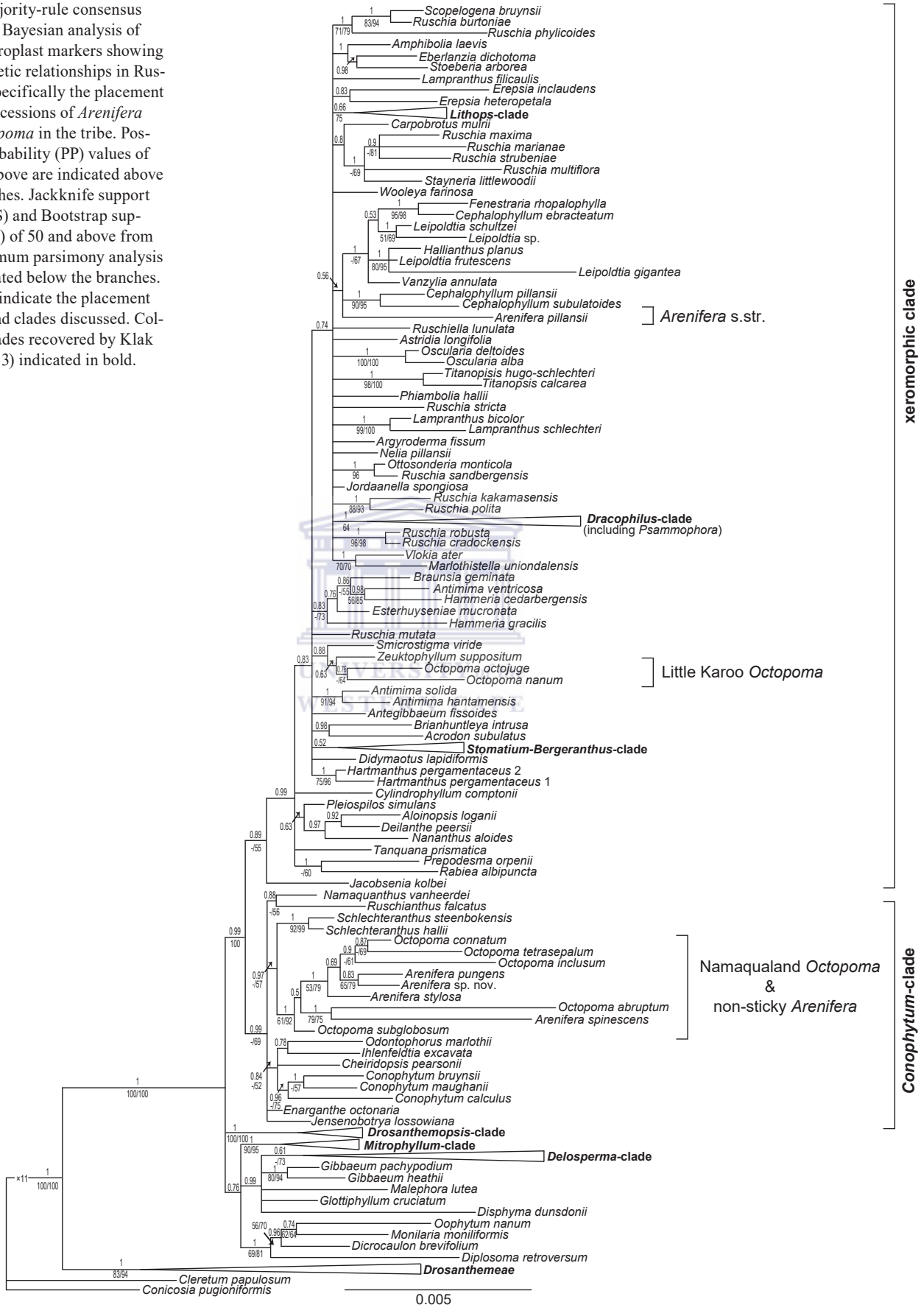

Fig. 1. Majority-rule consensus tree from Bayesian analysis of nine chloroplast markers showing phylogenetic relationships in Ruschieae, specifically the placement of new accessions of *Arenifera* and *Octopoma* in the tribe. Posterior probability (PP) values of 0.5 and above are indicated above the branches. Jackknife support values (JS) and Bootstrap supports (BS) of 50 and above from the maximum parsimony analysis are indicated below the branches. Brackets indicate the placement of taxa and clades discussed. Collapsed clades recovered by Klak & al. (2013) indicated in bold.

(Fig. 2D), whereas bladder cells in the other species were inconspicuous. Calcium oxalate crystals were deposited on the outer paraclinal walls in all species, but the crystals were poorly developed in *Arenifera pillansii* (Fig. 2D). In *Schlechteranthus* (*S. albiflorus*, *S. hallii*, *S. maximiliani*), the crystals were also deposited on the anticlinal walls of the epidermal cells (Fig. 2B). A hypodermis was only found in three species (*O. nanum*, *Octopoma octojuge*, *O. quadrisepalum*; Fig. 2A).

Tanniferous idioblasts were present in a ring below the epidermal cells in all the species, although the density of idioblasts varied. Similarly, raphide bundles were present, at different densities, in all the species.

Two types (Form I and Form II) of stomatal protection, described by Ihlenfeldt & Hartmann (1982), were identified. In both these forms, the leaf surface is sculptured by the tanniferous idioblasts below the epidermis, with elevations above the tanniferous idioblasts and depressions between the tanniferous idioblasts in all species (Fig. 2D, F). In the first form the stomata are distributed in the depressions, but are not protected further by any additional structures. This form was observed

Table 1. Summary of important morphological and anatomical characters for species of *Arenifera*, *Octopoma* and *Schlechteranthus*.

Species	Leaf mucro present	Leaf surface sticky	Number of locules	Closing body closing >½ of exit of locules	Calcium oxalate crystals deposited on outer paraclinal wall of epidermal cell	Hypodermis present	Bladder cells prominent	Outer wall of epidermal cells forming blunt papillae concentrated around the stomata	Epidermal cells anticlinally elongated	Stomata hidden by parastomal cells
Arenifera pillansii (L.Bolus) A.G.J.Herre	–	+	7–8		Poorly developed	–	+	–	–	–
Arenifera pungens H.E.K.Hartmann	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	–
Arenifera sp. nov.	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	+
Arenifera spinescens (L.Bolus) H.E.K.Hartmann	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	–
Arenifera stylosa (L.Bolus) H.E.K.Hartmann	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	+
Octopoma abruptum N.E.Br.	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	–
Octopoma connatum (L.Bolus) L.Bolus	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	–
Octopoma inclusum N.E.Br.	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	+
Octopoma nanum (L.Bolus) Klak	–	–	8	+	+	+	–	–	–	–
Octopoma octojuge N.E.Br.	–	–	8	+	+	+	–	–	–	–
Octopoma quadrisepalum (L.Bolus) H.E.K.Hartmann	–	–	8	+	+	+	–	–	–	–
Octopoma subglobosum (L.Bolus) L.Bolus	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	–
Octopoma tetrasepalum (L.Bolus) H.E.K.Hartmann	+	–	7–9	–	+	–	–	+ decreasing in density away from the stomata	–	+
Schlechteranthus albiflorus (L.Bolus) Klak	+	–	10–12	+	+ on anticlinal wall	–	–	+	+	+
Schlechteranthus hallii L.Bolus	+	–	10–12	+	+ on anticlinal wall	–	–	+	+	+
Schlechteranthus maximiliani Schwantes	+	–	10–12	+	+ on anticlinal wall	–	–	+	+	+

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in *Arenifera pillansii*, *A. pungens*, *A. spinescens*, *Octopoma abruptum*, *O. connatum*, *O. nanum*, *O. octojuge*, *O. quadrisepalum*, *O. subglobosum*, *O. tetrasepalum* (Fig. 2D, F). In the second form the stomata are sunken in the depressions and protected by parastomal cells which overarch the guard cells. This form was observed in *Arenifera* sp. nov., *A. stylosa*, *Octopoma inclusum*, *O. tetrasepalum*, *Schlechteranthus albiflorus*, *S. hallii*, *S. maximiliani* (Fig. 2C).

■ DISCUSSION

Polyphyly of *Octopoma*. — When described by Brown (1930), the genus *Octopoma* was contrasted only to the similarly multilocular genus *Leipoldtia* and distinguished by the connate leaves, contiguous expanding keels and absence of valve wings. Subsequently, additional species have been described, based mainly on the 8-locular capsules and connate leaves, however, the absence of valve wings and keel characters are no longer diagnostic for the genus. The artificial nature of the genus was noted by Hartmann (1996) and Klak (2010), who speculated that *Octopoma* may not be monophyletic. Two groups were identified by Hartmann (1996) based on capsule morphology, with the first group comprised of *O. octojuge*, *O. quadrisepalum* and *O. subglobosum* with valve wings present and large closing bodies. The second group, *O. abruptum*, *O. connatum*, *O. inclusum*, and *O. tetrasepalum*, possess small closing bodies and lack valve wings. Klak (2010), however, hypothesised a slightly different division based on geographical groupings, the first for those in the Little Karoo (*O. nanum*, *O. octojuge*, *O. quadrisepalum*) and the second for those in Namaqualand (*O. abruptum*, *O. connatum*, *O. inclusum*, *O. subglobosum*, *O. tetrasepalum*). Only two species (*O. octojuge*, *O. subglobosum*), both from Hartmann's (1996) first group but representing the two geographical groupings of Klak (2010), were included in the phylogeny of Klak & al. (2013). The two species were not recovered together but rather allied with very different clades. In all of our phylogenetic analyses (Fig. 1; Electr. Suppl.: Figs. S1, S2), *Octopoma* is confirmed as polyphyletic, with species allocated to one of two clades, corresponding to the two main geographical disjunctions as suggested by Klak (2010). The Namaqualand species (*O. connatum*, *O. inclusum*, *O. quadrisepalum*, *O. subglobosum*, *O. tetrasepalum*) were placed in the *Conophytum*-clade (PP = 0.99, BS = 69), while the Little Karoo species (*O. octojuge*, the type of the generic name, and *O. nanum*), were placed sister to *Zeuktophyllum* and *Smicrostigma* (PP = 0.88) in a subclade in the xeromorphic clade (Fig. 1; Klak & al., 2013).

The Little Karoo species of *Octopoma* (together with a third species not included in the phylogenies, *O. quadrisepalum*)

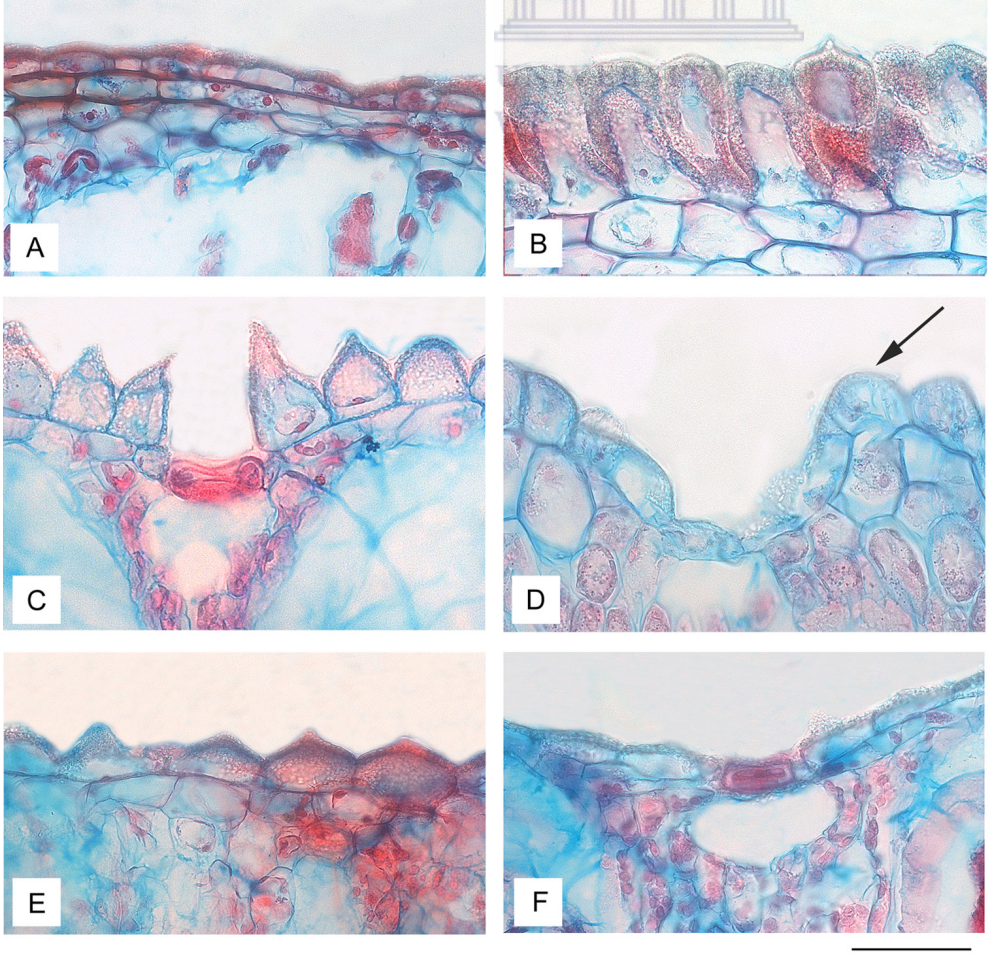

Fig. 2. Transverse sections through the leaves of *Arenifera*, *Octopoma* and *Schlechteranthus* species showing characters of taxonomic importance. **A,** Hypodermis found in southern *Octopoma* species, *O. nanum*; **B,** Prominently thickened and anticlinally elongated oblong epidermal cells in *Schlechteranthus* subg. *Schlechteranthus*, *S. hallii*; **C,** Stomata in depression, sunken and hidden by parastomal cell, *O. inclusum*; **D,** Bladder cells (arrow) in *A. pillansii*; **E,** Papillate epidermis in *Schlechteranthus* subg. *Microphyllus*, *Arenifera pungens* (= *Schlechteranthus pungens*); **F,** Stomata in depression, not sunken or hidden, *O. connatum*. — Vouchers: A, *Klak 2426* (BOL); B, *Powell 71* (NBG); C, *Powell 35* (NBG); D, *Bruyns 9136* (BOL); E, *Powell 28* (NBG); F, *Powell 10* (NBG). — Scale = 50 µm.

differ from their congeners in leaf characters, namely the absence of a prominent mucro, smooth epidermal cells, and the presence of a hypodermis in the lamina (Fig. 2A). The capsules are also distinguished by the larger closing bodies (blocking ⅝ of the locule). In contrast, the Namaqualand species have leaves with a prominent mucro, epidermal cells that form blunt papillae and a lamina without a hypodermis (Fig. 2C, F). The capsules also have smaller closing bodies blocking ⅓ of the locule.

The Little Karoo *Octopoma* (hereafter referred to as *Octopoma* s.str.) is recovered with *Zeuktophyllum* and *Smicrostigma*, both also Little Karoo endemics, forming a small, albeit weakly supported clade (PP = 0.88). These three genera (*Octopoma* s.str., *Smicrostigma*, *Zeuktophyllum*) share solitary flowers without a hypanthium, multilocular capsules with covering membranes (Hartmann, 2001) and sunken stomata (Ihlenfeldt & Hartmann, 1982), although only slightly sunken in *Zeuktophyllum*. In fact, *Zeuktophyllum calycinum* (L.Bolus) H.E.K.Hartmann was originally described in *Octopoma* (Hartmann, 2001). However, *Zeuktophyllum* differs from *Octopoma* s.str. in capsule morphology, with 10 locules (8 or 9 in *Octopoma* s.str.) and the presence of funicular hairs (absent in *Octopoma* s.str.). The presence of closing bodies distinguishes *Octopoma* s.str. further, as they are absent in both *Zeuktophyllum* and *Smicrostigma* (Hartmann, 2001).

Polyphyly of *Arenifera*. — *Arenifera* was recovered as polyphyletic in all three of the phylogenetic analyses (Fig. 1; Electr. Suppl.: Figs. S1, S2), with the type, *A. pillansii* (*Arenifera* s.str.), recovered in the xeromorphic clade (PP = 0.74, Fig. 1) and the remaining, non-sticky *Arenifera* species (*A. pungens*, *A. spinescens*, *Arenifera* sp. nov., *A. stylosa*) placed in the *Conophytum*-clade (PP = 0.99, BS = 69). *Arenifera pillansii* is readily distinguished from its congeners by the sticky, non-papillate leaf surface, prominent bladder cells (Fig. 2D), solitary flowers and 4-lobed calyces. The leaf surfaces of the other species are not sticky, with epidermal cells that form blunt papillae (similar to those found in Namaqualand *Octopoma* and *Schlechteranthus*), inconspicuous bladder cells and flowers in 3-flowered dichasia with 5- or 6-lobed calyces.

The prominently sticky leaves of *Arenifera pillansii* suggest an affinity with the similarly sticky-leaved genus *Psammophora* (Bolus, 1927; Herre, 1948; Hartmann, 1996). However, this relationship was not recovered in any of the phylogenetic trees. As in Klak & al. (2013), *Psammophora* is placed within the *Dracophilus*-clade (collapsed in Fig. 1), while *Arenifera pillansii* (*Arenifera* s.str.) is recovered within the xeromorphic clade (PP = 0.74, Fig 1). *Arenifera* s.str. is retained here as a monotypic genus pending further investigation of its allies.

Expansion of *Schlechteranthus*. — The non-sticky species of *Arenifera* and Namaqualand species of *Octopoma* were recovered as a subclade (hereafter referred to as the *O. subglobosum–A. spinescens* subclade, PP = 1.0, JK = 61, BS = 92) within the *Conophytum*-clade (PP = 97, BS = 69, Fig. 1), with species of both the non-sticky *Arenifera* and Namaqualand *Octopoma* interspersed within one another (Fig. 1). Excluding the spinescent inflorescences of *Arenifera* (Fig. 3E), these species are indistinguishable and share a number of morphological and anatomical characters. They are generally compact subshrubs (100–400 mm in height; Fig. 3A, H), with small leaves, epidermal cells forming blunt papillae, capsules with 7 to 9 locules with closing bodies that block ⅓ of the locule (Fig. 3D). Together this *O. subglobosum–A. spinescens* subclade (PP = 1, JK = 61, BS = 92) is sister to *Schlechteranthus* (PP = 0.97, BS = 57).

Schlechteranthus, as currently circumscribed (Klak & Bruyns, 2016), is poorly distinguished from the species of the *O. subglobosum–A. spinescens* subclade. *Schlechteranthus* is distinguished by the larger leaves (5–30 × 4.5–9.0 mm long), larger capsules (6–11 × 4–9 mm) with more locules (10–12) and larger closing bodies (blocking ¾ of the locule; Fig. 3C), whereas species of the *O. subglobosum–A. spinescens* subclade have smaller leaves (3.5–20.0 × 1.5–4.5 mm long), smaller capsules (2–6 × 2–6 mm) with fewer locules (7–9) and smaller closing bodies (blocking ⅓ of locule). The epidermal cells also differ, with those of *Schlechteranthus* anticlinally elongated with calcium oxalate crystals deposited on the anticlinal walls (Fig. 2B), while those of the *O. subglobosum–A. spinescens* subclade are isodiametric with few crystal deposits (Fig. 2C, E, F). The leaf surface of *Schlechteranthus* species appears to be only slightly papillate, with the outer wall of the epidermal cells forming blunt papillae that are only concentrated around the stomata or conspicuously raised throughout (as in *S. holgatensis* Klak). The leaf surface of species in the *O. subglobosum–A. spinescens* species appears more papillate, as the papillae extend away from the stomata, but decrease in density. *Schlechteranthus* species are also generally larger shrubs (up to 450 mm in height; Fig. 3F), while species of the *O. subglobosum–A. spinescens* subclade are generally smaller shrubs (100–400 mm in height) (Fig. 3A, H).

The species of *Schlechteranthus* and the *O. subglobosum–A. spinescens* subclade do, however, share epidermal cells that form blunt papillae, which is also shared with a number of other genera in the *Conophytum*-clade (*Cheiridopsis*, *Conophytum*, *Ihlenfeldtia*, *Odontophorus*). The species are distinguished from other genera in the *Conophytum*-clade by a combination of features. They are woody shrubs with prominently mucronate leaves (inconspicuous in *S. maximiliani*) and epidermal cells forming blunt to conspicuously raised papillae (Fig. 3G), white to magenta flowers with few to many filamentous staminodes surrounding the stamens in a loose cone, with anthers arranged in a cone (Fig. 3B), and multilocular capsules (7–12 locules). Upon assessment of generic circumscription in the *Schlechteranthus*-clade, it is apparent that there are two species groups; one comprised of species of the *O. subglobosum–A. spinescens* subclade, and the other comprised of species of *Schlechteranthus* s.str. However, the distinguishing characters for these two groups are largely size-dependant (apart from locule number), i.e., leaf size, capsule size, closing body size and general shrub size. Therefore, based on the phylogenetic results, morphological and anatomical examination of the species, the circumscription of *Schlechteranthus* s.str. is here expanded to include the species of the *O. subglobosum–A. spinescens* subclade. As such *Schlechteranthus* s.l. is defined as a genus of woody shrubs, with prominently (rarely inconspicuous) mucronate leaves and epidermal cells forming

blunt to conspicuously raised papillae, white to magenta flowers with few to many filamentous staminodes surrounding the stamens in a loose cone, with anthers arranged in a cone and multilocular capsules (7–12 locules). The two main subgroups within the expanded genus are accommodated as subgenera. Subgenus *Schlechteranthus* includes the species with large capsules (6–11 × 4–9 mm) with large closing bodies (blocking >½ of locule), and epidermal cells anticlinally elongated with calcium oxalate crystals deposited on the anticlinal walls, and subg. *Microphyllus* R.F.Powell includes the species with small capsules (2–6 × 2–6 mm), small closing bodies (blocking <½ of locule) and epidermal cells isodiametric, without crystals on the anticlinal walls.

Species of *Schlechteranthus* s.l. are further distinguished from species in the *Conophytum*-clade in their woody, shrubby habit. Although *Enarganthe* and *Namaquanthus* share this

Fig. 3. Morphological characters of *Schlechteranthus* s.l. **A,** Compact shrub, *Octopoma inclusum*; **B,** Flowers with anthers collected in a cone, *Octopoma subglobosum*; **C,** The larger capsule with large closing bodies, closing ¾ of seed exit in *Schlechteranthus* subg. *Schlechteranthus*, *S. maximiliani*; **D,** The smaller capsules of *Schlechteranthus* subg. *Microphyllus* with smaller closing bodies which close ⅓ of seed exit, *Octopoma connatum*; **E,** The 3-diachasia inflorescence forming spines in *Arenifera pungens*; **F,** The densely leafy, larger-leaved *Schlechteranthus hallii*; **G,** The papillate leaves with prominent mucro, *Octopoma subglobosum*; **H,** The spiny shrub *Arenifera pungens.* — Photographs: A & C–H, R.F. Powell; B, P. Burgoyne.

shrubby habit, *Schlechteranthus* s.l. is distinguished from *Namaquanthus* by the presence of closing bodies (absent in *Namaquanthus*) and from *Enarganthe* by the trigonous leaves that are fused at the base (leaves cylindrical and not fused at the base in *Enarganthe*). The large multilocular capsules and leaf mucro are also shared with some genera in the *Conophytum*-clade (*Cheiridopsis*, *Conophytum*, *Ihlenfeldtia*, *Odontophorus*), however, *Schlechteranthus* s.l. is distinguished by the non-sheathing leaves (persistent partly or fully sheathing in *Cheiridopsis*, *Conophytum* and *Ihlenfeldtia*) and the woody non-caespitose shrubby habit with stems that are never reduced (caespitose shrublets with highly reduced stems in *Cheiridopsis*, *Conophytum*, *Ihlenfeldtia* and *Odontophorus*).

Key to the multilocular (≥6) taxa of Ruschieae with an erect shrubby habit, xeromorphic leaf epidermis and capsules with closing bodies

1. Capsules 6–8(9)-locular …… 2
1. Capsules (9)10–18-locular …… 9
2. Leaves large and chunky, 25–70(–120) mm long, 5–30 mm broad and thick …… 3
2. Leaves small and slender, 3–25 mm long, 4–6 mm broad and thick …… 6
3. Flowers in annually enlarged, persistent inflorescences … …… ***Ottosonderia***
3. Inflorescences not persistent, but formed and ripening annually, new ones formed every year …… 4
4. Base of capsules shallow, bowl-shaped; closing bodies stalked and large, blocking the exit of the locules …… …… ***Antimima***
4. Base of capsules deep and funnel-shaped; closing bodies hook-shaped, small, not blocking exit of locules …… 5
5. Calyx 6-lobed; capsules mostly 6-locular, valves open and close repeatedly …… ***Astridia***
5. Calyx 4-lobed; capsules (5)6–8-locular (within a specimen), valves opening fully, but not closing completely again …… ***Stayneria***
6. Leaf surface sticky, with sand adhering to the surface with age; calyx 4-lobed …… ***Arenifera*** s.str.
6. Leaf surface never sticky, free of sand; calyx 4–6-lobed .7
7. Base of capsules shallow and bowl-shaped; capsules 6-locular …… ***Antimima***
7. Base of capsules deep and funnel-shaped; capsules 6–8(9)-locular …… 8
8. Leaves with smooth epidermis; hypodermis present; closing bodies large, blocking the exit of locules; old peduncles never forming spines …… ***Octopoma*** s.str.
8. Leaves with epidermal cells forming blunt to conspicuously raised papillae; hypodermis absent; closing bodies small, not blocking the exit of locules; sometimes old peduncles forming spines …… …… ***Schlechteranthus*** subg. ***Microphyllus***
9. Densely branched shrubs; leaves with a prominent mucro, rarely inconspicuous …… …… ***Schlechteranthus*** subg. ***Schlechteranthus***
9. Loosely branched shrubs; leaves without a mucro ….. 10
10. Leaves heterophyllous; branches rarely erect, more often spreading to climbing …… ***Vanzijlia***
10. Leaves homophyllous; branches erect or ascending … 11
11. Flowers pink (rarely white or yellow); base of capsule deep and funnel-shaped …… ***Leipoldtia***
11. Flowers yellow; base of capsule shallow and bowl-shaped …… ***Cephalophyllum***

■ TAXONOMIC TREATMENT

1. ***Arenifera*** A.G.J.Herre in Sukkulentenkunde 2: 35. 1948 – Type: *Arenifera pillansii* (L.Bolus) A.G.J.Herre. (≡ *Psammophora pillansii* L.Bolus).

Arenifera is here reduced to a monotypic genus restricted to the Richtersveld and characterised by its shrubby habit, sticky leaves without an apical mucro, prominent bladder cells, solitary flowers with 4-lobed calyces, with old peduncles forming blunt spines and multilocular capsules with covering membranes and conspicuous rodlet-shaped closing bodies.

Species. – *Arenifera pillansii* (L.Bolus) A.G.J.Herre.

2. ***Octopoma*** N.E.Br. in Gard. Chron., ser. 3, 87: 72, in clavi, 126, in clavi. 1930 – Type: *Octopoma octojuge* N.E.Br.

Octopoma is here reduced to include only three species centred in the Little Karoo. The genus is distinguished by the shrubby habit, leaves with a smooth epidermis, absence of a prominent mucro with a hypodermis, and capsules with 6–8 locules and large closing bodies (blocking ⅝ of locule).

Species. – *Octopoma nanum* (L.Bolus) Klak, *O. octojuge* N.E.Br., *O. quadrisepalum* (L.Bolus) H.E.K.Hartmann.

3. ***Schlechteranthus*** Schwantes in Monatsschr. Deutsch. Kakteen-Ges. 1: 16. 1929, emend. nov. R.F. Powell – Type: *Schlechteranthus maximiliani* Schwantes.

= *Polymita* N.E.Br. in Gard. Chron., ser. 3, 87: 72, in clavi. 1930 – Type: *Polymita pearsonii* N.E.Br. = *P. albiflora* (L.Bolus) L.Bolus.

The circumscription of *Schlechteranthus* is expanded here to include five species of *Octopoma* and four species of *Arenifera*. As such it now comprises 14 species, with a distribution centred in Namaqualand, extending from Clanwilliam and the Tanqua Karoo, northwards into the Richtersveld. The genus is distinguished by the shrubby habit, leaves with a prominent mucro (rarely inconspicuous) with epidermal cells forming blunt to conspicuously raised papillae, white to magenta flowers with few to many filamentous staminodes surrounding the stamens in a loose cone, with anthers arranged in a cone and 7–12-locular capsules. Two subgenera are recognised to accommodate the differences in epidermal cell shape, cuticle thickness, locule number and variation in shrub, leaf and capsule size.

3.1 ***Schlechteranthus*** subg. ***Schlechteranthus***

The subgenus is distinguished by the large leaves (5–30 × 4.5–9.0 mm), large capsules (6–11 × 4–9 mm) with 10–12 locules, and larger closing bodies that block ¾ of the locule. It is

further distinguished by the anticlinally elongated epidermal cells with calcium oxalate crystal deposits on the anticlinal walls. The outer walls of the epidermal cells form blunt to conspicuously raised papillae which are only concentrated around the stomata. This results in the leaf appearing only slightly papillate. Six species are accommodated in the subgenus (Klak & Bruyns, 2016) distributed from the Kamiesberg north into the Richtersveld.

Species. – *Schlechteranthus albiflorus* (L.Bolus) Klak, *S. diutinus* (L.Bolus) Klak, *S. hallii* L.Bolus, *S. holgatensis*, *S. maximiliani* Schwantes, *S. steenbokensis* (H.E.K.Hartmann) Klak.

3.2 ***Schlechteranthus*** subg. ***Microphyllus*** R.F.Powell, **subg. nov.** – Type: *Schlechteranthus pungens* (H.E.K.Hartmann) R.F.Powell (≡ *Arenifera pungens* H.E.K.Hartmann).

The subgenus can be distinguished by the smaller leaves (3.5–20 × 1.5–4.5 mm), smaller capsules (2–6 × 2–6 mm) with 7 to 9 locules and small closing bodies that block ⅓ of the locule. The epidermal cells also differ in that they are isodiametric with no crystals deposited on the anticlinal wall. The outer walls of the epidermal cells form blunt papillae which are concentrated around the stomata but extend away from the stomata, decreasing in density. This results in the leaf appearing more papillate than in subg. *Schlechteranthus.* Eight species are accommodated within the subgenus occurring from Matjiesfontein northwards into the Richtersveld.

Schlechteranthus abruptus (A.Berger) R.F.Powell, **comb. nov.** ≡ *Mesembryanthemum abruptum* A.Berger in Bot. Jahrb. Syst. 57: 638. 1922 ≡ *Octopoma abruptum* (A.Berger) N.E.Br. in Gard. Chron., ser. 3, 87: 126. 1930 – Holotype: South Africa, Western Cape Province, Brandewynrivier, between Clanwilliam and Calvinia, *Schlechter 10828* (BOL barcode BOL134021!).

= *Ruschia rupigena* L.Bolus, Notes Mesembryanthemum 3: 415. 1933 ≡ *Octopoma rupigenum* (L.Bolus) L.Bolus in J. S. African Bot. 33: 306. 1967 **syn. nov.** – Holotype: South Africa, Western Cape Province, near Pakhuis, Clanwilliam Div., common on rocks between Pakhuis and Buishoekfontein, *L. Bolus 1504/33* (BOL barcode BOL134031!).

Schlechteranthus connatus (L.Bolus) R.F.Powell, **comb. nov.** ≡ *Ruschia connata* L.Bolus, Notes Mesembryanthemum 1: 139. 1928 ≡ *Octopoma connatum* (L.Bolus) L.Bolus in J. S. African Bot. 29: 49. 1963 – Holotype: South Africa, Northern Cape Province, between Doornpoort and Brakfontein, *Pillans 5794* (BOL barcode BOL134023!).

Schlechteranthus inclusus (L.Bolus) R.F.Powell, **comb. nov.** ≡ *Mesembryanthemum inclusum* L.Bolus in Ann. Bolus Herb. 4: 40. 1926 ≡ *Octopoma inclusum* (L.Bolus) N.E.Br., Gard. Chron., ser. 3, 87: 126. 1930 – Holotype: South Africa, Northern Cape Province, Slopes overlooking the sea, south of Hondeklip Bay, Namaqualand, *Pillans 17758* (BOL barcode BOL134025!).

Schlechteranthus pungens (H.E.K.Hartmann) R.F.Powell, **comb. nov.** ≡ *Arenifera pungens* H.E.K.Hartmann in Bradleya 14: 37. 1996 – Holotype: South Africa, Northern Cape Province, Namaqualand, *Hartmann*, *Dehn*, *Gölling*, *Rust & Stüber 25739* (HBG barcode HBG900700 [photo!]).

Schlechteranthus spinescens (L.Bolus) R.F.Powell, **comb. nov.** ≡ *Ruschia spinescens* L.Bolus, Notes Mesembryanthemum 2: 175. 1930 ≡ *Arenifera spinescens* (L.Bolus) H.E.K.Hartmann in Bradleya 14: 38. 1996 – Holotype: South Africa, Western Cape Province, Whitehill near Matjiesfontein, Laingsburg, *Compton 19081* (BOL barcode BOL129577!).

Schlechteranthus stylosus (L.Bolus) R.F.Powell, **comb. nov.** ≡ *Ruschia stylosa* L.Bolus, Notes Mesembryanthemum 1: 144. 1928 ≡ *Arenifera stylosa* (L.Bolus) H.E.K.Hartmann in Bradleya 14: 38. 1996 – Holotype: South Africa, Northern Cape Province, hills N.E. of Arris Drift, *Pillans 5742* (BOL barcode BOL129579!).

Schlechteranthus subglobosus (L.Bolus) R.F.Powell, **comb. nov.** ≡ *Ruschia subglobosa* L.Bolus, Notes Mesembryanthemum 1: 140. 1928 ≡ *Octopoma subglobosum* (L.Bolus) L.Bolus in J. S. African Bot. 29: 49. 1963 – Holotype: South Africa, Northern Cape Province, hills on north side of O'kiep, Little Namaqualand, *Pillans 5844* (BOL barcode BOL134032!).

Schlechteranthus tetrasepalus (L.Bolus) R.F.Powell, **comb. nov.** ≡ *Ruschia tetrasepala* L.Bolus, Notes Mesembryanthemum 2: 373. 1932 ≡ *Octopoma tetrasepalum* (L.Bolus) H.E.K.Hartmann in Bradleya 16: 74. 1998 – Holotype: South Africa, Western Cape Province, between the town and [the] Sout River, *Luckhoff sub BOL 20203* (BOL barcode BOL134033!).

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Appendix 1. Voucher information for leaf material studied in transverse section.

Voucher information is listed as taxon, voucher information.

Arenifera A.G.J.Herre: ***A. pillansii*** (L.Bolus) A.G.J.Herre, *Bruyns 9136* (BOL); ***A. pungens*** H.E.K.Hartmann, *Powell 28* (NBG); ***Arenifera* sp. nov.**, *Powell 41* (NBG); ***A. spinescens*** (L.Bolus) H.E.K.Hartmann, *Klak 2424* (BOL); ***A. stylosa*** (L.Bolus). H.E.K.Hartmann, *Powell 75* (NBG); ***Octopoma*** N.E.Br.: ***O. abruptum*** N.E.Br., *Powell 5* (NBG); ***O. connatum*** (L.Bolus) L.Bolus, *Powell 10* (NBG); ***O. inclusum*** N.E.Br., *Powell 35* (NBG); ***O. nanum*** (L.Bolus) Klak, *Klak 2426* (BOL); ***O. octojuge*** N.E.Br., *Bohnen 8924* (NBG); ***O. quadrisepalum*** (L.Bolus) H.E.K.Hartmann, *Gwynn-Evans 155.2* (NBG); ***O. subglobosum*** (L.Bolus) L.Bolus, *Powell 33* (NBG); ***O. tetrasepalum*** (L.Bolus) H.E.K.Hartmann, *Klak 2411* (BOL); ***Schlechteranthus*** Schwantes: ***S. albiflorus*** (L.Bolus) Klak, *Van Jaarsveld 4236* (NBG); ***S. hallii*** L.Bolus, *Powell 71* (NBG); ***S. maximiliani*** Schwantes, *Powell 48* (NBG).

Appendix 2. New accessions for which cpDNA sequence data were obtained, with corresponding voucher information and GenBank accession numbers.

Taxon, voucher information, *atpB*, *psbJ*, *matK*, *rpl16*, *rps16*, *trnD*, *trnL*, *trnQ*, *trnS*.

Arenifera A.G.J.Herre: ***A. pillansii*** (L.Bolus) A.G.J.Herre, *Bruyns 9136* (BOL), KT248239, KT248248, KT248260, KT248267, KT248278, KT248290, KT248302, KT248314, KT248326. ***A. pungens*** H.E.K.Hartmann, *Powell 28* (NBG), KT248240, KT248249, KT281447, –, KT248279, KT248291, KT248303, KT248315, KT248327. ***Arenifera* sp.nov.**, *Powell 41* (NBG), –, KT248251, KT281448, KT248269, KT248281, KT248293, KT248305, KT248317, KT248329. ***A. spinescens*** (L.Bolus) H.E.K.Hartmann, *Klak 2424* (BOL), KT248241, KT248250, KT248261, KT248268, KT248280, KT248292, KT248304, KT248316, KT248328. ***A. stylosa*** (L.Bolus). H.E.K.Hartmann, *Powell 75* (NBG), KT248242, KT248252, KT248262, KT248270, KT248282, KT248294, KT248306, KT248318, KT248330. ***Leipoldtia*** L.Bolus: ***L. gigantea*** Klak, *Klak 1275* (BOL), KT248243, KT248253, KT248263, KT248271, KT248283, KT248295, KT248307, KT248319, KT248331. ***Leipoldtia* sp.**, *Klak 2406* (BOL), KT248244, KT248254, KT248264, KT248272, KT248284, KT248296, KT248308, KT248320, KT248332. ***Octopoma*** N.E.Br.: ***O. abruptum*** N.E.Br., *Powell 5* (NBG), KT248246, KT248258, KT281451, KT248276, KT248288, KT248300, KT248312, KT248324, KT248335. ***O. connatum*** (L.Bolus) L.Bolus, *Powell 10* (NBG), KT248255, –, KT281449, KT248273, KT248285, KT248297, KT248309, KT248321, –. ***O. inclusum*** N.E.Br., *Powell 35* (NBG), –, KT248256, KT281450, KT248274, KT248286, KT248298, KT248310, KT248322, KT248333. ***O. nanum*** (L.Bolus) Klak, *Klak 2426* (BOL), KT248245, KT248257, KT248265, KT248275, KT248287, KT248299, KT248311, KT248323, KT248334. ***O. tetrasepalum*** (L.Bolus) H.E.K.Hartmann, *Klak 2411* (BOL), KT248247, KT248259, KT248266, KT248277, KT248289, KT248301, KT248313, KT248325, KT248336.

APPENDIX E2. **Powell RF, Boatwright JS, Klak C, Magee AR.** Inclusion of *Ihlenfeldtia* and *Odontophorus* in *Cheiridopsis* (Ruschioideae; Aizoaceae) with insights into generic and subgeneric circumscriptions within the *Conophytum*-clade. *Botanical Journal of the Linnean Society.* **Accepted**.

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Inclusion of *Ihlenfeldtia* and *Odontophorus* in *Cheiridopsis* (Ruschioideae; Aizoaceae) with insights into generic and subgeneric circumscriptions within the *Conophytum*-clade

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ABSTRACT

Phylogenetic sampling in the *Conophytum*-clade was expanded to include a comprehensive sampling of almost all species in the clade (excluding *Conophytum,* which was representatively sampled) for six plastid regions (*matK, rpl16, rps16, trnL-F, trnQ-rps16, trnS-trnG*). The combined phylogenetic data was analysed using maximum parsimony, maximum likelihood and Bayesian inference. The expanded sampling recovered a strongly supported sister relationship between *Cheiridopsis* and *Conophytum* in the phylogenetic

analyses. *Cheiridopsis* was however not recovered as monophyletic, with *Ihlenfeldtia* and *Odontophorus* embedded within. In *Cheiridopsis,* three clades were recovered, largely corresponding to the three subgenera currently recognised. *Odontophorus* was recovered within the *Cheiridopsis* subgenus *Odontophoroides* clade and *Ihlenfeldtia* within the *Cheiridopsis* subgenus *Aequifoliae* clade. *Cheiridopsis* was here expanded to include *Ihlenfeldtia* and *Odontophorus,* with four new combinations in *Odontophorus* included. Three species previously classified in *Cheiridopsis* subgenus *Aequifoliae* (*C. glomerata, C. pillansii, C. purpurea*) were recovered rather within the subgenus *Odontophoroides* clade, with which they share the erect capsules with rounded tops, and were accordingly here included in this subgenus. A detailed study on morphological characters was conducted for all the species in the *Conophytum*-clade, to aid the assessment of generic circumscription and morphological variation within the clade. The old leaves form sheaths that enclose the emerging leaf pairs, was recovered as a synapomorphic character for the *Conophytum-Cheiridopsis* clade. Two capsule morphologies were found across the *Conophytum*-clade, i.e. simple capsules without covering membranes and closing bodies, and complex multilocular capsules with covering membranes and closing bodies. A study of taxonomically informative leaf anatomical sections revealed that the majority of genera in the *Conophytum*-clade possessed papillae on the epidermis, ranging from blunt papillae to trichomes, with the exception of *Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus,* which were glabrous. Pollen exine structure was mostly uniform, i.e. tricolpate pollen with poorly-defined colpi, but in *Namaquanthus,* the exine was extensively micro-reticulate, while in *Enarganthe* pollen was tricolpate with well-defined colpi, and in *Jensenobotrya* pollen was syncolpate with multiple colpi, further separating these morphologically distinct monotypic genera. A key to the genera in the *Conophytum*-clade is provided, as well as an updated key to the subgenera of *Cheiridopsis.* Revised generic and subgeneric descriptions for

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Cheiridopsis, with appropriate nomenclatural changes, a list of recognised species, as well as species richness and distribution maps, for the re-circumscribed genus and subgenera are included.

KEYWORDS: *Cheiridopsis* subgenus *Aequifoliae*; *Cheiridopsis* subgenus *Odontophoroides*; leaf anatomy; morphology; papillate; phylogeny; sheathing.

INTRODUCTION

The classification of the Aizoaceae is heavily reliant on capsule morphology, often in combination with leaf and floral characters (Hartmann, 1991, 2001). Ihlenfeldt (1960) argued that capsule morphology provided more natural groupings than previous classifications based on leaf and floral morphology (Rappa & Camarrone, 1953; Haworth, 1975). Hartmann (1991) recognised twelve morphological groupings based mainly on capsule characters, and generic circumscriptions of the family were assessed within these groups. The weight given to capsule characters has sometimes resulted in unnatural classifications, particularly those based on indiscrete characters. For example, the species of *Ihlenfeldtia* H.E.K.Hartmann were separated from *Cheiridopsis* N.E.Br., and placed in a group with *Tanquana* H.E.K.Hartmann & Liede and *Vanheerdia* L.Bolus (Hartmann, 1992), based solely on the smaller closing bodies in the capsules. Subsequent phylogenetic data however places *Ihlenfeldtia* in a clade with *Cheiridopsis* and *Odontophorus* N.E.Br. (Klak *et al.,* 2013; Powell *et al.,* 2016).

Cheiridopsis is a genus of caespitose plants, characterised by the old leaves that form a sheath enclosing the emerging leaf pair, either completely or partially (Hartmann & Dehn, 1987). The genus was revised by Hartmann & Dehn (1987), with 23 species classified into three subgenera. Subsequently, an additional 12 species were described, with several species known from only the type locality (Hammer, 1994, 1996; Hartmann, 2001; Hammer &

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Desmet, 2002; Klak & *et al.,* 2015). Additionally, two species of *Cheiridopsis* were separated to form the new genus, *Ihlenfeldtia* (Hartmann, 1992).

Odontophorus was included in the same capsule morphological grouping as *Cheiridopsis* (Hartmann, 1991), and was last revised by Hartmann (1976). As the generic name suggests, *Odontophorus* has distinctly toothed keels on the leaves, however, this character is shared with some species of *Cheiridopsis* (Hammer, 1996) and the similarity of leaf and capsule morphology of these genera has been noted previously (Hammer, 1994; Klak *et al.,* 2015). In fact, *Cheiridopsis* subgenus *Odontophoroides* H.E.K.Hartmann, is named for its similarity to species of *Odontophorus* (Hartmann & Dehn, 1987).

Cheiridopsis, Ihlenfeldtia and *Odontophorus* all share a caespitose habit, papillate leaves that form sheaths enclosing the emerging leaf pair, radiate flowers with free petaloid staminodes and large woody, multilocular capsules (Hartmann, 2001). The genera are endemic to the Greater Cape Floristic Region of southern Africa, with diversity centred in the Springbok Region (Hartmann, 1976; Hartmann & Dehn, 1987; Hartmann, 1992). The genera are currently differentiated solely based on indiscrete internal capsule characters, with *Ihlenfeldtia* distinguished from *Cheiridopsis* based on the smaller closing bodies (Hartmann, 1992), while *Odontophorus* has narrower valve wings than *Cheiridopsis* (Hartmann, 2001). However, species of *Cheiridopsis* subgenus *Aequifoliae* H.E.K.Hartmann also possess narrow valve wings (Hartmann & Dehn, 1987), highlighting the overlap in characters between these genera.

The phylogenetic analyses of the Ruschieae (Klak *et al.,* 2013), places *Cheiridopsis, Ihlenfeldtia* and *Odontophorus* in the *Conophytum*-clade. This clade includes nine genera: *Cheiridopsis, Conophytum* N.E.Br., *Enarganthe* N.E.Br., *Ihlenfeldtia, Jensenobotrya* A.G.J.Herre, *Namaquanthus* L.Bolus, *Odontophorus, Ruschianthus* L.Bolus, and *Schlechteranthus* Schwantes (Klak *et al.,* 2013; Powell *et al.,* 2016). The genera include five

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of the nine morphological groups recognised by Hartmann for the Ruschieae (1991), illustrating the diversity in leaf, floral and capsule morphology of the clade. Due to this diversity in morphological characters, it has as yet not been possible to find a clear synapomorphy for the clade (Klak *et al.,* 2013). The genera do, however, share mostly multilocular capsules of the complex type, with covering membranes and closing bodies, with the exception of *Conophytum*, *Jensenobotrya* and *Ruschianthus,* which have simple capsules without covering membranes and closing bodies (Hartmann, 2001). The leaves in the majority of the genera are papillate and in *Conophytum, Cheiridopsis, Ihlenfeldtia* and *Odontophorus,* the old leaves form a sheath that encloses the emerging leaf pair, either completely or partially (Hartmann, 2001). The sheath almost always fully encloses the emerging leaf pair during the dormant period in *Conophytum,* with the exception of *C. herreanthus* S.A.Hammer, where the sheath remains basal (Hammer, 2002). This species is unusual and was previously recognised as a monotypic genus (viz. *Herreanthus* Schwantes), but is currently included in *Conophytum* based on the diagnostic fused petaloid staminodes that form a tube (Hammer, 1994).

Recent phylogenetic data, based on plastid DNA sequences, has revealed new relationships between the genera in the *Conophytum*-clade (Klak *et al.,* 2013), resulting in reassessment of generic limits within the clade (Powell *et al.,* 2016). Generic circumscription in the *Schlechteranthus* clade of the *Conophytum*-clade (Klak *et al*., 2013) was recently assessed (Klak & Bruyns, 2016, Powell *et al*., 2016). This clade included *Octopoma* N.E.Br., *Polymita* N.E.Br. and *Schlechteranthus*, and subsequently *Arenifera* A.G.J.Herre, a genus not included in the subfamilial phylogenetic sampling (Klak *et al.,* 2013; Klak & Bruyns, 2016, Powell *et al*., 2016). Based on the phylogenetic, morphological and anatomical data, the genera in this clade were all included in a re-circumscribed *Schlechteranthus* with two

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subgenera erected: subgenus *Schlechteranthus* and subgenus *Microphyllus* R.F.Powell (Klak & Bruyns, 2016, Powell *et al*., 2016).

Leaf anatomy has been used to inform a range of classifications, from subfamilial to generic, and has been noted to be of great taxonomic value in the subfamily and family (Reule, 1937; Ihlenfeldt & Bittrich, 1985; Klak & Linder, 1998; Landrum, 2001). Leaf anatomical data was shown to be an important character for circumscribing an expanded *Schlechteranthus* with the diagnostic characters of the subgenera based on a combination of leaf epidermal structure and capsule characters (Powell *et al.,* 2016). An anatomical study on the large genus *Conophytum,* also revealed the importance of leaf anatomical characters in subgeneric classification (Opel, 2005), which supported the subgeneric sections presented by Hammer (2002).

Although leaf anatomy has often been used to classify taxa in the family, pollen exine structure is seldom used. Bittrich (1987) and Dupont (1977) surveyed the pollen exine structure across the Mesembryanthemoideae and Ruschioideae, respectively, and found variation in ornamentation of the exine. In the Ruschioideae, the majority of species were tricolpate, but it was found that *Jensenobotrya* had syncolpate pollen (Dupont, 1977). Although the study also included some species of *Conophytum,* the exine structure of the remaining genera in the *Conophytum*-clade is unknown (Dupont, 1977).

In the present study, phylogenetic sampling of *Cheiridopsis, Ihlenfeldtia* and *Odontophorus* is expanded to include the majority of species in these genera, while sampling in *Conophytum* is expanded to represent the large sections (seven of sixteen sections) in the genus. Generic circumscription in the *Conophytum*-clade is investigated using phylogenetic, morphological, anatomical and palynological data. The relationship between *Cheiridopsis, Ihlenfeldtia* and *Odontophorus* is assessed and the subgeneric circumscription of *Cheiridopsis* refined.

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MATERIALS AND METHODS

PHYLOGENETIC DATA

Phylogenetic sampling in the *Conophytum*-clade (Klak *et al.,* 2013) was expanded by 175 new sequences for six plastid gene regions. This included 24 of the 32 species of *Cheiridopsis,* the two species of *Ihlenfeldtia*, two of the four species of *Odontophorus* and five species of *Conophytum,* representing seven (of 16) sections in the genus: *Conophytum bilobum* (Marloth) N.E.Br. (section *Biloba* N.E.Br.), *C. bruynsii* S.A.Hammer (section *Minuscula* (Schwantes) Tischer ex S.A.Hammer), *C. calculus* (A.Berger) N.E.Br. (section *Cataphracta* Schwantes ex S.A.Hammer), *C. ficiforme* N.E.Br. (section *Conophytum* N.E.Br.), *C. friedrichiae* Schwantes (section *Ophthalmophyllum* (Dinter & Schwantes) G.D.Rowley), *C. herreanthus* (section *Herreanthus* (Schwantes) S.A.Hammer) and *C. wettsteinii* N.E.Br. (section *Wettsteinia* (Schwantes) Tischer ex S.A.Hammer). Section *Herreanthus* and section *Ophthalmophyllum* represent two previously recognised genera now included in *Conophytum.* Material was collected in the field and several rare species were obtained from greenhouse collections at the Karoo Botanical Gardens, the South African National Biodiversity Institute, Kirstenbosch as well as from the Sphaeroid Institute (California, USA). The voucher specimens for these samples are housed at the Compton Herbarium (NBG). Appendix 1 provides voucher information for the samples as well as the GenBank accession numbers for the sequences.

Total DNA was extracted from silica-dried leaf material (0.2 mg) using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. Six plastid gene regions were amplified and sequenced. Nuclear regions were excluded as they have been shown to be problematic, indicating gene duplication or multiple copies, as found in Klak *et al.* (2013). A portion of the *trnQ*^{UUG}*-rps16* intergenic spacer was

amplified using the primers *trnQ*^{UUG} and *rps16x1* (Shaw *et al.,* 2007). The *trnS-trnG* intergenic region was amplified using the primers *trnS* and *trnG* (Hamilton, 1999). The *trnL–F* region (consisting of the adjacent *trnL* intron and *trnL–F* intergenic spacer) was amplified with primers c and f (Taberlet *et al.,* 1991). The *rps16* region was amplified using the primers *rps16F* and *rps16R2* (Oxelman *et al.,* 1997). The *rpl16* intron was amplified using primers *rpl16* 71F (Jordan *et al.,* 1996) and *rpl16* 1516R (Kelchner & Clark, 1997). A portion of the *matK* gene was amplified using DNA barcoding primers 3F-Kim and 1R-Kim (Cuenoud *et al.,* 2002) for 19 species, while *matK* was sequenced at the Canadian Centre for DNA Barcoding (Guelph, Canada) for the remaining 11 species (*Cheiridopsis aspera* L.Bolus*, C. denticulata* (Haw.)N.E.Br.*, C. meyeri* N.E.Br.*, C. namaquensis* (Sond.) H.E.K.Hartmann*, C. peculiaris* N.E.Br.*, C. schlechteri* Schwantes*, Conophytum bilobum, C. ficiforme, C. friedrichiae, C. herreanthus, C. wettsteinii*), to contribute towards the International Barcode of Life project (iBOL, 2014).

Polymerase chain reactions (PCRs) were performed in 25 µl reactions containing 22.5 µl Thermo Scientific 1.1X ReddyMix PCR Master Mix (Thermo Fischer Scientific, Inc.), 0.8 µl Bovine Serum Albumin, 0.6 µl sterile distilled water, 0.3 µl of each primer and 0.5 µl of DNA template. The PCR reactions were carried out using the following thermal conditions: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 8 min. For samples that did not amplify successfully, the protocol was adjusted to include a temperature ramp following Shaw *et al.* (2005). Successfully amplified samples were cleaned using the ExoSAP protocol of Werle *et al.* (1994) using 5 units of Exonuclease I and 0.5 units of Shrimp Alkaline Phosphatase. Automated sequencing was carried out by Macrogen (Seoul, Korea). Electropherograms obtained from the sequences were manually

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checked and inconsistencies in the sequences were edited where necessary using MEGA version 6 (Higgins *et al.,* 1994).

The new sequences, together with published sequences of the *Conophytum*-clade from Klak *et al.* (2013) and Powell *et al.* (2016), were automatically aligned using the Clustal W function in MEGA version 6 (Higgins *et al.,* 1994; Tamura *et al.,* 2013). *Knersia diversifolia* (L.Bolus) H.E.K.Hartmann & Liede (= *Drosanthemum diversifolium* in Klak *et al.,* 2013) and *Jacobsenia vaginata* (L.Bolus) Ihlenf., were selected as outgroup taxa, as they were recovered as sister to the *Conophytum*-clade in the previous analyses (Klak *et al.,* 2013; Powell *et al.,* 2016). The alignment was checked manually and adjusted accordingly where necessary, with gaps positioned so as to minimize nucleotide mismatches. Hypervariable regions for *trnQ*^{UUG}*-rps16* (79 base pairs) and *trnS-trnG* (48 base pairs) were excluded from the analysis. Due to the low sequence divergence, the plastid matrices were analysed in combination, rather than separately, following previous phylogenetic studies in the family (Klak *et al.,* 2013; Powell *et al.,* 2016). The combined plastid dataset included a total of 4506 characters and were analysed using maximum parsimony, maximum likelihood and Bayesian inference.

The maximum parsimony (MP) algorithm was implemented in PAUP* version 4.0b4 (Swofford, 2000). Character transformations were unordered and equally weighted (Fitch, 1971). A heuristic search with 1000 random sequence additions, tree bisection reconnection (TBR) branch-swapping, and the MULPARS option selected, was performed. All character transformations were treated with equal likelihood and a maximum of 10 trees were saved in each replicate, to minimise swapping on local minima. Trees of the shortest length were saved and used as starting trees for a second round of TBR swapping with no limit on the number of trees saved, to ensure the shortest trees were recovered in the analysis. Node support was evaluated using the jackknife function in PAUP, with a full search, 1000

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replicates and a limit of 1000 trees per replicate (Farris *et al.,* 1996). Only jackknife support (JK) values greater than or equal to 50% were retained, and the following scale was used to evaluate support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Maximum likelihood (ML) analyses were performed using RAxML version 8.1.11 (Stamatakis, 2006) on the combined plastid data. The data was partitioned into 6 partitions and the analyses were completed on the CIPRES Portal, version 3.3 (Miller *et al.,* 2010), using the default settings. A ML tree with bootstrap node support (BS) was produced, with the following scale used to evaluate support percentages: 50–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Bayesian inference (BI) was performed on the combined plastid dataset using MrBayes 3.2.3 (Ronquist & Huelsenbeck, 2003). The analyses were run on the CIPRES Portal, version 3.3 (Miller *et al.,* 2010). Parameters were set in a Bayes block and the data were partitioned into 6 partitions. All parameters were unlinked (statfreq, revmat, shape, pinvar) between partitions. Separate matrices for the different gene regions were run in JModel test (Guindon & Gascuel, 2003; Darriba *et al.,* 2012) and the following models were suggested for the specific gene regions: HKY for *matK*, *trnL–F* and *rpl16* (Hasegawa *et al.,* 1985); F81 for *trnS-trnG* (Felsenstein, 1981) and F81+G for *rps16* and $trnQ^{UUG}$*-rps16* (Felsenstein, 1981) which were implemented accordingly to the specific regions in the analyses. Two simultaneous runs were completed for 3 500 000 generations with a sampling frequency of 100. The standard deviation between the split frequencies stabilised below 0.01, providing evidence that a sufficient number of generations had been completed. Using Tracer v.1.5 (Rambaut & Drummond, 2009), the stability of two runs were compared and the suboptimal trees were discarded as the "burn-in" phase. The remaining 7002 trees were used to construct a 50% majority rule consensus tree with posterior probabilities (PP). Only

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support values greater than or equal to 0.5 were retained, and the following scale used: 0.50–0.94, weak; and 0.95–1.0, strong.

A constraint topological analysis was conducted in PAUP (Swofford, 2000), using the constrained monophyly option. *Cheiridopsis* (including *Ihlenfeldtia* and *Odontophorus*) was constrained to monophyly, to test whether trees including the two outlying *Cheiridopsis* species (*C. pearsonii* N.E.Br. and *C. peculiaris*), placed outside of the main *Cheiridopsis* clade (see results section), were significantly different from those that did not include them in the genus. A Templeton-Wilcoxon test (Swofford, 2000) was run to test for a significant difference between the two trees produced under topological constrained and unconstrained parameters (Table 1).

LEAF ANATOMICAL DATA

Fresh leaf material was sampled from the field and greenhouse collections for anatomical study (Appendix 2). This included a representative sampling from the subgenera in *Cheiridopsis,* with seven species from subgenus *Cheiridopsis* N.E.Br.*,* six species from subgenus *Aequifoliae* and six species from subgenus *Odontophoroides.* Two species of *Ihlenfeldtia*, as well as one species of *Odontophorus* were also included. Fresh leaf material for *Enarganthe octonaria* (L.Bolus) N.E.Br. was obtained from field collections, while *Jensenobotrya lossowiana* A.G.J.Herre and *Namaquanthus vanheerdei* L.Bolus were sampled from greenhouse collections at Kirstenbosch. *Ruschianthus falcatus* L.Bolus was not relocated in the field, nor was a living collection of the species available. Leaf anatomical data for *Conophytum* and *Schlechteranthus* were obtained from literature (Opel, 2005; Powell *et al.,* 2016).

Fresh leaf material was fixed in formalin-aceto-alcohol (FAA). The FAA was prepared using 90 ml of 70% ethanol, 5 ml of 40% formalin and 5 ml of glacial acetic acid

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(De Neergaard *et al.,* 2001). The leaf material was embedded in parafin wax (Rudall, 1995), and 12–15 µm transverse sections from the central third of the leaf were cut using a Reichert-Jung autocut microtome (Model 2040). The sections were then double-stained with alcian blue and safranin. Permanent slides were made using Entellan, then viewed with a Zeiss compound microscope and photographed using an Olympus SC30 camera.

POLLEN EXINE STRUCTURE

Pollen samples were collected from greenhouse collections and herbarium specimens for 18 taxa across the *Conophytum*-clade (Appendix 3). These taxa were selected as they are representative of the genera in the *Conophytum*-clade, as well as representing the subgeneric classifications within the clade. This data was supplemented by previous palynological studies of the subfamily (Dupont, 1977).

The pollen was prepared for viewing using a light microscope and Glycerin jelly slides following Erdtman (1960). Glycerin jelly was prepared following Kearns *et al.* (1993). A small amount of the jelly was placed on a cleaned slide and the pollen was dusted onto it. A cover slip was then placed over the jelly and the slide was placed on a heated plate until just melted. The pollen was then viewed using a Zeiss compound microscope.

MORPHOLOGICAL CHARACTER MAPPING

Specimens of the *Conophytum*-clade examined for morphological study included the complete collections available at BOL, NBG and SAM.

The morphological character of sheathing (old leaves forming a sheath enclosing the emerging leaf pair) versus non-sheathing leaves, as well as sheath type, was coded manually and mapped onto the BI consensus tree, using the trace character history function in Mesquite version 3.04 (Maddison & Madisson, 2015).

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DISTRIBUTIONAL DATA

Distribution data for the species of *Cheiridopsis* s.l. (as treated in the taxonomic treatment, i.e. including *Ihlenfeldtia* and *Odontophorus*) was obtained from the 599 databased specimens in BOL, NBG and PRE. The distribution data of the species of *Cheiridopsis* was mapped in ArcMap (ESRI, 2011) for the genus collectively, as well as separately for the subgenera. A Quarter Degree Square (Edwards & Leistner, 1971; Leistner & Morris, 1976) grid was overlaid onto the distribution data and the Spatial Join tool (ESRI, 2011) was used to join the two datasets. The number of species per Quarter Degree Square (QDS) was calculated from the count data in the Spatial Join (ESRI, 2011). The Spatial Join analysis and count data was calculated separately for *Cheiridopsis* and each of the subgenera, with separate maps for the genus and subgenera produced.

RESULTS

PHYLOGENETIC ANALYSES

The combined plastid matrix for the six gene regions consisted of 4506 unambiguously aligned positions with 350 variable characters and 114 parsimony informative characters. In the MP analysis, 899 trees were retained with a tree length of 540 steps (consistency index (CI) = 0.90; retention index (RI) = 0.81). The phylogenies produced in the MP, ML and BI analyses were all consistent with one another in topology, with slight differences in resolution and support values (Fig. 1, Electronic Appendix 1 & 2). The phylogenetic tree recovered from the MP analysis was poorly resolved and showed relatively low nodal support values when compared to the other analyses (Electronic Appendix 1). The phylogenetic tree produced by the ML analysis provided a better resolved topology with relatively strong

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support values, especially within clades (Electronic Appendix 2). The BI analysis, however, provided the most resolved phylogenetic tree, with the strongest support values (Fig. 1).

Two main clades were recovered, one including the species of *Cheiridopsis, Conophytum, Ihlenfeldtia, Odontophorus* (PP=0.95, BS=54; Fig. 1, Fig. 2, Electronic Appendix 2) and the other contained the species of *Schlechteranthus* (PP=1, BS=100, JK=92; Fig. 1, Electronic Appendix 1 & 2). The placement of *Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus* remained unresolved within the *Conophytum*-clade (Fig. 1, Electronic Appendix 1 & 2).

Three clades were recovered within the *Conophytum–Cheiridopsis* clade, one comprising the species of *Conophytum* (PP=0.99, BS=71; Fig. 1, Electronic Appendix 1 & 2), the second including species of *Cheiridopsis, Ihlenfeldtia,* and *Odontophorus* (PP=1, BS=81, JK=54; Fig. 1, Electronic Appendix 1 & 2) and the third with *Cheiridopsis pearsonii* and *C. peculiaris* (PP=0.83, BS=59; Fig. 1, Electronic Appendix 1 & 2).

Cheiridopsis is rendered paraphyletic by the inclusion of *Ihlenfeldtia* and *Odontophorus,* as well as by the unresolved affinity of *C. pearsonii* and *C. peculiaris*. The sampled *Odontophorus* species were placed in a clade with the species of *Cheiridopsis* subgenus *Odontophoroides* (PP=1, BS=65, JK=73; Fig. 1, Electronic Appendix 1 & 2) and the genus was recovered as polyphyletic in this clade, with *O. angustifolius* L.Bolus placed sister to *C. pilosula* L.Bolus (PP=0.92, BS=91, JK=65; Fig. 1, Electronic Appendix 1 & 2) and *O. marlothii* N.E.Br. recovered as sister to *C. umdausensis* L.Bolus and *C. pillansii* L.Bolus (PP=0.84, BS=69, JK=51, Fig. 1, Electronic Appendix 1 & 2). The subgenus *Odontophoroides* clade also included three species of subgenus *Aequifoliae*, i.e. *C. glomerata* S.A.Hammer, *C. pillansii* and *C. purpurea* L.Bolus (Fig. 1, Electronic Appendix 1 & 2). The two species of *Ihlenfeldtia* were placed in a clade with species of subgenus *Aequifoliae*, but were unresolved within the clade (PP=0.99, BS=65, JK=51; Fig. 1, Electronic Appendix 1 &

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2). The remaining clade within the *Cheiridopsis* clade, is represented by species of subgenus *Cheiridopsis* (PP=0.99, BS=86, JK=61; Fig. 1, Electronic Appendix 1 & 2), excluding *C. pearsonii* and *C. peculiaris*

The constrained monophyly of *Cheiridopsis* placed *Cheiridopsis pearsonii* and *C. peculiaris* directly in the subgenus *Cheiridopsis* clade, with no significant difference between the constrained and unconstrained phylogenies (Templeton: p = 0.15, Winning-sites: p = 0.50) and a negligible difference of 2 steps in tree length for the constrained (tree length = 542 steps) and unconstrained (tree length = 540 steps) trees (Table 1).

LEAF ANATOMY

Leaf anatomical characters for the species examined are summarised in Table 2. A range of papillae formed by the epidermal cells were found across the taxa sampled, with the outer walls of epidermal cells in *Cheiridopsis aspera, C. caroli-schmidtii* (Dinter & A.Berger) N.E.Br., *C. gamoepensis* S.A.Hammer, *C. imitans* L.Bolus, *C. minor* (L.Bolus) H.E.K.Hartmann, *C. meyeri, C. namaquensis, C. peculiaris, C. umdausensis* forming blunt papillae (Fig. 2A), defined as low protuberances in the centre of the epidermal cell (Opel, 2005). The epidermal cells in the remainder of the species (*Cheiridopsis acuminata* L.Bolus, *C. denticulata, C. herrei* L.Bolus, *C. glomerata, C. pilosula, C. ponderosa* S.A.Hammer, *C. purpurea* and *C. verrucosa* L.Bolus, *Ihlenfeldtia vanzylii* (L.Bolus) H.E.K.Hartmann and *Odontophorus marlothii*) produced trichomes, which are defined as outgrowths of epidermal cells that are longer than broad (Opel 2005) (Fig. 2B–C). *Enarganthe octonaria, Jensenobotrya lossowiana* and *Namaquanthus vanheerdei* had flat epidermal cells, suggesting no papillae and a glabrous leaf surface (Fig. 2D–F). Calcium oxalate crystals were present as deposits on the outer epidermal cell walls in all species, with no evident difference across the species. Tanniferous idioblasts were present in a ring below the epidermal cells in

all the species, with raphide bundles scattered throughout the epidermis, at varying densities, across the species.

Cheiridopsis acuminata, C. pilosula, C. ponderosa and *Odontophorus angustifolius* possessed stomata sunken in the depressions, formed by the underlying tanniferous idioblasts, and protected by parastomal cells that overarch the guard cells. This type of stomatal protection was identified and described by Ihlenfeldt & Hartmann (1982) and referred to as Form II (Fig. 2H, I). Form I (Ihlenfeldt & Hartmann, 1982) was found in the remainder of the species, with the stomata placed in depressions, formed by the underlying tanniferous idioblasts below the epidermis, which sculpt the leaf surface into elevation and depressions (Fig. 2G).

POLLEN EXINE STRUCTURE

Three types of pollen exine structure were found within the *Conophytum*-clade: (1) tricolpate pollen with poorly-defined colpi, (2) tricolpate pollen with well-defined colpi, and (3) syncolpate pollen (summarised in Table 2). Tricolpate pollen grains with poorly-defined colpi were the most common, observed in *Cheiridopsis, Conophytum, Ihlenfeldtia, Namaquanthus, Odontophorus* and *Schlechteranthus* (Table 2). Although the exine in *Namaquanthus* was unique in that it was extensively micro-recticulate. Tricolpate pollen with well-defined colpi was found only in *Enarganthe*. Similarly, syncolpate pollen grains with numerous colpi were found only in *Jensenobotrya* (Table 2; Dupont, 1977).

MORPHOLOGICAL CHARACTER MAPPING

The selected morphological characters (sheathing and sheathing type) were traced with 1 step onto the BI consensus tree. Old leaves which forms sheaths enclosing the emerging leaf pair during dormancy, was reconstructed as a synapomorphy for the clade including *Cheiridopsis,*

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Conophytum, Ihlenfeldtia and *Odontophorus* (Fig. 3A). The complete sheath type, where the sheath fully encloses the leaves during dormancy, was recovered as a synapomorphy for *Conophytum,* with a loss in *C. herreanthus.* However, due to the poor resolution within this genus, it is unclear if the loss of this sheathing type represents a reversal in *C. herreanthus.* This sheath type was also found in *Cheiridopsis meyeri, C. minor, C. namaquensis* and *C. peculiaris* (Fig. 3B). The majority of *Cheiridopsis* taxa however, had sheaths that do not fully enclose the leaves during dormancy, only partially protecting the leaves, and this character was recovered as a synapomorphy for the genus (Fig. 3B).

DISTRIBUTIONAL DATA

The distribution of *Cheiridopsis* follows the winter-rainfall pattern and extends from southern Namibia to Langebaan on the south-western coast of South Africa and eastwards to Pofadder (Fig. 4A). Species diversity is centred in the Springbok-Steinkopf Region, with 14 (Springbok) to 15 (Steinkopf) species per QDS. *Cheiridopsis* subgenus *Cheiridopsis* is confined to South Africa and has the most southern distribution, extending to Langebaan in the Western Cape, with the centre of diversity in the Springbok Region, with 5 species within the QDS (Fig. 4B). *Cheiridopsis* subgenus *Odontophoroides* has the smallest distribution range, with species extending from the South Africa-Namibia border, southwards to Garies. Steinkopf is the largest hotspot for the subgenus, with 5 species within the QDS (Fig. 4C) and a smaller hotspot in the Richtersveld, containing 4 species in the QDS (Fig. 4C). *Cheiridopsis* subgenus *Aequifoliae* is the only subgenus that extends into Namibia, with the southern extent of the distribution to Clanwilliam and eastwards to Pofadder, with two centres of diversity in the Springbok-Steinkopf Region (5 species within the QDS) and the Richtersveld (3 species within the QDS) (Fig. 4D).

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DISCUSSION

TAXONOMICALLY INFORMATIVE CHARACTERS IN THE *CONOPHYTUM*-CLADE

Expanded phylogenetic sampling has often been shown to improve phylogenetic signal, providing comprehensive results from which to assess generic circumscription (Hillis, 1998; Pick *et al.,* 2010; Echternacht *et al.,* 2013). This was again found in the present study, where the expanded sampling of the *Conophytum*-clade, which included 35 of the 48 (73%) species in the clade (excluding the large genus *Conophytum,* which was representatively sampled), uncovered novel phylogenetic relationships, improved support for existing relationships and allowed for assessment of monophyly of the genera (Fig 1, Klak *et al.,* 2013; Powell *et al.,* 2016). *Cheiridopsis* and *Conophytum* were recovered as sister taxa (PP=0.95; Fig. 1) and although this relationship was recovered previously (Powell *et al.,* 2016), the expanded sampling in the present study improved support for the relationship (Powell *et al.,* 2016, PP=0.84), while in the subfamilial phylogeny Klak *et al.* (2013) recovered these genera in separate clades.

Our greatly expanded sampling of *Cheiridopsis* (including 75% of the currently recognised species) has shown for the first time that *Ihlenfeldtia* and *Odontophorus* are embedded within *Cheiridopsis* (PP=1, BS=81, JK=54; Fig. 1). Despite the expanded phylogenetic sampling, the position of the four monotypic genera, i.e. *Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus,* remained unresolved in the *Conophytum*-clade (Fig. 1, Electronic Appendix 1 & 2). In Klak *et al.* (2013), *Enarganthe* was sister to *Conophytum, Namaquanthus* was sister to *Schlechteranthus,* while *Jensenobotrya* and *Ruschianthus* were unresolved. The phylogenetic placement of *Enarganthe* and *Namaquanthus* in Klak *et al.* (2013) was unsupported by morphological characters, as these genera, as well as *Jensenobotrya* and *Ruschianthus,* display a combination of unique

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morphological characters that distinguish them from all other genera in the subfamily. In addition, *Jensenobotrya* and *Ruschianthus* are geographically isolated from other members of the subfamily, with *Jensenobotrya* only found on gneiss cliffs off the coast of Namibia, at Dolphin head, surrounded by Namib dunes (Burgoyne, 1998) and *Ruschianthus* is known only from limestone outcrops at high altitudes (1200-1400 m) on the summit of mountains in the Rosh Pinah area in Namibia (BOL records). The unresolved placement, morphological and anatomical data illustrates the uniqueness of these monotypic genera and as such, *Enarganthe, Jensenobotrya, Namaquanthus* and *Ruschianthus* are retained as monotypic genera.

The diversity in capsule morphology within the *Conophytum*-clade can be seen by the inclusion of genera from five of the twelve morphological groups recognised by Hartmann (1991). When assessing the capsule morphology across the *Conophytum*-clade, two main capsule morphologies were present, (1) a simple capsule without covering membranes and closing bodies (Fig. 5A, B) and (2) a complex capsule with covering membranes and closing bodies (Fig. 5C, D) (Klak *et al.,* 2013). The simple capsule type corresponded with a lower locule number, as the genera with simple capsules, i.e. *Jensenobotrya, Ruschianthus* and *Conophytum* possessed 5 locules, although a few species of *Conophytum* may have up to 8 locules. The majority of genera in the *Conophytum*-clade are, however, multilocular (>6 locules) with complex capsules (Fig. 5C, D) that include covering membranes and closing bodies (Hartmann, 2001), although in *Enarganthe* and *Namaquanthus* closing bodies are diagnostically absent (Bolus, 1927, 1954). In addition, the size of the closing bodies has also been used to define genera within the *Conophytum*-clade; however, this is often variable, even within a genus. In *Schlechteranthus,* both large closing bodies (blocking ¾ of the locule) and small closing bodies (blocking $^{1}/_{3}$ of the locule) are present (Powell *et al.,* 2016). The smaller closing bodies were used to distinguish *Ihlenfeldtia* from *Cheiridopsis*, leading to

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the hypothesis of a close phylogenetic relationship between *Ihlenfeldtia, Tanquana* and *Vanheerdia* (Hartmann, 1992) rather than *Ihlenfeldita* with *Cheiridopsis.* However, *Ihlenfeldtia* shares a compact caespitose habit, with leaves that form sheaths enclosing the emerging leaf pair, and large woody, multilocular capsules (Table 2) with *Cheiridopsis* (Hammer, 1996; Hartmann, 2001). This relationship is supported by the phylogenetic results of the present study, which show that *Ihlenfeldtia* is embedded within *Cheiridopsis* (PP=1, BS=81, JK=54; Fig. 1, Electronic Appendix 1 & 2). Valve wings are also often used as a diagnostic character for distinguishing genera (Hartmann, 2001), but were shown to be variable in the *Conophytum*-clade (ranging from absent to broad, with the full range of variation found in *Schlechteranthus*), as well as across the subgenera in *Cheiridopsis* (Hartmann & Dehn, 1987; Powell *et al.,* 2016). The width of valve wings has been used to distinguish *Odontophorus* from *Cheiridopsis,* with *Odontophorus* possessing narrow valve wings (Hartmann, 2001). However, species in *Cheiridopsis* subgenus *Aequifoliae* also possess narrow valve wings (Hartmann & Dehn, 1987), and therefore this character is not suitable to differentiate these genera. *Cheiridopsis* and *Odontophorus* also share a mostly caespitose habit, with leaves forming sheaths enclosing the emerging leaf pair and large multilocular, woody capsules (Hammer, 1994; Hartmann, 2001). Although capsule characters have proven to be informative in the *Conophytum*-clade, continuous indiscrete internal capsule characters are not useful, as they are often variable within a genus. The sole reliance on these indiscrete characters to distinguish genera, such as *Cheiridopsis* from *Ihlenfeldtia* and *Odontophorus,* has previously been shown to be unsuitable (Klak & Bruyns, 2016; Powell *et al.,* 2016) and is further supported here by the phylogenetic placement of *Ihlenfeldtia* and *Odontophorus* in *Cheiridopsis* (Fig. 1, Electronic Appendix 1 & 2). Valve wings can however sometimes be useful in distinguishing genera, for examples, in

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Jensenobotrya, the valve wings are unusual, as they are only distally attached to the capsule and so spread like ribbons when the capsule is opened (Herre, 1951; Hartmann, 2001).

The development of sheaths that protect the emerging leaves during the summer dormant period was recovered as a synapomorphy for the *Cheiridopsis–Conophytum* clade (Fig. 3A). In most species of *Cheiridopsis* (as well as in *Conophytum herreanthus*) the sheath only partially encloses the emerging leaf pair during the dormant period (Fig. 3B, Fig. 6E; Hammer, 1996, 2002; Hartmann, 2001). In *Conophytum* (and secondarily in a few species of *Cheiridopsis,* viz. the *Cheiridopsis meyeri-C. namaquensis* clade and *Cheiridopsis peculiaris*), the sheath completely envelops the emerging leaf pair (Fig. 3B, Fig. 6C, D; Hammer, 1996). Sheathing is also found in other genera in the subfamily, such as *Antimima* N.E.Br. and *Mitrophyllum* Schwantes (Hartmann, 2001), as well as in the Mesembryanthemoideae (Bittrich, 1987; Klak *et al.,* 2013). However, in *Conophytum* and *Cheiridopsis*, the protective sheath is retained despite the genera possessing xeromorphic leaves (Fig. 2A, D; Klak *et al.,* 2013).

Leaf anatomical characters have been used to inform generic circumscription in the family (Reule, 1937; Ihlenfeldt & Bittrich, 1985; Klak & Linder, 1998), as well as subgeneric classification in the *Conophytum*-clade (Opel, 2005; Powell *et al.,* 2016). Although epidermal shape was mostly uniform across the clade, cell shape was used to distinguish the subgenera in *Schlechteranthus* (Powell *et al.,* 2016). The epidermal cells are papillate, ranging from blunt papillae to trichomes (Fig. 2A-C), with the exception of *Enarganthe, Jensenobotrya* and *Namaquanthus,* where leaf surfaces are glabrous (Fig. 2D-F). The stomata across the *Conophytum*-clade were found in depressions (Fig. 2G), while in seven *Schlechteranthus* species and three *Cheiridopsis* species (*Cheiridopsis acuminata – Odontophorus angustifolius* clade, Fig. 1), stomata were sunken and protected by parastomal cells (Fig. 2H, I; Powell *et al.,* 2016). Although this stomatal type was variable within *Schlechteranthus,* in

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Cheiridopsis the species with this stomatal protection formed a strongly supported clade (PP=0.92, BS=91, JK=65; Fig. 1).

Surveys of pollen exine structure in Ruschioideae (Dupont, 1977) and Mesembryanthemoideae (Bittrich, 1987) showed that there was variation in this character in both subfamilies. However, the sampling did not include surveys of all the genera in the subfamilies, and with the absence of a phylogenetic hypothesis, patterns in exine structure could not be sufficiently assessed. However, in the present study, pollen exine structure was comprehensively surveyed for all the genera in the *Conophytum*-clade as well as for the subgeneric groupings (Table 2), which allowed for assessment of the variation across the clade. Although the exine structure was mostly uniform (tricolpate with poorly-defined colpi) in the *Conophytum*-clade, differences were found in *Enarganthe* (tricolpate with well-defined colpi) and *Jensenobotrya* (syncolpate), which distinguished these genera from others in the clade (Table 2).

CHEIRIDOPSIS, IHLENFELDTIA AND *ODONTOPHORUS*

Ihlenfeldtia and *Odontophorus* are strongly recovered within a paraphyletic *Cheiridopsis* in all of the phylogenetic analyses (Fig. 1, Electronic Appendix 1 & 2). This is hardly surprising as they share several morphological characters, and are currently distinguished solely on the basis of continuous indiscrete internal capsule characters, i.e. the size of closing bodies or the width of valve wings. Based on the phylogenetic, morphological and anatomical evidence, we here include the species of *Ihlenfeldtia* and *Odontophorus* into an expanded *Cheiridopsis* s.l. In its new circumscription, *Cheiridopsis* s.l. is easily recognised by the mostly compact, caespitose habit, leaves that form sheaths that enclose the emerging leaf pair (either partially or fully) and large woody multilocular capsules, with 8 to 18 locules. The three subgenera

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currently recognised are largely recovered in the phylogenetic analyses (Fig. 1, Electronic Appendix 1 & 2), although some minor adjustments are required.

Cheiridopsis* subgenus *Cheiridopsis

With the exception of *Cheiridopsis pearsonii* and *C. peculiaris*, all of the species with decumbent capsules (Fig. 5E) and heterophyllous leaves were recovered as a monophyletic group (Fig. 1, Electronic Appendix 1 & 2) corresponding to Hartmann & Dehn's (1987) concept of subgenus *Cheiridopsis*. When the genus is constrained to monophyly, *Cheiridopsis pearsonii* and *C. peculiaris* are included in the subgenus *Cheiridopsis* clade, with a constrained phylogenetic tree only 2 steps longer and not significantly different (P=0.15) from the unconstrained tree. The fact that *C. pearsonii* and *C. peculiaris* are placed directly in the subgenus, without specifically constraining them to the subgenus, provides strong support for their inclusion in subgenus *Cheiridopsis. Cheiridopsis peculiaris* is somewhat unusual, as the name eludes, due to the strange shell-shaped leaves, but shares decumbent capsules and heterophyllous leaves with subgenus *Cheiridopsis* (Hartmann & Dehn, 1987). The sheath is also unusual within *Cheiridopsis,* in that it completely encloses the emerging leaf pair during the dormant period. However, similar sheaths can also be found in other species within the subgenus, i.e. the species of the *C. meyeri–C. namaquensis* clade (Fig. 3B, Fig. 6C). *Cheiridopsis pearsonii* shares the decumbent capsules with undulate covering membranes and large closing bodies with the rest of the species in the subgenera, and is most similar to *C. namaquensis* in leaf shape (Hartmann, 2001).

Cheiridopsis* subgenus *Odontophoroides

Species recovered in the *Cheiridopsis* subgenus *Odontophoroides* clade all share erect capsules (Fig. 5G), with rounded tops (Fig. 1, Electronic Appendix 1 & 2). This clade

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includes the two species of *Odontophorus* sampled, and the placement of these species is supported by the similar capsule characters and the isophyllous leaves (Hartmann, 2001). *Odontophorus* is recovered as polyphyletic (Fig. 1, Electronic Appendix 1 & 2), with *O. angustifolius* included in the *C. acuminata–O.angustifolius* clade, characterised by sunken stomata hidden by parastomal cells (Fig. 3I). This complex stomatal protection was only found in these three species in the *Cheiridopsis* s.l. clade (PP=0.92, BS=91, JK=65; Fig. 1).

Three species (*Cheiridopsis glomerata, C. pillansii, C. purpurea*), previously treated in subgenus *Aequifoliae*, were recovered within the subgenus *Odontophoroides* clade in all of the phylogenetic analyses (Fig. 1, Electronic Appendix 1 & 2). These species were placed by Hartmann & Dehn (1987) in subgenus *Aequifoliae* based on the internodes, keeled leaves and awned valve wings. However, they share the erect capsules with rounded tops, diagnostic of subgenus *Odontophoroides* (Fig. 5G), and so we transfer them here to subgenus *Odontophoroides*. As a result, the magenta flowering, *C. glomerata* (Fig. 6B) and *C. purpurea*, are now placed together with the only other magenta flowered species in the genus, *C. speciosa.*

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Cheiridopsis* subgenus *Aequifoliae

The *Cheiridopsis* subgenus *Aequifoliae* clade is characterised by species that possess erect capsules with flat to centrally elevated tops (Fig. 5F) and isophyllous leaves (Fig. 1, Electronic Appendix 1 & 2). This clade differs from Hartmann & Dehn's (1987) concept of *C.* subgenus *Aequifoliae* by the exclusion of *C. glomerata, C. pillansii, C. purpurea* (discussed earlier) and the inclusion of both species of *Ihlenfeldtia* (Fig. 1, Electronic Appendix 1 & 2). The placement of *Ihlenfeldtia* in subgenus *Aequifoliae* is further supported by the shared presence of trichomes (Fig. 2C) which are found in all species of subgenus *Aequifoliae* (Fig 2B).

KEY TO THE GENERA OF THE *CONOPHYTUM*-CLADE

1. Old leaves do not form a sheath that encloses the emerging leaf pair during dormancy.....2

2. Capsules 5-locular, without covering membranes 3

3. Flowers green, never opening fully as restricted by adjacent leaf pair; leaves sickle-shaped; pollen unknown; caespitose, with leaves held upright.....***Ruschianthus***

3'. Flowers white to pink, opening fully; leaves shortly clavate to almost globose; pollen syncolpate; shrub, with leaves hanging down cliffs like bunches of grapes.....***Jensenobotrya***

2'. Capsules multilocular (>6 locules), with covering membranes 4

4. Leaves not fused at the base, trigonous to club-shaped; flowers without filamentous staminodes .....***Enarganthe***

4'. Leaves fused at least $^{1}/_{3}$ of their length at the base, leaves finger-shaped, triquetrous to trigonous; flowers with or without filamentous staminodes 5

5. Capsules without closing bodies; finger-shaped leaves which almost form a U-shape ....***Namaquanthus***

5'. Capsules with closing bodies; leaves triquetrous to trigonous.....***Schlechteranthus***

1'. Old leaves form sheaths that encloses the emerging leaf pair during dormancy 6

6. Petaloid staminodes fused forming a tube; capsules soft and papery, 3–8-locular, without covering membranes and closing bodies..... ***Conophytum***

6. Petaloid staminodes free, not forming a tube; capsules hard and woody, 8–18-locular, with covering membranes and closing bodies.....***Cheiridopsis***

TAXONOMIC TREATMENT

1. *Cheiridopsis* N.E.Br., Gard. Chron 3: 412 (1925), emend. nov. R.F. Powell *Type: C. rostrata* N.E.Br.

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Ihlenfeldtia H.E.K.Hartmann, Bot. Jahrb. Syst. 114 (1992), **syn. nov.** *Type: I. excavata* (L.Bolus) H.E.K.Hartmann = *Cheiridopsis excavata* L.Bolus.

Odontophorus N.E.Br., Gard. Chron 3: 12 (1927), **syn. nov.** *Type: O. marlothii* N.E.Br. = *Cheiridopsis marlothii* (N.E.Br.) R.F.Powell.

Succulent, perennial shrubs, compact to caespitose or rarely mat-forming, 50–300 mm in height. *Leaves* isophyllous, with subsequent leaf pairs equal along stem or heterophyllous, with subsequent leaf pairs unequal along stem; triquetrous to trigonous, sometimes rhombic, 4–150 mm long; leaf surface sometimes rough, formed by elevations above subhypodermal idioblasts or smooth; mucronate, sometimes with dentate margins or keels, epidermis papillate, with epidermal cells forming blunt papillae or trichomes; old leaves forming sheaths which usually only partially enclose the emerging leaf pair (fully enclose the emerging leaf pair in only a few species, i.e. *C. meyeri, C. minor, C. namaquensis, C. peculiaris*). *Flowers* solitary; pedicels 30–90 mm; radiate, 30–70 mm in diameter; petaloid staminodes free, cream to yellow, rarely orange, red, or magenta, sometimes magenta-suffused. *Capsules* woody, pedicels decumbent or erect, top flat to centrally elevated or rounded, base funnel-shaped to semi-globose; 8 to 18 locules; covering membranes undulate in radial direction, often with elevations in subcentral position, sometimes as prominent bulges, humps or tubes, very rarely flat; closing bodies usually large, blocking at least ¾ of locule, seldom smaller, blocking at least ½ of locule; valve wings broad, equal in width to valve, or narrow, $^{1}/_{3}$ width of valve, sometimes reduced to awns. *Seeds* flat to round, light to darker brown, smooth to papillate.

Diagnostic characters and distribution: *Cheiridopsis* now includes 38 species classified into three subgenera, *Cheiridopsis, Aequifoliae* and *Odontophoroides.* The genus is characterised by the old leaves that form sheaths enclosing the emerging leaf pair partially, but sometimes

fully (Fig. 6C, E) and the large multilocular woody capsules, with 8 to 18 locules (Fig. 5C, D). The species of *Cheiridopsis* are also compact to caespitose, rarely mat-forming. The diversity of the genus is centred in the Springbok-Steinkopf region (Fig. 4A) and distribution follows the winter-rainfall region of southern Africa. The distribution extends from southern Namibia, southwards to Langebaan and eastwards to Pofadder (Fig. 4A).

2. KEY TO THE SUBGENERA IN *CHEIRIDOPSIS*

1. Pedicel decumbent, leaves heterophyllous ..... **3.1. *Cheiridopsis* subgenus *Cheiridopsis***

1. Pedicel erect; leaves isophyllous 2

2. Capsule top rounded..... **3.2. *Cheiridopsis* subgenus *Odontophoroides***

2. Capsule top flat to centrally elevated ..... **3.3. *Cheiridopsis* subgenus *Aequifoliae***

3. TAXONOMIC TREATMENT OF SUBGENERA

3.1. *Cheiridopsis* subgenus *Cheiridopsis*. *Type: C. rostrata* N.E.Br.

Compact perennial shrubs, sometimes mat-forming. *Leaves* heterophyllous, with subsequent leaf pairs unequal along stem; surface smooth, with no elevations; epidermal cells forming blunt papillae; sheaths partially or fully enclosing the emerging leaf pair during dormancy. *Flowers* cream to yellow, rarely orange, red. *Capsules* with decumbent pedicels; top flat to rounded, base funnel-shaped; valve wings narrow, $^{1}/_{3}$ width of valve or rarely rectangular, often narrowing in an awn distally; covering membranes prominently undulate, often with additional protrusions in subcentral position; closing bodies large, blocking at least ¾ of the locule. *Seeds* mostly smooth, rarely papillate.

Diagnostic characters and distribution: *Cheiridopsis* subgenus *Cheiridopsis* includes 14 species that are distinguished by their decumbent capsules (Fig. 5E) and heterophyllous

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leaves. Some species (*C. meyeri, C. minor, C. namaquensis, C. peculiaris*) can be distinguished by the sheath that fully encloses the emerging leaf pair during the dormant period (Fig. 6C). The species occur from the Richtersveld, southwards to Langebaan, with the centre of diversity in the Springbok-Steinkopf Region (Fig. 4B).

Recognised species: *Cheiridopsis amabilis* S.A.Hammer, *C. delphinoides* S.A.Hammer, *C. derenbergiana* Schwantes, *C. gamoepensis* S.A.Hammer, *C. imitans* L.Bolus, *C. meyeri* N.E.Br., *C. minor* (L.Bolus) H.E.K.Hartmann, *C. namaquensis* (Sond.) H.E.K.Hartmann, *C. pearsonii* N.E.Br., *C. peculiaris* N.E.Br., *C. rostrata* (L.) N.E.Br., *C. schlechteri* Tischer, *C. turbinata* L.Bolus, *C. umbrosa* S.A.Hammer & Desmet.

3.2. *Cheiridopsis* subgenus *Odontophoroides* H.E.K.Hartmann, Biblioth. Bot. 136: 59. 1983. *Type: C. acuminata* L.Bolus.

Compact perennial shrubs, sometimes mat-forming. *Leaves* isophyllous, with subsequent pairs equal along stem; surface often rough with elevations above the subhypodermal idioblasts or smooth; epidermal cells forming blunt papillae or trichomes; sheath always only partially enclosing the emerging leaf pair during dormancy. *Flowers* cream, yellow, magenta, or magenta-suffused. *Capsules* on erect pedicels; top rounded, base semi-globose; valve wings broad, as wide as valve, to narrow, $^{1}/_{3}$ width of valve, sometimes ending in an awn; covering membranes undulate; closing bodies large, blocking at least ¾ of locule. *Seeds* sparsely to densely papillate.

Nomenclatural changes:

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Cheiridopsis angustifolia (L.Bolus) R.F.Powell, **comb. nov.** *Odontophorus angustifolius* L.Bolus, Notes Mesembryanthemum 3: 128. 1938. Type: South Africa, Northern Cape Province, Kosies, *Van Heerde 21845* (BOL, holo!).

Cheiridopsis marlothii (N.E.Br.) R.F.Powell, **comb. nov.** *Odontophorus marlothii* N.E.Br., Gard. Chron. 3:12. 1927. Type: South Africa, Northern Cape Province, Ezelsfontein, between Spektakel and O'kiep, *Marloth 6947* (BOL, lecto!).

Cheiridopsis nana (L.Bolus) R.F.Powell, **comb. nov.** *Odontophorus nanus* L.Bolus Notes Mesembryanthemum 2: 59. 1929. Type: South Africa, Northern Cape Province, Eenriet, upper slopes, about 7 miles N.E. of Steinkopf, Little Namaland [Namaqualand], *Pearson 4071* (BOL, holo!).

Cheiridopsis pusilla (S.A.Hammer) R.F.Powell, **comb. nov.** *Odontophorus pusillus* S.A.Hammer Piante Grasse 15(4): 86. 1996. Type: *Van Jaarsveld 11461* (BOL, holo!).

Diagnostic characters and distribution: Cheiridopsis subgenus *Odontophoroides* is the largest subgenus with 16 species and is characterised by the isophyllous leaves (equal subsequent pairs) and the erect capsules with rounded tops (Fig. 5G). Often the leaves are also characterised by a rough surface (Fig. 6F), but this is not found in all species in the subgenus. Although this subgenus has the largest number of species, it has the most restricted distribution from the northern border of South Africa at Sendelingsdrif, in the Richtersveld, to Wallekraal, with hotspots in the Springbok-Steinkopf Region and the Richtersveld (Fig. 4C).

Recognised species: Cheiridopsis acuminata L.Bolus, *C. alba-oculata* Klak & Helme, *C. angustifolia* (L.Bolus) R.F.Powell, *C. aspera* L.Bolus, *C. glomerata* S.A.Hammer, *C.*

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marlothii (N.E.Br.) R.F.Powell, *C. nana* (L.Bolus) R.F.Powell, *C. pillansii* L.Bolus, *C. pilosula* L.Bolus, *C. ponderosa* S.A.Hammer, *C. purpurea* L.Bolus, *C. pusilla* (S.A.Hammer) R.F.Powell, *C. rudis* L.Bolus, *C. speciosa* L.Bolus, *C. umdausensis* L.Bolus, *C. velox* S.A.Hammer.

3.3. *Cheiridopsis* subgenus *Aequifoliae* H.E.K.Hartmann, Biblioth. Bot. 136: 59. 1983. *Type: C. denticulata* (Haw.) N.E.Br.

Compact perennial shrubs. *Leaves* isophyllous, with subsequent pairs equal along stem; surface smooth with no elevations, epidermal cells forming trichomes, sheath always only partially enclosing the emerging leaf pair during dormancy. *Flowers* cream to yellow, rarely orange, red. *Capsules* on erect pedicels; top flat to centrally elevated, but never rounded, base funnel-shaped; valve wings reduced to awns; covering membranes undulate with low distal rims; closing bodies usually large, blocking at least ¾ of locule, seldom smaller, blocking at least ½ of locule. *Seeds* smooth.

Nomenclatural changes:

Cheiridopsis excavata L.Bolus, Notes Mesembryanthemum 3:48. 1937. *Ihlenfeldtia excavata* (L.Bolus) H.E.K.Hartmann Bot. Jahrb. Syst. 114(1): 48. 1992. *Type*: South Africa, Northern Cape Province, South of Breekpoort, Namaland, *van Heerde 21836* (BOL, holo!).

Cheiridopsis vanzylii L.Bolus, Notes Mesembryanthemum 2:116. 1929. *Ihlenfeldtia vanzylii* (L.Bolus) H.E.K.Hartmann, Bot. Jahrb. Syst. 114(1): 49. 1992. Type: South Africa, Northern Cape Province, Pofadder, *Fuller 4* (BOL, holo!).

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Diagnostic characters and distribution: *Cheiridopsis* subgenus *Aequifoliae* is the smallest subgenus in *Cheiridopsis,* with eight species. The subgenus shares the isophyllous leaves (equal subsequent pairs) and erect pedicels with subgenus *Odontophoroides* but differs by the capsule tops that are flat or centrally elevated on the top (Fig. 5I). The subgenus has the most northern distribution extending from southern Namibia to Clanwilliam and eastwards to Pofadder, with two centres of diversity in the Springbok-Steinkopf Region and the Richtersveld (Fig. 4D).

Recognised species: *Cheiridopsis brownii* Schick & Tisch., *C. caroli-schmidtii* (Dinter & A.Berger) N.E.Br., *C. denticulata* (Haw.) N.E.Br., *C. excavata* L.Bolus, *C. herrei* L.Bolus, *C. robusta* (Haw.) N.E.Br., *C. vanzylii* L.Bolus, *C. verrucosa* L.Bolus.

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FIGURE LEGENDS

Figure 1. Majority Rule Consensus tree from Bayesian analysis of six plastid markers, indicating phylogenetic relationships in the *Conophytum*-clade. Posterior probability (PP) values above 0.5 are indicated above the branches. Jackknife support values (JS) and Bootstrap supports (BS) above 50, from the maximum parsimony and maximum likelihood analyses, are indicated below the branches. Brackets indicate the placement of taxa and clades discussed, with embedded genera indicated in bold.

Figure 2. Transverse sections through the leaves of taxa in the *Conophytum*-clade illustrating characters of taxonomic importance. (A) epidermal cells forming blunt papillae in *Cheiridopsis caroli-schmidtii*; (B) epidermal cells forming trichomes in *Cheiridopsis verrucosa*; (C) trichomes in *Ihlenfeldtia vanzylii*; (D) glabrous epidermis of: (D) *Namaquanthus vanheerdei*; (E) *Enarganthe octonaria*; (F) *Jensenobotrya lossowiana;* (G) stomata in depression, not sunken or hidden in *Cheiridopsis robusta*; (H) stomata in depression, sunken and hidden by parastomal cell in *Cheiridopsis acuminata*; (I) sunken stomata in *Odontophorus angustifolius*. Vouchers: (A) *Powell 105* (NBG); (B) *Powell 99* (NBG); (C) *KBG222/98* (KBG); (D) *van Jaarsveld 2475* (NBG); (E) *Powell 45* (NBG); (F) *SUG 12618* (NBG); (G) *Powell 66* (NBG); (H) *Powell 68* (NBG); (I) *EVJ 106/87* (NBG). Scale: A–F = 200µm; G–I = 50 µm.

Figure 3. Character reconstruction of sheathing and sheathing type on the Bayesian inference consensus tree. (A) sheathing genera of the Conophytum-clade, indicated by the black branches; (B) sheath type mapped onto the Bayesian inference consensus tree, complete sheath refers to species were the sheath fully encloses the emerging pair during the dormant period (black branches) and in the partial sheath, the sheath only encloses part of the emerging leaf pair during dormancy (grey branches). The white branches indicate species that do not sheath.

Figure 4. Number of species per Quarter Degree Square (QDS) (Leistner & Morris, 1976) and distribution of *Cheiridopsis* s.l. and subgenera (as treated in the taxonomic treatment) in the Greater Cape Floristic region, South Africa. (A) distribution and number of species per QDS for the genus *Cheiridopsis* s.l.; (B) distribution and number of species per QDS in

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subgenus *Cheiridopsis*; (C) distribution and number of species per QDS in subgenus *Odontophoroides* (including species of previously recognised *Odontophorus*)*;* (D) distribution and number of species per QDS in subgenus *Aequifoliae* (including species of previously recognised *Ihlenfeldtia*).

Figure 5. Capsules in *Conophytum* and *Cheiridopsis*. (A) closed simple capsule of *Conophytum wettsteinii*; (B) open simple capsule of *Conophytum wettsteinii* showing the absence of covering membranes and closing bodies; (C) closed multi-locular capsule of *Cheiridopsis denticulata;* (D) open capsule of *Cheiridopsis denticulata* showing the complex internal structures, i.e. covering membranes (cm) and closing bodies (cb), indicated by the white arrows; (E) decumbent capsules of *Cheiridopsis* subgenus *Cheiridopsis,* in *Cheiridopsis namaquensis;* (F) erect capsule of *Cheiridopsis denticulata* with flat to centrally elevated tops as in *Cheiridopsis* subgenus *Aequifoliae;* (G) *Cheiridopsis pilosula* illustrating the erect capsule with rounded tops, typical of *Cheiridopsis* subgenus *Odontophoroides.*

Figure 6. Leaf and floral characters of *Cheiridopsis* and *Conophytum*. (A) new leaves of *Conophytum wettsteinii* breaking out of their papery sheath, with a magenta flower; (B) partial sheath enclosing the emerging leaf pair, with a magenta flower, in *Cheiridopsis glomerata;* (C) leaves of *Cheiridopsis meyeri* completely enclosed by a white papery sheath during the dormant period, indicated by the arrow; (D) leaves of *Conophytum uviforme* completely enclosed in a sheath during the dormant period, indicated by the arrow; (E) *Cheiridopsis purpurea* with the partial sheath, common to many *Cheiridopsis* species which only encloses part of the leaves during the dormant period; (F) *Cheiridopsis aspera* with the prominent rough leaf surface often found in subgenus *Odontophoroides.*

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Table 1. Results of the topological constraint analysis, p-values reported from the Templeton-Wilcoxon test.

		Templeton				Winning-sites	
Tree	Length	Rank sums	N	z	P	Counts	P
1 (constrained topology)	542	3.0	2	-1.4142	0.1573	2	0.50
2 (unconstrained topology)	540	0.0	-	-	-	0	-

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Table 2: Summary of important generic anatomical, morphological and palynological characters in the *Conophytum*-clade.

	Schlechteranthus	***Conophytum***	***Cheiridopsis* s.s.**	***Ihlenfeldtia* s.s.**	***Odontophorus s.s.***	***Enarganthe***	***Jensenobotrya***	***Namaquanthus***	***Ruschianthus***
Habit	Woody shrub	Caespitose	Caespitose	Caespitose	Caespitose, sometimes mat-forming	Woody shrub	Woody shrub	Woody shrub	Caespitose
Leaf shape	Triquetrous to trigonous	Globose to ovoid, rarely square	Triquetrous to trigonous	Triquetrous to trigonous	Triquetrous to trigonous	Clavate to trigonous	Shortly clavate to almost globose	Finger-shaped	Sickle-shaped
Degree of leaf length fused	$^1/_6$ to ¾ fused	Usually completely fused, sometimes top ¼ free, rarely completely free	$^1/_6$ to $^5/_6$ fused	$^1/_5$ to ¼ fused	$^1/_5$ to ¼fused	Leaf pairs not fused	$^1/_5$ fused	$^1/_6$ fused	$^1/_6$ fused
Old leaf forming sheath	No	Yes	Yes	Yes	Yes	No	No	No	No
Epidermal papillae*	Blunt papillae	Blunt pappilae to trichomes	Blunt pappilae to trichomes	Blunt pappilae to trichomes	Blunt pappilae to trichomes	Flat epidermal cells, no papillae	Flat epidermal cells, no papillae	Flat epidermal cells, no papillae	Epidermal cells very slightly elevated
Stomatal protection†	Form I and II	Form I and II, and stomata sometimes elevated	Form I and II	Form I	Form I and II	Form I	Form I	Form I	Form I
Petaloid staminodes	Free at base, never forming a tube	Fused, forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube
Flower colour	White to magenta	White, cream, yellow, orange, red or magenta	White, cream, yellow, orange or magenta	Yellow, often purplish suffused, rarely creamy white, red with age	Yellow, often cream-coloured, sometimes white or basally white	Bright reddish-purple	Pink	Bright purple	Green
Filamentous staminodes	Present	Present or absent	Present	Absent	Absent	Absent	Absent	Present	Present
Pollen exine structure	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi, distinctly micro-reticulate	Syncolpate	Tricolpate with well-defined colpi	Unknown
No of locules	7-12	3-8	8-18	10-15	10	8	5	(8-)12(-17)	5
Covering membranes	Present	Absent	Present	Present	Present	Present	Absent	Present	Absent
Closing bodies	Present	Absent	Present	Present	Present	Absent, placenta sometimes forms hood over locule	Absent	Absent	Absent

Footnotes:

* as defined by Opel, (2005): blunt papillae are low protuberances in the centre of the epidermal cell and trichomes are defined as outgrowths of epidermal cells that are longer than broad.

† Form I – stomata placed in depressions formed by underlying tanniferous idioblats; Form II – stomtata sunken below epidermal surface, in depressions and further protected by overarching parastomal cells (Ihlenfeldt & Hartmann, 1982).

Appendix 1. Accessions for which cpDNA sequence data were obtained, with corresponding voucher information and GenBank accession numbers. The information is listed as follows: *taxon: matK, rpl16, rps16, trnL-F, trnQ^{UUG}-rps16, trnS-trnG;* voucher information.

Conophytum N.E.Br: *C. bilobum* (Marloth) N.E.Br.:

Powell 19 (NBG). *C. bruynsii* S.A.Hammer: KF132665; KF132179; AJ532795; AJ558090; KF131886; KF133146; *Bruyns 6784* (BOL). *C. calculus* (A.Berger) N.E.Br.: KF132666; KF132180; KF132451; KF132793; KF131887; KF133147; *Klak 1641* (BOL). *C. ficiforme* N.E.Br.: *Powell 3* (NBG). *C. friedrichiae* Schwantes: *KBG 312/89* (KBG). *C, herreanthus* S.A.Hammer: *KBG 103/91* (KBG). *C. maughanii* N.E.Br.: KF132667; KF132181; KF132452; KF132794; KF131888; KF133148; *Bruyns 8913* (BOL). *Conophytum wettsteinii* N.E.Br.: *Powell 50* (NBG). ***Cheiridopsis*** N.E.Br.: *C. acuminata* L.Bolus:

Powell 68 (NBG). *C. alba-oculata* Klak & Helme:

Klak 2308 (BOL). *C. aspera* L.Bolus:

Powell 14 (NBG). *C. brownii* Schick & Tisch.:

Powell 61 (NBG). *C. caroli-schmidtii* N.E.Br.:

Powell 105 (NBG). *C. denticulata* N.E.Br.:

Powell 9 (NBG). *C. derenbergiana* Schwantes:

Powell 79 (NBG). *C. gamoepensis* S.A.Hammer:

Powell 89 (NBG). *C. glomerata* S.A.Hammer:

Powell 96 (NBG). *C. herrei* L.Bolus: *Powell 100* (NBG). *C. meyeri* N.E.Br.: *Powell 16* (NBG). *C. minor* (L.Bolus) H.E.K.Hartmann: *Powell 94* (NBG). *C. namaquensis* (Sond.) H.E.K.Hartmann: *Powell 11* (NBG). *C. pearsonii* N.E.Br.: KF132664; KF132178; KF132450; KF132792; KF131885; KF133145; *Bruyns 9504* (BOL). *C. peculiaris* N.E.Br.:

Powell 15 (NBG). *C. pillansii* L.Bolus:

Powell 45 (NBG). *C. pilosula* L.Bolus:

Powell 69 (NBG). *C. ponderosa* S.A.Hammer:

Powell 103 (NBG). *C. purpurea* L.Bolus:

Powell 85 (NBG). *C. robusta* N.E.Br.:

Powell 66 (NBG). *C. rostrata* N.E.Br.:

Powell 88 (NBG). *C. speciosa* L.Bolus:

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Powell 107 (NBG). *C. umdausensis* L.Bolus:
Powell 102 (NBG). *C. verrucosa* L.Bolus:
Powell 99 (NBG). ***Drosanthemum*** Schwantes: *D. diversifolium* L.Bolus: KF132633; KF132149; KF132435; KF132778; KF131856; KF133116; *Klak 1743* (BOL). ***Enarganthe*** N.E.Br: *E. octonaria* (L.Bolus) N.E.Br: KF132684; KF132198; AJ532805; AJ439023; JN896433; JN896380; *Klak 491* (BOL). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. excavata* (L.Bolus) H.E.K.Hartmann: KF132701; KF132214; KF132477; KF132818; KF131921; KF133182; *Wisura 1926* (BOL). *I. vanzylii* (L.Bolus) H.E.K.Hartmann:
KGB 222/98 (KBG). ***Jacobsenia*** L.Bolus & Schwantes: *J. vaginata* (L.Bolus) Ihlenf.: KF132703; KF132215; KF132479; KF132820; KF131923; KF133184; *Wisura 882* (BOL). ***Jensenobotrya*** A.G.J.Herre: *J. lossowiana* A.G.J.Herre: KF132704; KF132216; KF132480; KF132821; KF131924; KF133185; *Van Jaarsveld 21145* (BOL). ***Namaquanthus*** L.Bolus *N. vanheerdei* L.Bolus: KF132724; –; AJ532810; AJ439049; KF131944; KF133205; *Klak 251* (BOL). ***Odontophorus*** N.E.Br: *O. angustifolius* L.Bolus:
EVJ 106/87 (NBG). *O. marlothii* N.E.Br.: KF132731; KF132243; AJ532823; AJ558101; KF131951; KF133212; *Klak 862* (BOL). ***Ruschianthus*** L.Bolus *R. falcatus* L.Bolus: KF132758; KF132266; KF132518; KF132857; KF131978; KF133240; *Rawe s.n.*(BOL). ***Schlechteranthus*** Schwantes: *S. abruptus* (A.Berger) R.F.Powell: KT281451, KT248276, KT248288, KT248312, KT248324, KT248335; *Powell 5* (NBG). *S. connatus* (L.Bolus) R.F.Powell: KT281449, KT248273, KT248297, KT248309, KT248321; *Powell 10* (NBG). *S. hallii* L.Bolus: *Klak 259* (BOL). *S. inclusus* (N.E.Br.) R.F.Powell: KT281450, KT248274, KT248286, KT248310, KT248322, KT248333; *Powell 35* (NBG). *S. pungens* (H.E.K.Hartmann) R.F.Powell: KT281447, —, KT248279, KT248303, KT248315, KT248327; *Powell 28* (NBG). *S.* sp.nov.: KT281448, KT248269, KT248281, KT248305, KT248317, KT248329; *Powell 41* (NBG). *S. spinescens* (L.Bolus) R.F.Powell *Klak 2424* (BOL). *S. steenbokensis* (H.E.K.Hartmann) Klak: *Bruyns 8267* (BOL). *S. subglobosus* (L.Bolus) R.F.Powell: *Bruyns 8240* (BOL). *S. tetrasepalus* (L.Bolus) R.F.Powell: KT248266, KT248277, KT248289, KT248313, KT248325, KT248336; *Klak 2411* (BOL).

Appendix 2. Voucher information for leaf material studied in transverse section. Voucher information is listed as taxon, collector, collection number and herbarium.

Cheiridopsis acuminata L.Bolus*, Powell 68* (NBG);*Cheiridopsis aspera* L.Bolus*, Powell 14* (NBG); *Cheiridopsis denticulate* N.E.Br.*, Powell 9* (NBG); *Cheiridopsis caroli-schmidtii* N.E.Br.*, Powell 105* (NBG); *Cheiridopsis gamoepensis* S.A.Hammer*, Powell 89* (NBG); *Cheiridopsis glomerata, Powell 96* (NBG); *Cheiridopsis imitans, Powell 91* (NBG); *Cheiridopsis herrei, Powell 100* (NBG); *Cheiridopsis meyeri, Powell 16* (NBG); *Cheiridopsis minor, Powell 94* (NBG); *Cheiridopsis namaquensis, Powell 11* (NBG); *Cheiridopsis peculiaris, Powell 15* (NBG); *Cheiridopsis pillansii, Powell 45* (NBG); *Cheiridopsis pilosula, Powell 69* (NBG); *Cheiridopsis ponderosa, Powell 103* (NBG); *Cheiridopsis purpurea, Powell 85* (NBG); *Cheiridopsis robusta, Powell 66* (NBG); *Cheiridopsis rostrata, Powell 88* (NBG); *Cheiridopsis schlechteri, Powell 17* (NBG); *Cheiridopsis speciosa, Powell 107* (NBG); *Cheiridopsis verrucosa, Powell 99* (NBG); *Cheiridopsis umdausensis, Powell 102* (NBG*); Enarganthe octonaria, Powell 45* (NBG); *Ihlenfeldtia excavata, Powell 55* (NBG); *Ihlenfeldtia vanzylii, KBG 222/98* (KBG); *Jensenobotrya lossowiana, SUG 12618* (NBG); *Namaquanthus vanheerdei, EVJ 2475* (NBG); *Odontophorus angustifolius, EVJ 106/87* (NBG).

Appendix 3. Voucher information for pollen material studied under the light microscope. Voucher information is listed as taxon, collector, collection number and herbarium. *Conophytum frutescens*, *Wisura 862* (NBG); *Conophytum hians, Wilkins s.n*. (NBG); *Conophytum uviforme, Powell 39* (NBG); *Cheiridopsis denticulata,van Heerde s.n.* (BOL); *Cheiridopsis derenbergiana, Wisura 2053* (NBG); *Cheiridopsis namaquensis, van Heerde 69* (BOL); *Cheiridopsis pillansii, Wisura 1617* (NBG); *Cheiridopsis pilosula, van Jaarsveld 2548* (NBG); *Enarganthe octonaria, van Jaarsveld 4206* (NBG); *Ihlenfeldtia excavata, van Heerde 30* (NBG); *Namaquanthus vanheerdei, van Jaarsveld 2475* (NBG); *Octopoma connatum, Helme 3226* (BOL); *Odontophorus marlothii, Hall NBG 624/57* (NBG); *Odontophorus nanus, Smithers s.n.* (BOL); *Schlechteranthus albiflorus, Van Berkel 462* (NBG); *Schlecheranthus hallii, Burgoyne 7340* (NBG); *Schlechteranthus spinescens, Desmet & Ellis 1345* (NBG).

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Appendix 1. Accessions for which cpDNA sequence data were obtained, with corresponding voucher information and GenBank accession numbers. The information is listed as follows: *taxon: matK, rpl16, rps16, trnL-F, trnQ*^{UUG}*-rps16, trnS-trnG;* voucher information.

Conophytum N.E.Br: *C. bilobum* (Marloth) N.E.Br.:
Powell 19 (NBG). *C. bruynsii* S.A.Hammer: KF132665; KF132179; AJ532795; AJ558090; KF131886; KF133146; *Bruyns 6784* (BOL). *C. calculus* (A.Berger) N.E.Br.: KF132666; KF132180; KF132451; KF132793; KF131887; KF133147; *Klak 1641* (BOL). *C. ficiforme* N.E.Br.: *Powell 3* (NBG). *C. friedrichiae* Schwantes: *KBG 312/89* (KBG). *C, herreanthus* S.A.Hammer: *KBG 103/91* (KBG). *C. maughanii* N.E.Br.: KF132667; KF132181; KF132452; KF132794; KF131888; KF133148; *Bruyns 8913* (BOL). *Conophytum wettsteinii* N.E.Br.: *Powell 50* (NBG). ***Cheiridopsis*** N.E.Br.: *C. acuminata* L.Bolus:
Powell 68 (NBG). *C. alba-oculata* Klak & Helme:
Klak 2308 (BOL). *C. aspera* L.Bolus:
Powell 14 (NBG). *C. brownii* Schick & Tisch.:
Powell 61 (NBG). *C. caroli-schmidtii* N.E.Br.:
Powell 105 (NBG). *C. denticulata* N.E.Br.:
Powell 9 (NBG). *C. derenbergiana* Schwantes:
Powell 79 (NBG). *C. gamoepensis* S.A.Hammer:
Powell 89 (NBG). *C. glomerata* S.A.Hammer:
Powell 96 (NBG). *C. herrei* L.Bolus: *Powell 100* (NBG). *C. meyeri* N.E.Br.: *Powell 16* (NBG). *C. minor* (L.Bolus) H.E.K.Hartmann: *Powell 94* (NBG). *C. namaquensis* (Sond.) H.E.K.Hartmann: *Powell 11* (NBG). *C. pearsonii* N.E.Br.: KF132664; KF132178; KF132450; KF132792; KF131885; KF133145; *Bruyns 9504* (BOL). *C. peculiaris* N.E.Br.:
Powell 15 (NBG). *C. pillansii* L.Bolus:
Powell 45 (NBG). *C. pilosula* L.Bolus:
Powell 69 (NBG). *C. ponderosa* S.A.Hammer:
Powell 103 (NBG). *C. purpurea* L.Bolus:
Powell 85 (NBG). *C. robusta* N.E.Br.:
Powell 66 (NBG). *C. rostrata* N.E.Br.:
Powell 88 (NBG). *C. speciosa* L.Bolus:
Powell 107 (NBG). *C. umdausensis* L.Bolus:
Powell 102 (NBG). *C. verrucosa* L.Bolus:
Powell 99 (NBG). ***Drosanthemum*** Schwantes: *D. diversifolium* L.Bolus: KF132633; KF132149; KF132435; KF132778; KF131856; KF133116; *Klak 1743* (BOL). ***Enarganthe*** N.E.Br: *E. octonaria* (L.Bolus) N.E.Br: KF132684; KF132198; AJ532805; AJ439023; JN896433; JN896380; *Klak 491* (BOL). ***Ihlenfeldtia*** H.E.K.Hartmann: *I. excavata* (L.Bolus) H.E.K.Hartmann: KF132701; KF132214; KF132477; KF132818; KF131921; KF133182; *Wisura 1926* (BOL). *I. vanzylii* (L.Bolus) H.E.K.Hartmann:

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KGB 222/98 (KBG). ***Jacobsenia*** L.Bolus & Schwantes: *J. vaginata* (L.Bolus) Ihlenf.: KF132703; KF132215; KF132479; KF132820; KF131923; KF133184; *Wisura 882* (BOL). ***Jensenobotrya*** A.G.J.Herre: *J. lossowiana* A.G.J.Herre: KF132704; KF132216; KF132480; KF132821; KF131924; KF133185; *Van Jaarsveld 21145* (BOL). ***Namaquanthus*** L.Bolus *N. vanheerdei* L.Bolus: KF132724; –; AJ532810; AJ439049; KF131944; KF133205; *Klak 251* (BOL). ***Odontophorus*** N.E.Br: *O. angustifolius* L.Bolus:

EVJ 106/87 (NBG). *O. marlothii* N.E.Br.: KF132731; KF132243; AJ532823; AJ558101; KF131951; KF133212; *Klak 862* (BOL). ***Ruschianthus*** L.Bolus *R. falcatus* L.Bolus: KF132758; KF132266; KF132518; KF132857; KF131978; KF133240; *Rawe s.n.*(BOL). ***Schlechteranthus*** Schwantes: *S. abruptus* (A.Berger) R.F.Powell: KT281451, KT248276, KT248288, KT248312, KT248324, KT248335; *Powell 5* (NBG). *S. connatus* (L.Bolus) R.F.Powell: KT281449, KT248273, KT248297, KT248309, KT248321; *Powell 10* (NBG). *S. hallii* L.Bolus: *Klak 259* (BOL). *S. inclusus* (N.E.Br.) R.F.Powell: KT281450, KT248274, KT248286, KT248310, KT248322, KT248333; *Powell 35* (NBG). *S. pungens* (H.E.K.Hartmann) R.F.Powell: KT281447, —, KT248279, KT248303, KT248315, KT248327; *Powell 28* (NBG). *S.* sp.nov.: KT281448, KT248269, KT248281, KT248305, KT248317, KT248329; *Powell 41* (NBG). *S. spinescens* (L.Bolus) R.F.Powell *Klak 2424* (BOL). *S. steenbokensis* (H.E.K.Hartmann) Klak: *Bruyns 8267* (BOL). *S. subglobosus* (L.Bolus) R.F.Powell: *Bruyns 8240* (BOL). *S. tetrasepalus* (L.Bolus) R.F.Powell: KT248266, KT248277, KT248289, KT248313, KT248325, KT248336; *Klak 2411* (BOL).

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Appendix 2. Voucher information for leaf material studied in transverse section. Voucher information is listed as taxon, collector, collection number and herbarium.

Cheiridopsis acuminata L.Bolus*, Powell 68* (NBG);*Cheiridopsis aspera* L.Bolus*, Powell 14* (NBG); *Cheiridopsis denticulate* N.E.Br.*, Powell 9* (NBG); *Cheiridopsis caroli-schmidtii* N.E.Br.*, Powell 105* (NBG); *Cheiridopsis gamoepensis* S.A.Hammer*, Powell 89* (NBG); *Cheiridopsis glomerata, Powell 96* (NBG); *Cheiridopsis imitans, Powell 91* (NBG); *Cheiridopsis herrei, Powell 100* (NBG); *Cheiridopsis meyeri, Powell 16* (NBG); *Cheiridopsis minor, Powell 94* (NBG); *Cheiridopsis namaquensis, Powell 11* (NBG); *Cheiridopsis peculiaris, Powell 15* (NBG); *Cheiridopsis pillansii, Powell 45* (NBG); *Cheiridopsis pilosula, Powell 69* (NBG); *Cheiridopsis ponderosa, Powell 103* (NBG); *Cheiridopsis purpurea, Powell 85* (NBG); *Cheiridopsis robusta, Powell 66* (NBG); *Cheiridopsis rostrata, Powell 88* (NBG); *Cheiridopsis schlechteri, Powell 17* (NBG); *Cheiridopsis speciosa, Powell 107* (NBG); *Cheiridopsis verrucosa, Powell 99* (NBG); *Cheiridopsis umdausensis, Powell 102* (NBG*); Enarganthe octonaria, Powell 45* (NBG); *Ihlenfeldtia excavata, Powell 55* (NBG); *Ihlenfeldtia vanzylii, KBG 222/98* (KBG); *Jensenobotrya lossowiana, SUG 12618* (NBG); *Namaquanthus vanheerdei, EVJ 2475* (NBG); *Odontophorus angustifolius, EVJ 106/87* (NBG).

Appendix 3. Voucher information for pollen material studied under the light microscope. Voucher information is listed as taxon, collector, collection number and herbarium.

Conophytum frutescens, *Wisura 862* (NBG); *Conophytum hians, Wilkins s.n*. (NBG); *Conophytum uviforme, Powell 39* (NBG); *Cheiridopsis denticulata,van Heerde s.n.* (BOL); *Cheiridopsis derenbergiana, Wisura 2053* (NBG); *Cheiridopsis namaquensis, van Heerde 69* (BOL); *Cheiridopsis pillansii, Wisura 1617* (NBG); *Cheiridopsis pilosula, van Jaarsveld 2548* (NBG); *Enarganthe octonaria, van Jaarsveld 4206* (NBG); *Ihlenfeldtia excavata, van Heerde 30* (NBG); *Namaquanthus vanheerdei, van Jaarsveld 2475* (NBG); *Octopoma connatum, Helme 3226* (BOL); *Odontophorus marlothii, Hall NBG 624/57* (NBG); *Odontophorus nanus, Smithers s.n.* (BOL); *Schlechteranthus albiflorus, Van Berkel 462* (NBG); *Schlecheranthus hallii, Burgoyne 7340* (NBG); *Schlechteranthus spinescens, Desmet & Ellis 1345* (NBG).

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Table 1. Results of the topological constraint analysis, p-values reported from the Templeton-Wilcoxon test.

		Templeton				Winning-sites	
Tree	Length	Rank sums	N	z	P	Counts	P
1 (constrained topology)	542	3.0	2	-1.4142	0.1573	2	0.50
2 (unconstrained topology)	540	0.0	-	-	-	0	-

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Table 2: Summary of important generic anatomical, morphological and palynological characters in the *Conophytum*-clade.

	Schlechteranthus	***Conophytum***	***Cheiridopsis* s.s.**	***Ihlenfeldtia* s.s.**	***Odontophorus* s.s.**	***Enarganthe***	***Jensenobotrya***	***Namaquanthus***	***Ruschianthus***
Habit	Woody shrub	Caespitose	Caespitose	Caespitose	Caespitose, sometimes mat-forming	Woody shrub	Woody shrub	Woody shrub	Caespitose
Leaf shape	Triquetrous to trigonous	Globose to ovoid, rarely square	Triquetrous to trigonous	Triquetrous to trigonous	Triquetrous to trigonous	Clavate to trigonous	Shortly clavate to almost globose	Finger-shaped	Sickle-shaped
Degree of leaf length fused	$^1/_6$ to ¾ fused	Usually completely fused, sometimes top ¼ free, rarely completely free	$^1/_6$ to $^5/_6$ fused	$^1/_5$ to ¼ fused	$^1/_5$ to ¼fused	Leaf pairs not fused	$^1/_5$ fused	$^1/_6$ fused	$^1/_6$ fused
Old leaf forming sheath	No	Yes	Yes	Yes	Yes	No	No	No	No
Epidermal papillae*	Blunt pappilae	Blunt pappilae to trichomes	Blunt pappilae to trichomes	Blunt pappilae to trichomes	Blunt pappilae to trichomes	Flat epidermal cells, no papillae	Flat epidermal cells, no papillae	Flat epidermal cells, no papillae	Epidermal cells very slightly elevated
Stomatal protection†	Form I and II	Form I and II, and stomata sometimes elevated	Form I and II	Form I	Form I and II	Form I	Form I	Form I	Form I
Petaloid staminodes	Free at base, never forming a tube	Fused, forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube	Free at base, never forming a tube
Flower colour	White to magenta	White, cream, yellow, orange, red or magenta	White, cream, yellow, orange or magenta	Yellow, often purplish suffused, rarely creamy white, red with age	Yellow, often cream-coloured, sometimes white or basally white	Bright reddish-purple	Pink	Bright purple	Green
Filamentous staminodes	Present	Present or absent	Present	Absent	Absent	Absent	Absent	Present	Present
Pollen exine structure	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi	Tricolpate with poorly defined colpi, distinctly micro-reticulate	Syncolpate	Tricolpate with well-defined colpi	Unknown
No of locules	7-12	3-8	8-18	10-15	10	8	5	(8-)12(-17)	5
Covering membranes	Present	Absent	Present	Present	Present	Present	Absent	Present	Absent
Closing bodies	Present	Absent	Present	Present	Present	Absent, placenta sometimes forms hood over locule	Absent	Absent	Absent

Footnotes:
* as defined by Opel, (2005): blunt papillae are low protuberances in the centre of the epidermal cell and trichomes are defined as outgrowths of epidermal cells that are longer than broad.
† Form I – stomata placed in depressions formed by underlying tanniferous idioblats; Form II – stomtata sunken below epidermal surface, in depressions and further protected by overarching parastomal cells (Ihlenfeldt & Hartmann, 1982).

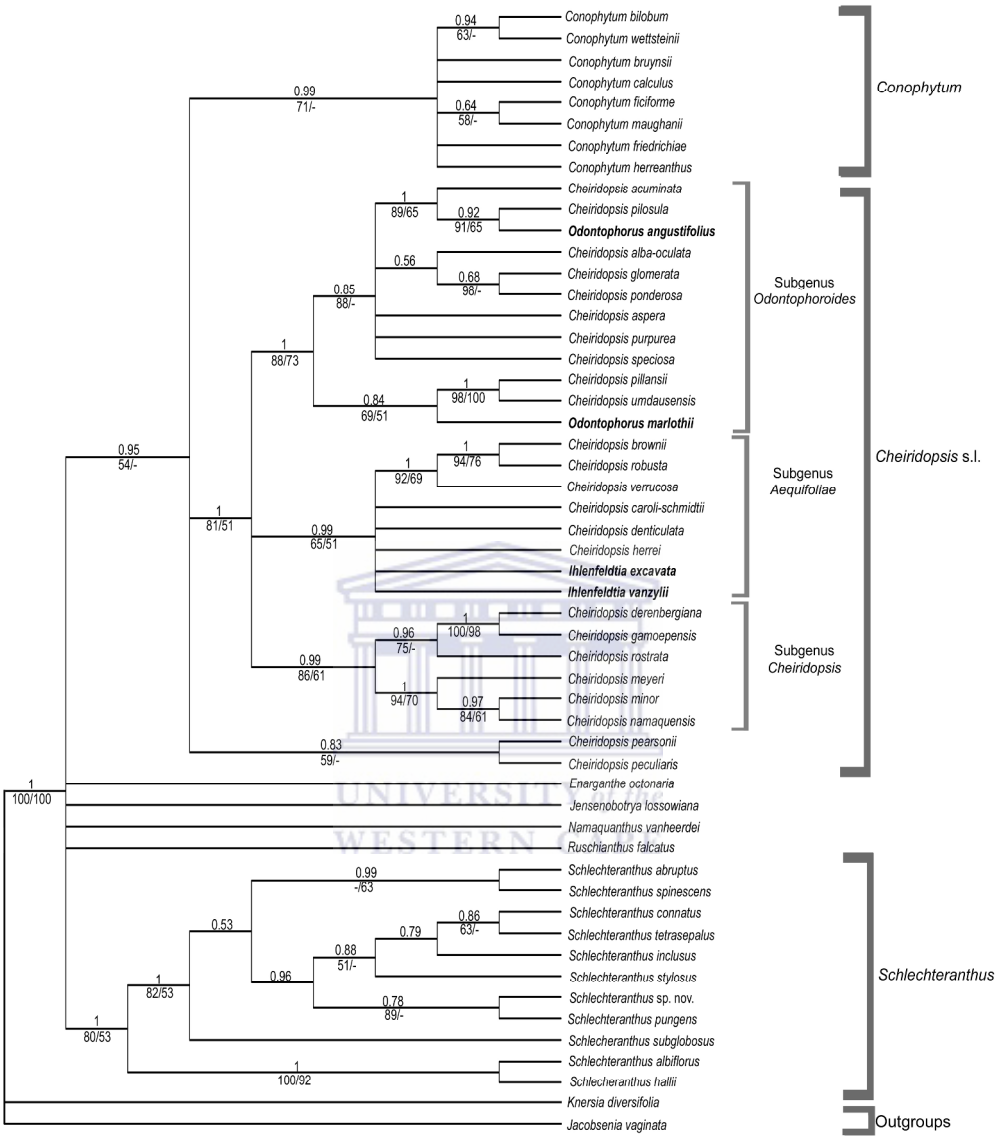

Figure 1. Majority Rule Consensus tree from Bayesian analysis of six plastid markers, indicating phylogenetic relationships in the Conophytum-clade. Posterior probability (PP) values above 0.5 are indicated above the branches. Jackknife support values (JS) and Bootstrap supports (BS) above 50, from the maximum parsimony and maximum likelihood analyses, are indicated below the branches. Brackets indicate the placement of taxa and clades discussed, with embedded genera indicated in bold.

149x177mm (600 x 600 DPI)

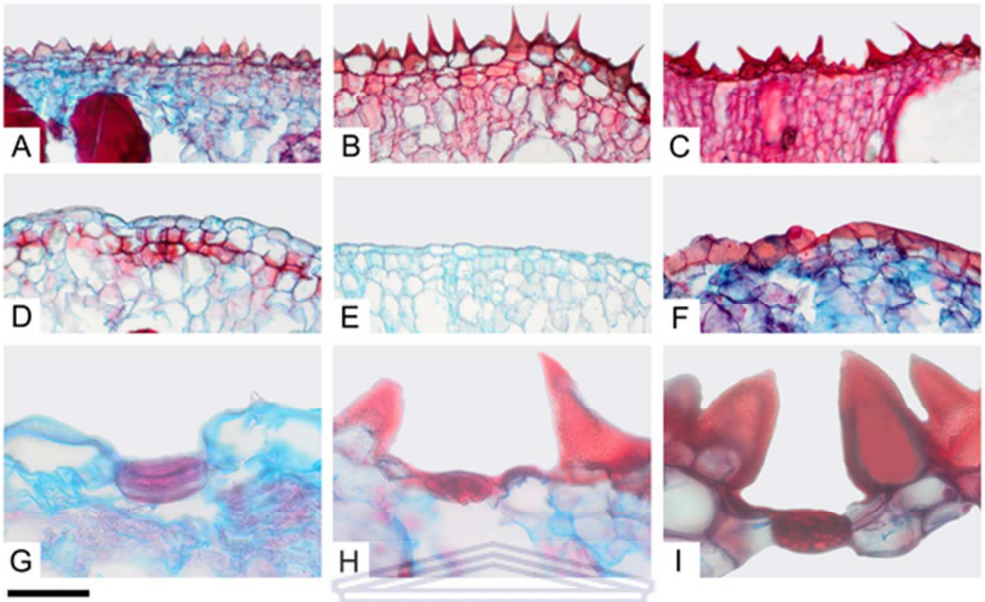

Figure 2. Transverse sections through the leaves of taxa in the Conophytum-clade illustrating characters of taxonomic importance. (A) epidermal cells forming blunt papillae in Cheiridopsis caroli-schmidtii; (B) epidermal cells forming trichomes in Cheiridopsis verrucosa; (C) trichomes in Ihlenfeldtia vanzylii; (D) glabrous epidermis of: (D) Namaquanthus vanheerdei; (E) Enarganthe octonaria; (F) Jensenobotrya lossowiana; (G) stomata in depression, not sunken or hidden in Cheiridopsis robusta; (H) stomata in depression, sunken and hidden by parastomal cell in Cheiridopsis acuminata; (I) sunken stomata in Odontophorus angustifolius. Vouchers: (A) Powell 105 (NBG); (B) Powell 99 (NBG); (C) KBG222/98 (KBG); (D) van Jaarsveld 2475 (NBG); (E) Powell 45 (NBG); (F) SUG 12618 (NBG); (G) Powell 66 (NBG); (H) Powell 68 (NBG); (I) EVJ 106/87 (NBG). Scale: A–F = 200µm; G–I = 50 µm.

50x31mm (300 x 300 DPI)

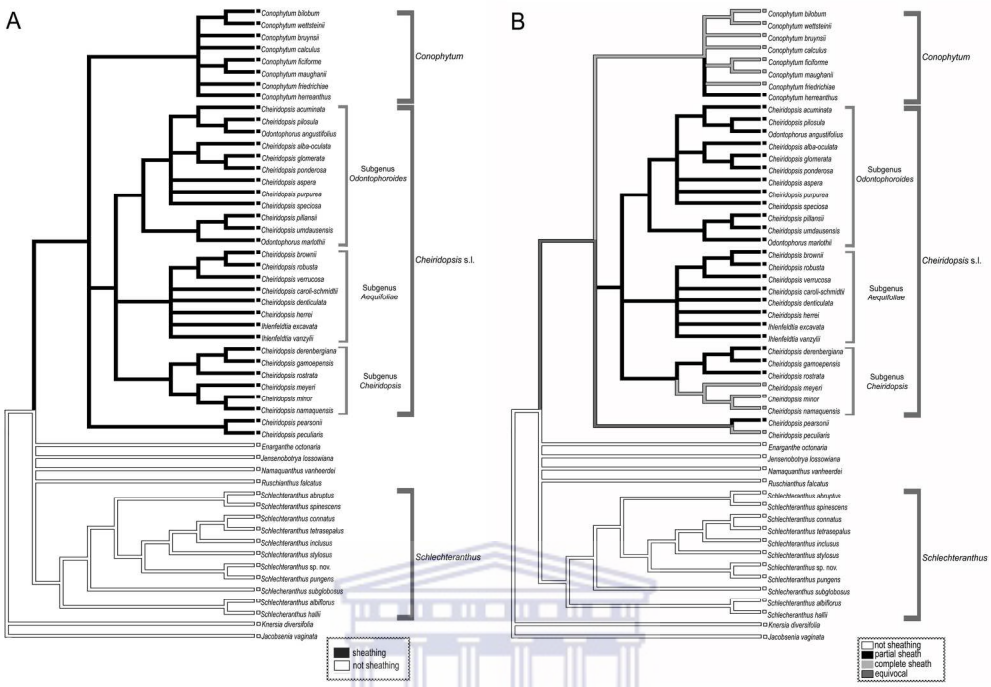

Figure 3. Character reconstruction of sheathing and sheathing type on the Bayesian inference consensus tree. (A) sheathing genera of the Conophytum-clade, indicated by the black branches; (B) sheath type mapped onto the Bayesian inference consensus tree, complete sheath refers to species were the sheath fully encloses the emerging pair during the dormant period (black branches) and in the partial sheath, the sheath only encloses part of the emerging leaf pair during dormancy (grey branches). The white branches indicate species that do not sheath.

180x127mm (300 x 300 DPI)

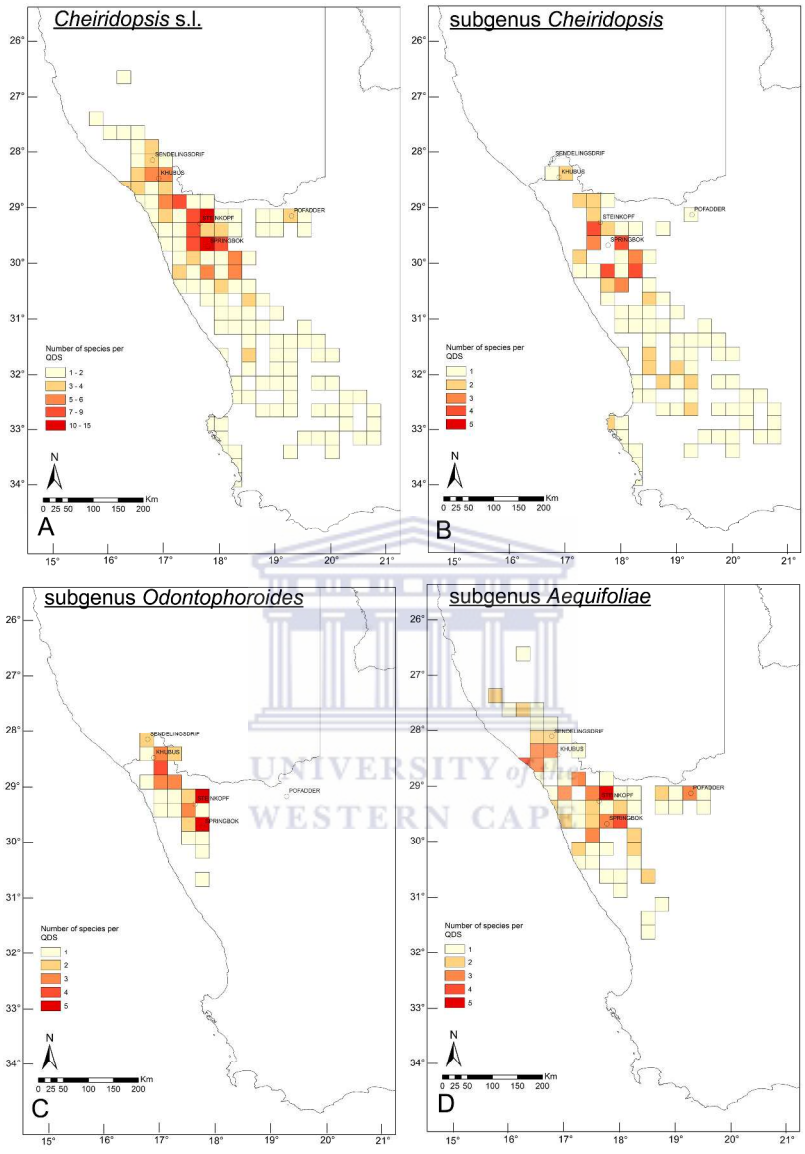

Figure 4. Number of species per Quarter Degree Square (QDS) (Leistner & Morris, 1976) and distribution of Cheiridopsis s.l. and subgenera (as treated in the taxonomic treatment) in the Greater Cape Floristic region, South Africa. (A) distribution and number of species per QDS for the genus Cheiridopsis s.l.; (B) distribution and number of species per QDS in subgenus Cheiridopsis; (C) distribution and number of species per QDS in subgenus Odontophoroides (including species of previously recognised Odontophorus); (D) distribution and number of species per QDS in subgenus Aequifoliae (including species of previously recognised Ihlenfeldtia).

199x266mm (600 x 600 DPI)

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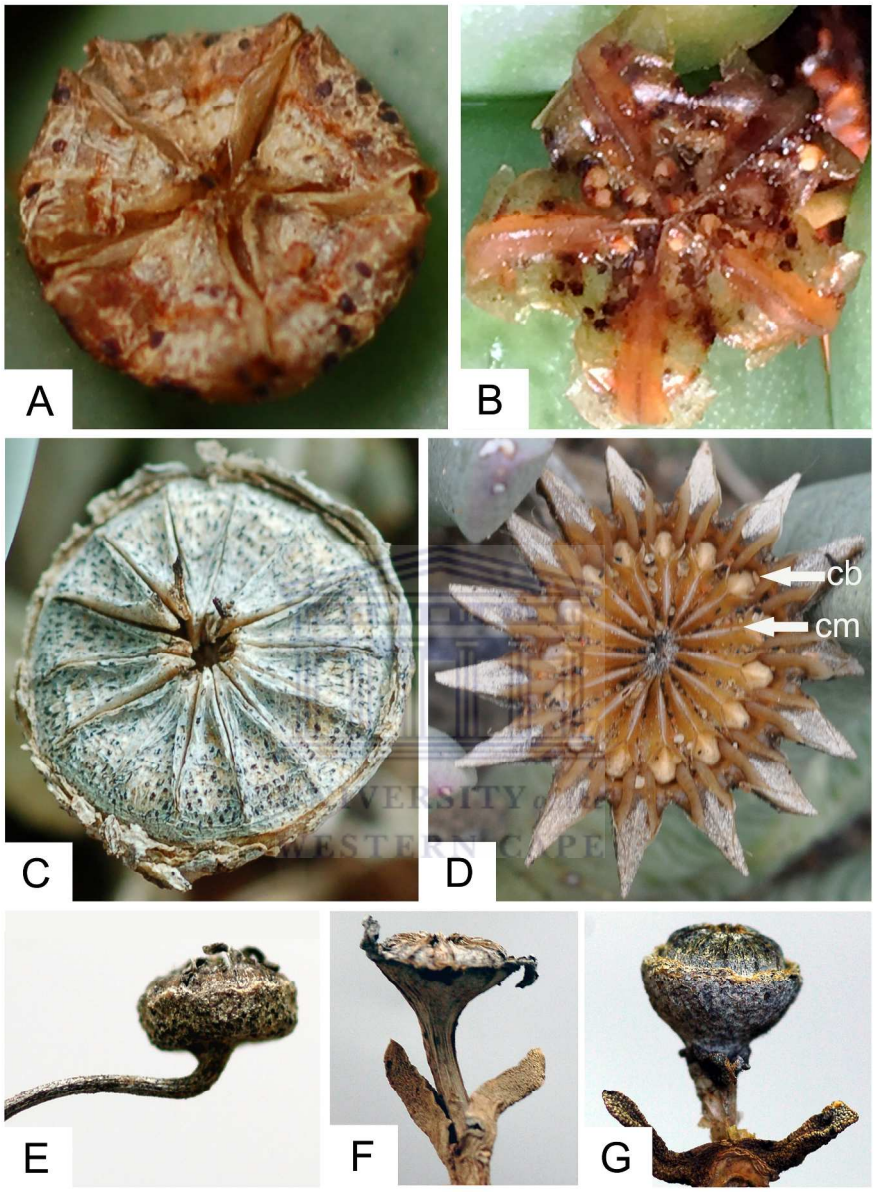

Figure 5. Capsules in Conophytum and Cheiridopsis. (A) closed simple capsule of Conophytum wettsteinii; (B) open simple capsule of Conophytum wettsteinii showing the absence of covering membranes and closing bodies; (C) closed multi-locular capsule of Cheiridopsis denticulata; (D) open capsule of Cheiridopsis denticulata showing the complex internal structures, i.e. covering membranes (cm) and closing bodies (cb), indicated by the white arrows; (E) decumbent capsules of Cheiridopsis subgenus Cheiridopsis, in Cheiridopsis namaquensis; (F) erect capsule of Cheiridopsis denticulata with flat to centrally elevated tops as in Cheiridopsis subgenus Aequifoliae; (G) Cheiridopsis pilosula illustrating the erect capsule with rounded tops, typical of Cheiridopsis subgenus Odontophoroides.

121x163mm (600 x 600 DPI)

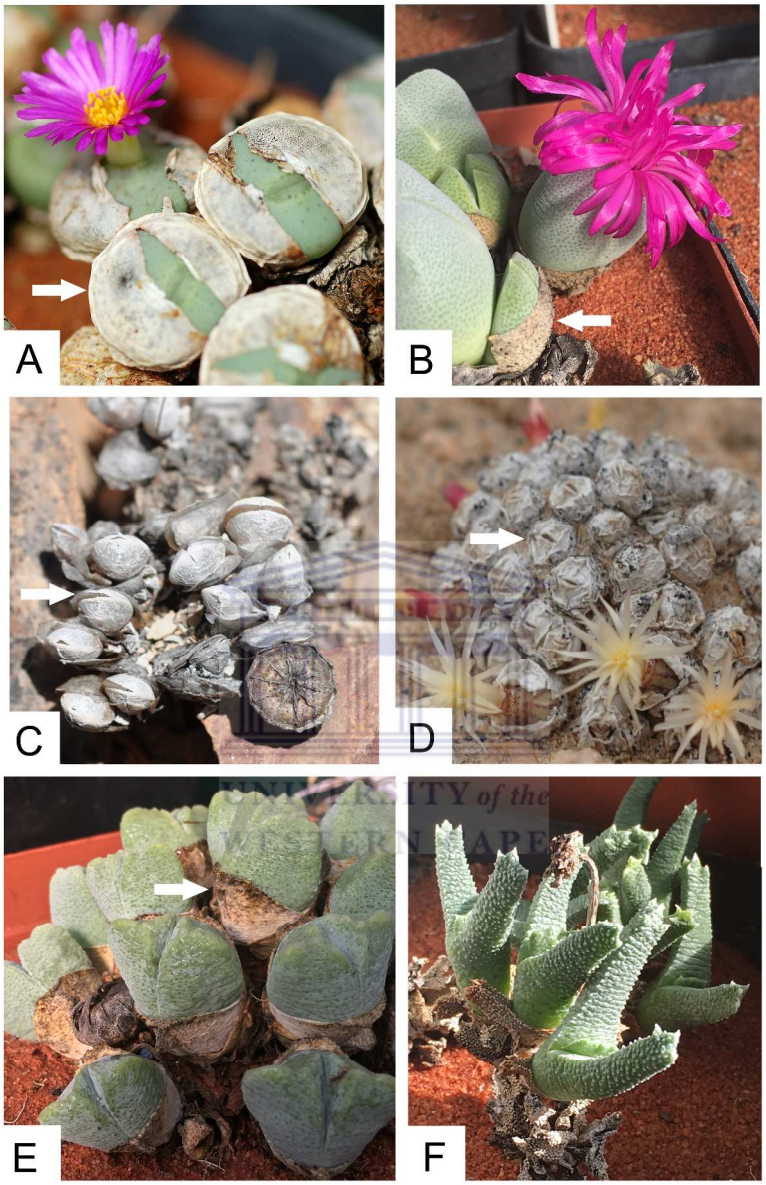

Figure 6. Leaf and floral characters of Cheiridopsis and Conophytum. (A) new leaves of Conophytum wettsteinii breaking out of their papery sheath, with a magenta flower; (B) partial sheath enclosing the emerging leaf pair, with a magenta flower, in Cheiridopsis glomerata; (C) leaves of Cheiridopsis meyeri completely enclosed by a white papery sheath during the dormant period, indicated by the arrow; (D) leaves of Conophytum uviforme completely enclosed in a sheath during the dormant period, indicated by the arrow; (E) Cheiridopsis purpurea with the partial sheath, common to many Cheiridopsis species which only encloses part of the leaves during the dormant period; (F) Cheiridopsis aspera with the prominent rough leaf surface often found in subgenus Odontophoroides.

189x291mm (600 x 600 DPI)

APPENDIX E3. **Powell RF, Klak C, Boatwright JS, Magee AR.** A taxonomic revision of *Schlechteranthus* subgenus *Microphyllus* (Ruschieae; Aizoaceae). *Systematic Botany*. **Submitted**.

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A Taxonomic Revision of *Schlechteranthus* subgenus *Microphyllus* (Ruschieae; Aizoaceae)

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Abstract—*Schlechteranthus* subgenus *Microphyllus* is a recently erected subgenus, which is revised here for the first time. The subgenus is comprised of nine succulent species, including a new species ***S. parvus***, which are endemic to the arid part of the Greater Cape Floristic Region. *Schlechteranthus* subgenus *Microphyllus* can be distinguished from subgenus *Schlechteranthus* by the smaller leaves and smaller capsules, with 7 to 9 locules and small closing bodies that block $^{1}/_{3}$ of the locule. Differences in leaf shape, degree of fusion, and arrangement, as well as inflorescence and spine structure were identified as important characters in distinguishing species in the subgenus. *Schlechteranthus parvus, S. pungens, S. spinescens* and *S. stylosus* all share the presence of spines, absence of bracteoles, and cymose inflorescences, while the prominently lengthened primary axis in *S. connatus, S. subglobosus,* and *S. tetrasepalus* grouped these species. Maps illustrating species richness hotspots within a Quarter Degree Square were produced for the genus and subgenera respectively. A key to the subgenera in *Schlechteranthus* and a comprehensive taxonomic treatment of subgenus *Microphyllus* is presented, including a key to the species, descriptions, figures illustrating diagnostic characters and distribution maps.

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***Keywords*—**distribution, Greater Cape Floristic Region, inflorescence structure, new species, papillate leaves, spines, succulent shrubs.

Schlechteranthus Schwantes is a genus of succulent shrubs included in the diverse and speciose tribe Ruschieae (Aizoaceae), with species endemic to the arid parts of the Greater Cape Floristic Region (Jürgens 1991; Hartmann 1996, 1998, 2001; Manning and Goldblatt 2012; Snijman 2013; Klak and Bruyns 2016). The genus is placed in the *Conophytum*-clade (Klak et al. 2013; Powell et al. 2016) and is distinguished by a combination of characters from other genera in the clade: A shrubby habit, leaves with a papillate epidermis and a prominent mucro, white to magenta cone-type flowers, with stamens arranged in a cone, and 7- to 12-locular capsules with pointed expanding keels. The generic circumscription of *Schlechteranthus* was recently assessed resulting in the expansion of the genus and erection of subgenus *Microphyllus* R. F. Powell (Klak and Bruyns 2016; Powell et al. 2016).

The phylogenetic analysis of Klak et al. (2013) placed *Schlechteranthus* sister to the previously recognised genus *Polymita* N. E. Br. An assessment of generic circumscription of these genera, led to the expansion of *Schlechteranthus* to include *Polymita*, with a taxonomic revision for these species presented in Klak and Bruyns (2016). *Schlechteranthus* and *Polymita* were placed sister to *Octopoma subglobosum* (L. Bolus) L. Bolus in the tribal phylogeny produced by Klak et al. (2013), but as only two species of *Octopoma* N. E. Br. were sampled, generic relationships between these species were unclear.

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Powell et al. (2016) expanded the phylogenetic sampling of *Octopoma* to include seven of the eight species, as well as the genus *Arenifera* A.G.J.Herre, which had not been sampled in any previous phylogenetic analyses. The results indicated that both *Octopoma* and *Arenifera* were polyphyletic, with five *Octopoma* and four *Arenifera* species placed sister to *Schlechteranthus* in the *Conophytum*-clade, while the type species of the genera were placed in the xeromorphic clade (Klak et al. 2013; Powell et al. 2016). The assessment of generic circumscriptions based on phylogenetic, morphological and anatomical data, of these species, specifically in relation to their sister genus *Schlechteranthus*, revealed two groups, which

were recognised as subgenera (Powell et al. 2016). Subgenus *Schlechteranthus* includes species of *Schlechteranthus* and the previously recognised genus *Polymita*, while subgenus *Microphyllus* includes the species previously included in *Arenifera* and *Octopoma*. The subgenera are distinguished from one another by differences in epidermal cell shape, cuticle thickness, locule number and variation in shrub, leaf and capsule size (Powell et al. 2016).

The species of subgenus *Microphyllus* were previously included in *Eberlanzia* Schwantes, *Mesembryanthemum* L. and *Ruschia* Schwantes (Berger 1922; Bolus 1926, 1928, 1930, 1932; Brown 1930), but were subsequently moved into *Arenifera* based on the shared inflorescence structure, capsule shape and presence of spines and into *Octopoma* based on the shared 8-locular capsules (Hartmann 1996; Hartmann 1998). The need for a taxonomic revision of these species was recognised even prior to the erection of the subgenus, with *Arenifera* and *Octopoma* identified as priority genera for taxonomic revision by Von Staden et al. (2013).

This study presents the first taxonomic revision for *Schlechteranthus* subgenus *Microphyllus* and includes the descriptions of nine species, including one new species, as well as a key to the species, notes on their ecology and comprehensive distribution maps.

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MATERIALS AND METHODS

***Examination of morphological characters*—**The complete collections of *Schlechteranthus* (160 specimens) housed at BOL, NBG (including SAM) and PRE were examined. In addition, all of the species of subgenus *Microphyllus* were studied *in situ*. During field visits, material was collected for herbarium and living collections of each species. These specimens are housed as voucher specimens at NBG and form part of the living collection at the Karoo Botanical Gardens. The herbarium specimens and living

collections were used for examination of leaf, floral and capsule characters to compare and identify diagnostic morphological characters across the subgenus.

***Phylogenetic analyses*—**A molecular and morphological cladistic analysis for subgenus *Microphyllus* was conducted using the molecular phylogenetic data produced in Powell et al. (2016) and eight coded morphological characters for the morphological analyses. Both the molecular and morphological data was analysed for the species in the subgenus, as well as for two selected outgroup taxa from subgenus *Schlechteranthus*. The two outgroup taxa (*S. hallii* L. Bolus and *S. steenbokensis* (H. E. K. Hartmann) Klak)), were selected as these species of subgenus *Schlechteranthus* were included in the molecular phylogenetic analysis of Powell et al. (2016).

The molecular and morphological dataset was analysed separately using Bayesian inference in MrBayes version 3.2.3 (Ronquist and Huelsenbeck 2003). The molecular data was analysed following Powell et al. (2016), with the combined plastid dataset partitioned into 10 partitions, with all parameters unlinked, the GTR + G + I model implemented (Huelsenbeck and Rannala 2004) and two simultaneous runs completed for 10^7 generations. For the morphological dataset, the standard model (Ronquist et al., 2011) was implemented and two simultaneous runs were completed for 15^5 generations. In both analyses, the split frequencies stabilised below 0.01 and suboptimal trees were discarded as the "burn-in" phase. A 50% majority-rule consensus tree was constructed from the remaining trees in the separate analyses and the posterior probability (PP) values are reported on the trees, respectively.

The maximum parsimony algorithm was implemented in PAUP* version 4.0b4 (Swofford 2000), for both the molecular and morphological data separately, with characters equally weighted and unordered. A heuristic search with 1,000 random sequence additions, tree bisection-reconnection (TBR) branch-swapping and 10 trees held per replicate was performed. Internal support was assessed with 1,000 bootstrap (BS) replicates under the same

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search parameters. The strict consensus tree with bootstrap support values for the molecular and morphological data is presented in Appendix 1 and 2, respectively.

The coded morphological characters were reconstructed onto the Bayesian inference consensus trees of both the molecular and morphological analysis, using the parsimony trace character history function in Mesquite version 3.04 (Maddison and Madisson 2015).

***Distribution and species richness maps*—**Data on the distribution of the species are presented as maps. These data were collected from the examined herbarium specimens as well as specimens produced from field collections. The distribution for each species was recorded using the Quarter Degree Reference System (Edwards and Leistner 1971; also outlined in Leistner and Morris 1976). The basic unit in this system is the one-degree square of latitude and longitude (a Quarter Degree Square), which is designated by a degree reference number (i.e. degrees of latitude and longitude of the north-west corner) and the district name of that grid. This grid reference system was used to produce maps for each of the species in subgenus *Microphyllus*.

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Species richness maps, indicating the number of species per Quarter Degree Square (QDS) were produced for the genus as well as the subgenera, with the distribution of the genus and subgenera mapped separately in ArcMap (ESRI 2011). A QDS grid was overlaid onto the distribution data and the Spatial Join tool (ESRI 2011) was used to join the two datasets. The number of species per QDS was calculated for the genus and subgenera from the count data in the Spatial Join (ESRI 2011), and the data were presented on the maps accordingly.

RESULTS AND DISCUSSION

Habit—*Schlechteranthus* subgenus *Microphyllus* consists of woody shrubs or shrublets, some of which are markedly compact, including *S. connatus* (L. Bolus) R. F. Powell, *S. inclusus* (L. Bolus) R. F. Powell (Fig. 1H), *S. parvus*, and *S. tetrasepalus* (L. Bolus) R. F. Powell, only reaching 150 mm in height, while the larger shrubs, *S. pungens* (H. E. K. Hartmann) R. F. Powell and *S. stylosus* (L. Bolus) R. F. Powell, grow up to 400 mm tall. The species share an upright to spreading habit, although *S. connatus* is often decumbent.

***Branches and leaf arrangement*—**Branches are up to 9(–13) mm in width, with the widest branches found in the very compact *S. tetrasepalus.* The upper branches are usually brown, but in *S. pungens* they are distinctively white, while in *S. spinescens* (L. Bolus) R. F. Powell they are often whitish-grey.

The leaves are usually crowded towards the tips of the branches in subgenus *Microphyllus*, however, in *S. subglobosus* (L. Bolus) R. F. Powell the opposite leaf pairs are evenly spaced along the branches with visible internodes (Fig. 1E). In *S. inclusus* the leaves are decussate and tightly packed along the branch, so that the internodes are not visible (Fig. 1G). In addition, the old blackened leaves remain on the branch in *S. inclusus* (Fig. 1G), in contrast to all the other species in the subgenus, where old leaves fall off the branch.

***Leaves*—**The leaves in species of subgenus *Microphyllus* are triquetrous to fatly trigonous and mucronate (Fig. 2), although in *S. parvus* the mucro is not prominent. Leaves display various shapes in outline, including globose, ovoid, oblong to lanceolate (Fig. 2). Leaf size ranges include 1.5–20.0 x 1.5–5.0 mm and the leaf pair bases are fused from $^{1}/_{5}$ to ¾ of the leaf length (Fig. 2). Leaf colour is usually green, ranging from bright to deep green and sometimes yellow-green. The leaves are slightly papillate, due to the epidermal cells forming blunt papillae, defined as low protuberances in the centre of the epidermal cell (Opel 2005). These blunt papillae result in a slightly velvety appearance of the leaf, but never appear hairy

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to the naked eye. In *S. abruptus* (A. Berger) R. F. Powell, the protuberances are slightly extended, creating a hair-like structure, which results in a prominently velvety leaf. The leaf surface of *S. subglobosus* is also slightly different from the rest of the species due to the stout, dome-shaped epidermal cells, which result in a rough leaf surface.

***Inflorescence structure*—**Flowers in species of subgenus *Microphyllus* are either solitary or arranged in cymose inflorescences. The non-spiny species, i.e. *S. abruptus, S. connatus, S. inclusus, S. subglobosus,* and *S. tetrasepalus,* all share solitary flowers (Fig. 1C, F), with the flowers on a central primary axis, as found in species of subgenus *Schlechteranthus* (Fig. 3B; Table 1; Appendix 3).

The central primary axis is sometimes prominently extended, ranging from 1 ½ times to 10 times longer than the capsule length (as found in *S. connatus* (Fig. 1C), *S. subglobosus* and *S. tetrasepalus*). This character was recovered as a synapomorphy for these species within the morphological phylogeny of the subgenus (Fig. 3B; Table 1; Appendix 3). In the remaining species, as well as in species of subgenus *Schlechteranthus,* the primary axis is proportionally equal to the length of the capsule (Fig. 3B; Table 1; Appendix 3).

The length of the primary axis also affects the position of the bracteole, with the bracteole positioned more than $^{1}/_{2}$ way down the primary axis in *S. connatus* (Fig. 1C) and *S. tetrasepalus,* while in *S. abruptus* and *S. inclusus* the bracteoles are positioned at the base of the capsule (less than ¼ of the way down the primary axis (Fig. 1G). The bracteoles of *S. subglobosus* are positioned the lowest, more than $^{2}/_{3}$ down the primary axis. Furthermore, in *S. inclusus,* the bracteoles are prominently cup-shaped (Fig. 1F). The bracteole is absent in *S. parvus, S. pungens, S. spinescens,* and *S. stylosus,* grouping these species in the morphological phylogeny (Fig. 3B; Table 1; Appendix 3).

Schlechteranthus parvus, S. pungens, S. spinescens, and *S. stylosus* share a cymose inflorescence with spines. Both these characters, together with the loss of the bracteole, were

recovered as synapomorphies in the morphological phylogeny for these species in the subgenus (Fig. 3B; Table 1; Appendix 3). The arrangement of the spines is directly linked to the inflorescence structure, as the spines are derived from the old peduncles and in some species (*S. parvus, S. stylosus, S. pungens*), additional spines are derived from aborted axillary buds. In *S. spinescens*, the spines are arranged in a simple cyme on the primary axis, with blunt spines derived solely from old peduncles where the capsules have fallen off (Fig. 4A; Table 1; Appendix 3). Although all the species share this spine type, this is the only spine formation found in *S. spinescens.* In *S. parvus, S. pungens*, and *S. stylosus* (Figs. 3, 4B, C; Table 1; Appendix 3), there are additional sharp spines derived from aborted axillary buds, which are arranged in compound cymes (Figs. 1B, 4B, C). In *S. parvus* and *S. stylosus*, the spines are arranged in a simple cyme on the secondary axes (Fig. 4B), while in *S. pungens,* the spines are arranged in a compound cyme on the secondary axes and in a simple cyme on the tertiary axes (Fig. 4C).

***Floral morphology*—**The calyx is always green, with 4–6 sepals and the petaloid staminodes are more or less equal, although inner row is sometimes slightly shorter. The flowers are white to magenta and sometimes a combination thereof, i.e. magenta tips with a white central eye. Filamentous staminodes are occasionally absent (*S. tetrasepalus,* sometimes absent in *S. stylosus*) and the number of filamentous staminodes varies from a few (up to only 3) to many, in which case they form a complete series around the stamens. *Schlechteranthus spinescens* and *S. parvus* have filamentous staminodes that form a complete series around the stamens, which is a useful character to distinguish *S. parvus* (Fig. 5A) from *S. stylosus.* Some species have few to many filamentous staminodes, such as *S. connatus* and *S. pungens,* forming a variable complete to incomplete series around the stamens. The remaining species, *S. abruptus, S. inclusus, S. stylosus,* and *S. subglobosus,* always have only a few filamentous staminodes (sometimes absent in *S. stylosus*), forming an incomplete series

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around the stamens. The stamens are always collected into a cone in the centre of the flower (Fig. 1D), which is found in all species of *Schlechteranthus*. If filamentous staminodes are present (absent in some species), these surround the stamens and together then also form a cone. The flowers include 6–9 stigmas, with the nectaries in a ring.

***Capsule morphology*—**The capsules are 7–9 locular (Fig. 1A), as opposed to the 10–12 locules found in subgenus *Schlechteranthus* (Powell et al. 2016). Capsule shape may be useful to distinguish some species in subgenus *Microphyllus*. The top of the capsules is usually convex, but sometimes only slightly so, with a somewhat flattened apex, as found in *S. subglobosus* (Fig. 6A). The base of the capsule is funnel-shaped in all the species (Fig. 6A), with the exception of *S. connatus*, where the base is subglobose (Fig. 6B). The subglobose base, in combination with the convex top, results in an almost globose capsule in *S. connatus*. The capsules possess complete covering membranes with small closing bodies that block $^{1}/_{3}$ of the locule (Figs. 1A, 6C). The small size of the closing bodies was recovered as a synapomorphy for this subgenus (Fig. 3; Table 1; Appendix 3). Closing rodlets are conspicuous in all species, with the rodlets protruding distally in *S. connatus* and *S. tetrasepalus.* Valve wings may be present or absent, and if present, they are usually narrow ($^{1}/_{4}$ to $^{1}/_{3}$ width of valve). Valve wings are absent in *S. abruptus, S. connatus, S. inclusus, S. parvus,* and *S. stylosus.*

***Phylogenetic relationships and character evolution*—**The trees recovered from the analyses of the molecular plastid data and morphological coded data are strongly incongruent with very different topologies and groupings of the species (Fig. 3). The subgenus *Microphyllus* is, however, recovered as monophyletic in both analyses (PP=1, BS=100 in the molecular tree; PP=1, BS=89 in the morphological tree), supported by the 7 to 9-locular capsules with small closing bodies.

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In the molecular analysis (Fig. 3A), the recovered clades are not supported by morphological characters; however, there is a slight distinction in geographical distributions of the species. The two species recovered in a well-supported clade, *S. abruptus* and *S. spinescens* (PP=1, BS=81), have a slightly more southern centre of distribution (Fig. 7B, F) while the distributions of the majority of species (*S. connatus*, *S. inclusus*, *parvus*, *S. pungens*, *S. spinescens*, *S. styosus*, *S. tetrasepalus*), recovered in a strongly supported clade (PP=1, BS=67), are centred in Namaqualand (Fig. 7A, C–E, G–I). The distribution of the remaining unresolved species, *S. subglobosus* (Fig. 3A), is also centred in Namaqualand with the distribution of this species restricted to this region (Fig. 7D).

In contrast, the weak geographical pattern was not recovered in the morphological analysis, as the trees are incongruent (Fig. 3). In the morphological analysis of subgenus *Microphyllus*, the clade including *S. parvus*, *S. pungens*, *S. spinescens*, and *S. stylosus* (PP=1, BS=98) is supported by three synapomorphic characters (Fig. 3B), i.e. the presence of spines, flowers arranged in a cymose inflorescence and the loss of the bracteole (Table 1; Appendix 3). These characters, when mapped onto the molecular tree, are shown to have converged twice, with a subsequent loss of these characters in *S. connatus*, *S. inclusus*, and *S. tetrasepalus* (Fig. 3A). The convergence of spines is not unusual in the Aizoaceae, as spines are shown to have evolved numerous times in the Ruschioideae, for example in *Antimima* N. E. Br., *Drosanthemum* Schwantes and *Leipoldtia* L. Bolus (Hartmann and Stüber 1993). Spine type, specifically the type where spines are derived from aborted buds (Table 1; Appendix 3), supported the grouping of *S. parvus*, *S. pungens*, and *S. stylosus* (PP=0.90, BS=66) in the morphological analysis (Fig. 3B), but was recovered as a convergence in the molecular analysis (Fig. 3A). The lengthened primary axis (Table 1; Appendix 3), grouped *S. connatus*, *S. subglobosus*, and *S. tetrasepalus* (PP=0.90, BS=67) in the morphological analysis (Fig. 3B), while it was recovered as a convergence in the molecular analysis for the

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clade including *S. connatus* and *S. tetrasepalus* (PP=0.85, BS=56) and for the unresolved *S. subglobosus* (Fig. 3A).

In light of the incongruence between the two phylogenetic datasets, we have arranged the species in the taxonomic treatment according to the sequence suggested by the morphological analysis (Fig. 3B), so that morphologically similar taxa are arranged together, for ease of identification.

TAXONOMIC TREATMENT

KEY TO THE SUBGENERA OF *SCHLECHTERANTHUS*:

1. Capsules > 6 mm in diameter, with 10 to 12 locules, closing bodies blocking more than half of the locule; leaves ≥ 4.5 mm wide; epidermal cells anticlinally elongated, with calcium oxalate crystals deposited on the anticlinal walls .....**subg. *Schlechteranthus***

1. Capsules < 6 mm in diameter, with 7 to 9 locules, closing bodies blocking less than half of the locule; leaves ≤ 4.5 mm wide; epidermal cells isodiameteric without crystals on the anticlinal wall.....**subg. *Microphyllus***

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SCHLECHTERANTHUS SUBGENUS MICROPHYLLUS R. F. Powell, Taxon 65: 259: 2016. TYPE: *Schlechteranthus pungens* (H. E. K. Hartmann) R. F. Powell.

Arenifera A. G. J. Herre *p.p.* excluding type, Sukkulentenkunde 2: 35. 1948.

Octopoma N. E. Br. *p.p*. excluding type, Gard. Chron. Ser. III. 87: 126, in clavi. 1930.

Low-growing, succulent, compact, upright to spreading shrub, often dome-shaped, 100–400 mm tall, with sparse to dense growth. *Branches* woody, decumbent or spreading to upright, branching from the base, up to 9 (–13) mm wide, occasionally covered by persistent old leaves; upper branches brown, or whitish grey to white. *Leaves* triquetrous to fatly trigonous; globose, ovoid, oblong or lanceolate in outline, 1.5–20.0 x 1.5–5.0 mm; margins

thickened; leaf pairs fused for 1/5–¾ of length; mucronate, although mucro sometimes not prominent; slightly papillate, resulting in a slightly velvety to prominently velvety, or rough leaf surface; bright green to deep green, sometimes yellow-green. *Flowers* solitary or in spinescent cymose inflorescences, spines formed either by old peduncles or aborted axillary buds; on central primary axis 10–55 mm long, equal to capsule length or up to 10 times longer; bracteole present or absent, sometimes cup-shaped; flowers cone-type, with stamens collected into a cone; calyx lobes green, 4–6; petaloid stamindoes ± equal, inner row sometimes shorter, white to magenta; filamentous staminodes absent or few to many, forming a complete or incomplete series around the stamens; stigmas 6 to 9, nectaries in a ring. *Fruit* a loculicidal capsule, with 7 to 9 locules, top prominently to slightly convex, base funnel-shaped, sometimes cup-shaped, 2.0–6.0 x 2.0–6.0 mm; covering membranes complete; closing bodies small, blocking 1/3 of locule; closing rodlet conspicuous, sometimes protruding distally; valve wings narrow, ¼ to 1/3 of valve width, sometimes absent. *Seeds* obovate to pear-shaped; smooth to slightly pustulate.

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***Diagnostic characters*—**Subgenus *Microphyllus* can be distinguished from subgenus *Schlechteranthus* by the smaller leaves (3.5–5.0 x 4–6 mm vs. 5–30 x 3.5–9.0 mm), smaller capsules (2–6 x 2–6 mm vs. 6–11 x 4–9 mm), with only 7 to 9 locules (vs. 10 to 12 locules) and small closing bodies that block 1/3 of the locule (vs. larger closing bodies that block ¾ of locule). In addition, the epidermal cells are isodiametric with no crystals deposited on the anticlinal wall, whereas in subgenus *Schlechteranthus* the epidermal cells are anticlinally elongated with calcium oxalate crystal deposits on the anticlinal walls. The epidermal cells of subgenus *Microphyllus* form blunt papillae which are found throughout the epidermis, but concentrated around the stomata, while in subgenus *Schlechteranthus*, the papillate epidermal cells are only found surrounding the stomata. *Schlechteranthus holgatensis* is an exception in subgenus *Schlechteranthus*, as it is densely papillate throughout the leaf surface. Nine species

are currently recognised in subgenus *Microphyllus* and six species in subgenus *Schlechteranthus* (Klak and Bruyns 2016).

Species of subgenus *Microphyllus* may be confused with other species of Ruschioideae which also have mulitlocular capsules and spines, as found in *Leipoldtia*, however, species of subgenus *Microphyllus* are distinguished from these species by the mucronate leaves, narrow valve wings (broad in *Leipoldtia*), small or large closing bodies (always large in *Leipoldtia*) and 7–9 locular capsules (c. 10-locular capsules in *Leipoldtia*).

Distribution and ecology—*Schlechteranthus* is distributed from Khubus to Uniondale, with hotspots around Bitterfontein, Khubus, Kleinsee, Springbok, and Steinkopf (Fig. 8A). The distribution of subgenus *Schlechteranthus* extends from north of Khubus to just north of Bitterfontein, with only two hotspots north of Kleinsee (Fig. 8B; Klak and Bruyns 2016). Subgenus *Microphyllus* has a much wider distribution range, from the border of Namibia, southwards to Clanwilliam with an eastern record at Uniondale (Fig. 8C). Interestingly, the highest species richness of subgenus *Microphyllus* is found around Bitterfontein (Fig. 8C), in contrast to subgenus *Schlechteranthus*, which does not occur as far south. There are smaller hotspots for subgenus *Microphyllus* around Bitterfontein, Steinkopf, and between Steinkopf and Khubus (Fig. 8C).

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The species occur in a wide range of ecological habitats such as gentle rocky slopes, alluvial banks and rocky soils, and on a number of geologies including calcrete, gneiss, quartz, red loamy sand, sandstone, and shale. The species flower from mid-winter to spring, i.e. August to October.

328 **KEY TO THE SPECIES OF SUBGENUS *MICROPHYLLUS*:**

329 1. Flowers solitary; primary axis bracteate, old peduncles or aborted axillary buds not
330 forming spines; valve wings absent or very narrow, up to ¼ of valve width 2
331 2. Leaves markedly globose in outline; leaves positioned at regular intervals along the
332 branch; capsules slightly convex, flattened at apex; branches upright to spreading; leaves
333 usually bright green..... **4. *S. subglobosus***
334 2. Leaves ovoid to lanceolate in outline; leaves crowded towards the tips of the branches or
335 stacked tightly along the branch; capsules convex, not flattened at apex; branches decumbent
336 or upright to spreading; leaves green to dark green, never bright green.....3
337 3. Primary axis 1½–10 times longer than capsule length; capsules often held in a row above
338 the terminal leaf pairs; bracteoles more than ⅓ way down the primary axis.....4
339 4. Leaves ovoid in outline, < 5 mm long, opposite leaf pairs fused ⅔ of length; capsule bases
340 funnel-shaped; branches upright.....**3. *S. tetrasepalus***
341 4. Leaves lanceolate in outline, > 8 mm long, opposite leaf pairs fused ¼ of length; capsule
342 bases rounded; branches decumbent..... **5. *S. connatus***
343 3. Primary axis equal in length to capsule length; capsules not held in a row above terminal
344 leaf pairs; bracteoles usually at base of capsules but always less than ¼ way down the
345 primary axis 5
346 5. Leaves decussate, with old leaves persisting on branch; opposite leaf pairs fused ¾ of
347 length, ovoid in outline, ≤ 11 mm long.....**1. *S. inclusus***
348 5. Leaves crowded towards the tips of the branch, with old leaves falling off branch; opposite
349 leaf pairs fused ¼ of length, lanceolate in outline, ≥12 mm long.....**2. *S. abruptus***
350 1. Flowers in cymose inflorescences; primary axis ebracteate, old peduncles or aborted
351 axillary buds forming spines; valve wings narrow, up to $^1/_3$ of valve width 6

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6. Blunt spines derived solely from old peduncles where capsules have fallen off; inflorescences in simple cymes..... **6. *S. spinescens***

6. Sharp spines derived from aborted axillary buds; inflorescences in compound cymes.... 7

7. Spines arranged in compound cyme on secondary axes; upper branches white; opposite leaf pairs fused for ¼ to ⅓ of length..... **7. *S. pungens***

7. Spines arranged in simple cyme on secondary axes; upper branches brown; opposite leaf pairs fused for ⅓ to ¾ of length.....8

8. Leaves ovoid in outline, prominently mucronate, leaf pairs fused for ≤ ½ their length; filamentous staminodes few, not forming a complete series around the stamens; large shrub to 400 mm tall..... **8. *S. stylosus***

8. Leaves subglobose to globose in outline, not prominently mucronate, leaf pairs fused for ≥ ½ their length; filamentous staminodes many, forming a complete series around the stamens; shrublet to 150 mm tall..... **9. *S. parvus***

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1. SCHLECHTERANTHUS INCLUSUS (L. Bolus) R. F. Powell, Taxon 65: 259. 2016.

Mesembryanthemum inclusum L. Bolus, Ann. Bolus Herb. 4: 40. 1926. *Octopoma inclusum* (L. Bolus) N. E. Br., Gard. Chron. 3: 126. 1930; Hartmann, Bradleya 16: 72. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. *Ruschia restituta* (L. Bolus) G. D. Rowley, Natl. Cact. Succ. J. 33: 8. 1978. TYPE: SOUTH AFRICA. Northern Cape Province: slopes overlooking the sea, south of Hondeklip Bay, Namaqualand [3017DA], 1924, *Pillans sub BOL 17758* (lectotype. designated by Hartmann (1998): BOL!).

Dense upright shrublets, to 100 mm tall. *Branches* to 8 mm wide; upper branches brown; leaves present along the branch, prominently stacked, with old black leaves remaining on branch. *Leaves* triquetrous, ovoid in outline; 4.0–11.0 x 3.0–3.5 mm; leaf pairs fused for ¾ of length; mucro prominent; slightly velvety; green, often blackened at base. *Flowers* solitary, on central primary axis to 11 mm long, equal proportionally to capsule length, bracteoles present less than ¼ way down axis, prominently cup-shaped, often holding capsule base; spines absent; filamentous staminodes few, forming an incomplete series around the stamens. *Fruit* 8-locular; top slightly convex, base funnel-shaped; 4.0 x 3.0 mm; closing rodlets conspicuous; valve wings absent. Figure 9.

***Diagnostic characters*—**This species is easily distinguished by the decussate leaves stacked along the branch and the old blackened leaves that remain on the branch (Fig. 1G). However, it may be confused with *S. tetrasepalus*, which shares a compact habit and leaf pairs with a high degree of fusion (Fig. 2F, G). *Schlechteranthus inclusus* is, however, further distinguished by the short primary axis which is equal in length to the length of the capsule, and the cup-shaped bracteoles (central axis at least 1½ times longer than capsule, leaves crowded towards the tip of the branches, with old leaves falling off, and bracteoles not cup-shaped in *S. tetrasepalus*).

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***Distribution and ecology*—**This species has a coastal distribution, found on the west coast of South Africa between Kleinsee and the Groen River mouth (Fig. 7A). *Schlechteranthus inclusus* is associated with a range of geologies including calcrete, granite, and gneiss, but always in shallow soil. The distribution of this species is closely linked to alluvial habitats, often occurring on rocky patches in or alongside river courses. It flowers from August to September.

***Additional specimens examined*—**South Africa. NORTHERN CAPE: 2917 (Springbok): Namaqualand, Kleinsee, Farm Drooge Kraal 180, lower south-eastern slopes of

Naroegas se Berg, below trig beacon (–AD), *Desmet and Smale 3284* (NBG). 3017 (Hondeklipbaai): east of Koingnaas, on south slope (–AB), *Klak 474* (BOL); South of Koingnaas along road to Hondeklip Bay, at Swartlintjiesrivier (–AB), *Klak 2359* (BOL); Close to turnoff to Hondeklipbaai from Kleinsee (–AB), *Powell 35* (NBG); Spoegrivier cave, on rocks above the river (–AD), *Klak 1836* (BOL); Nama'land, 2 miles north of Kamieskroon (–BB), *Littlewood sub KGB 584/59* (BOL); Gemsbokvlei, 10 miles east of Port Nolloth (–BC), *Van Heerde 27400* (BOL); North of Flamink Berg, Walle Kraal, Namaqualand (–BC), *Pillans 17911* (BOL); South of Groenrivier mouth, near the lighthouse (–DC), *Klak 1832* (BOL); Cape Province, Namaqualand, 6 miles north of Garies (–DD), *Hall sub NBG 1016/60* (BOL); Garies district, farm Tweerivier (–DD), *Klak 2411* (BOL). Precise locality unknown: South border of Little Namaqualand, *Peers sub NBG 1394/33* (BOL).

2. SCHLECHTERANTHUS ABRUPTUS (A. Berger) R. F. Powell, Taxon 65: 259. 2016.

Mesembryanthemum abruptum A. Berger, Bot. Jahrb. Syst. 57: 638. 1922. *Octopoma abruptum* (A. Berger) N. E. Br., Gard. Chron. 3: 126. 1930; Hartmann, Bradleya 16: 72. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 189. 2001; Klak in Manning and Goldblatt, Pl. Gr. C.F.R. 1: 296. 2012. *Ruschia abrupta* (A. Berger) G. D. Rowley, Natl. Cact. Succ. J. 33: 8. 1978. TYPE: SOUTH AFRICA. Western Cape Province: Brandewynrivier, between Clanwilliam and Calvinia [3118DD], Oct 1897, *R. Schlechter 10828* (isotype: BOL!).

Ruschia rupigena L. Bolus, Notes Mesembryanthemum 3: 520. 1935. *Ruschia rupicola* L. Bolus, Notes Mesembryanthemum 2: 415. 1933, *nomen illegit*. non Schwantes Z. Sukkulentenk. 2: 187. 1926. *Octopoma rupigena* (L. Bolus) L. Bolus J. S. African Bot. 33: 306. 1967; Hartmann, Bradleya 16: 73. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Manning and Goldblatt, Pl. Gr. C.F.R. 1: 297. 2012.

TYPE: SOUTH AFRICA. Western Cape Province: near Pakhuis, Clanwilliam Div., common on rocks between Pakhuis and Buishoekfontein [3218BB], Sep 1933, *L. Bolus sub NBG 1504/33* (holotype: BOL!).

Sparse, upright to spreading shrub, to 250 mm tall. *Branches* to 9 mm wide; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branch. *Leaves* triquetrous, lanceolate in outline; 12.0–20.0 x 2.5–5.0 mm; leaf pairs fused for ¼ of length; mucro prominent; prominently velvety as a result of extended papillae of epidermal cells; green. *Flowers* solitary; on central primary axis to 11 mm long, equal proportionally to capsule length, bracteoles present less than ¼ way down axis; spines absent; filamentous staminodes few, forming an incomplete series around the stamens. *Fruit* 8-locular; top slightly convex, base widely funnel-shaped; 5.0 x 3.5 mm; closing rodlets conspicuous; valve wings absent. Figure 10.

Diagnostic characters—*Schlechteranthus abruptus* is most likely to be confused with *S. connatus* as the species share a similar leaf shape and size, but can be distinguished by the shape of the capsule, which has a funnel-shaped base (capsule base subglobose in *S. connatus*) and the primary axis which also not lengthened (prominently lengthened in *S. connatus*). The bracteole position in *S. abruptus* is also distinct, as it is almost always found at the base of the capsule, but no more than ¼ way down the primary axis (more than ½ way down the primary axis in *S. connatus*). *Schlechteranthus abruptus* can also be distinguished by its prominently velvety leaf surface, which is unique to this species in the subgenus, and otherwise only found in *S. holgatensis* Klak in subgenus *Schlechteranthus* (Klak and Bruyns 2016).

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Distribution and ecology—*Schlechteranthus abruptus* is found in the area between Clanwilliam and Garies (Fig. 7B). The species is found on shallow rocky sandstone soils and flowers from July to August.

Additional specimens examined—South Africa. WESTERN CAPE: 3017 (Hondeklipbaai): north of Garies, Kys (–BD), *Klak 468* (BOL); Klip Vlei, between Kamieskroon and Garies, Namaqualand (–DD), *Thorne 49957* (SAM). 3218 (Clanwilliam): inter Pakhuis et Nardouw (–BB), *Leipoldt s.n.* (BOL). 3219 (Wuppertal): Klipfontein, north of Boontjieskloof (–AA), *Esterhuysen 32204* (BOL); On Pakhuis Pass, just before turnoff to Wuppertal (–AA), *Powell 5* (NBG); Pakhuis, near turnoff to Wuppertal (–AA), *Klak 433* (BOL). Precise locality unknown: near Clanwilliam, *L. Bolus sub BOL 23833* (BOL).

3. SCHLECHTERANTHUS TETRASEPALUS (L. Bolus) R. F. Powell, Taxon 65: 259. 2016.

Ruschia tetrasepala L. Bolus, Notes Mesembryanthemum 2: 373. 1932. *Octopoma tetrasepalum* (L. Bolus) H. E. K. Hartmann, Bradleya 16: 74. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. TYPE: SOUTH AFRICA. Western Cape Province: between the town and Sout River [3118DD], Apr 1932, *Luckhoff sub BOL 20203* (holotype: BOL!).

Dense upright shrublet, to 130 mm tall. *Branches* to 7(–13) mm wide; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, ovoid in outline; 3.0–5.0 x 1.5–4.0 mm; leaf pairs fused for $^{2}/_{3}$ of length; mucro prominent; slightly velvety; green. *Flowers* solitary; on central primary axis to 13 mm long, at least 1½ times longer than capsule length, bracteoles present more than $^{1}/_{3}$ way down axis; spines absent; filamentous staminodes absent. *Fruit* 7 to 8-locular; top convex, base

funnel-shaped; ca. 5.5 x 4.0 mm; closing rodlets conspicuous, protruding distally; valve wings absent.

***Diagnostic characters*—**This species is most similar to *S. inclusus* in its compact habit, leaves with a high degree of fusion (Fig. 2F, G), and solitary flowers, but is distinguished by the longer primary axis, which is at least 1½ times longer than the capsule, often resulting in the capsules held in a row above the terminal leaf pairs (primary axis equal in length to capsule and capsules not held in a row in *S. inclusus*). In *S. tetrasepalus,* the old leaves do not remain on the branch, leaving branches bare, while in *S. inclusus,* the old leaves remain on the branch.

***Distribution and ecology*—**This species occurs on gravel sandstone patches on alluvial banks of the Groen River (Fig. 7C) and in the Knersvlakte in quartz. *Schlechteranthus tetrasepalus* flowers in September.

***Additional specimens examined*—**South Africa. WESTERN CAPE: 3017 (Hondeklipbaai): Tweeriviere, Groen River (–DD), *Bruyns 10856* (BOL). 3118 (Vanrhynsdorp): Knersvlakte, Farm Moedverloor (–AD), *Klak 933, Klak 2418* (BOL).

4. SCHLECHTERANTHUS SUBGLOBOSUS (L. Bolus) R. F. Powell, Taxon 65: 259. 2016. *Ruschia subglobosa* L. Bolus, Notes Mesembryanthemum 1: 140. 1928. *Mesembryanthemum reductum* (L. Bolus) N. E. Br., Gard. Chron. 3: 33. 1930. *Octopoma subglobosa* (L. Bolus) L. Bolus, J. S. African Bot. 29: 49. 1963; Hartmann, Bradleya 16: 73. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 190. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. TYPE: SOUTH AFRICA. Northern Cape Province: hills on north side of O'kiep, Little Namaqualand [2917DB], Oct 1926, *Pillans 5844* (holotype: BOL!).

Sparse spreading shrub, to 200 mm tall. *Branches* to 4 mm wide; upper branches brown; leaves positioned at regular intervals along the branches, old leaves not remaining on the branches. *Leaves* fatly trigonous, markedly globose in outline; 2.5–9.0 x 1.5–3.0 mm; leaf pairs fused for $^1/_3$ of length; mucro very prominent as result of leaf shape; rough, as a result of stout dome-shaped epidermal cells; bright green. *Flowers* solitary; on central primary axis to 25 mm long, at least 5 times longer than capsule length, bracteoles present more than $^2/_3$ way down axis; spines absent; filamentous staminodes few, forming an incomplete series around the stamens. *Fruit* 7 to 8-locular; top slightly convex, flattened at the apex, base narrowly funnel-shaped; ca. 4.0 x 2.5 mm; closing rodlets conspicuous; valve wings narrow, up to $^1/_4$ width of valve. Figure 11.

Diagnostic characters—*Schlechteranthus subglobosus* is a distinct species and, although it shares a globose leaf shape with *S. parvus* (Fig. 2D, I), the leaf pairs are only fused for $^1/_3$ of length with a very prominent mucro (fused for ¼ of length with obscure mucro in *S. parvus*) and the flowers are solitary (flowers in cymose inflorescence in *S. parvus*). The leaves of *S. subglobosus* are regularly spaced along the branch (Fig. 1E), which is similar to *S. inclusus*, while the rest of the species in the subgenus have the leaves crowded towards the tips. In *S. subglobosus*, however, the leaves are positioned at regular intervals along the branch with the internodes visible between the leaves (Fig. 1E), while in *S. inclusus* the leaves are tightly packed and decussate, with no visible internodes (Fig. 1G). *Schlechteranthus subglobosus* can further be distinguished from the other species in the subgenus by its bright green leaves with a rough surface and very prominent mucro as a result of the fatly trigonous leaf shape, while the leaves in the remaining species are triquetrous green to dark green leaves, with a slightly velvety to velvety leaf surface and a prominent mucro (obscure in *S. parvus*) in the rest of the subgenus).

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Distribution and ecology—*Schlechteranthus subglobosus* has a narrow distribution with a specific habitat, found only on gentle rocky slopes, usually gneiss, on hills between Springbok and Kamieskroon (Fig. 7D). The species flowers from August to September.

Additional specimens examined—South Africa. NORTHERN CAPE: 2917 (Springbok): On road between Goodhouse and Concordia (–BD), *Powell 33* (NBG); Namqualand, Ratelpoort (–BD), *Hall*, sub *NBG 1770/48* (BOL); 3 km east of Nababeep and 1.5 km north of Divide copper mine (–DB), *Hilton-Taylor 2080* (BOL); 2 km east of Springbok, O'kiep (–DB), *Klak 2418* (BOL); Namaqualand, near Springbok (–DB), *L. Bolus* sub BOL 19008 (BOL); Khamieskroon District, Ybeep (–DD), *Bruyns 7610* (BOL); Namaqualand District, Kamiesberg mountain range at pass on Droëdap road (–DD), *Burgoyne 11265* (NBG); Little Namaqualand, three miles south of Mesklip (–DD), *Salter 4598* (BOL); Little Namaqualand, Mesklip (–DD), *Esterhuysen 7769* (BOL); Namaqualand, Farm De Draay 346 (–DD), *Le Roux 4126b* (NBG). 2918 (Gamoep): Springbok District, Kweekfontein (–CA), *Bruyns 8240* (BOL); Namaqualand District, on road from Gamoep to Springbok, 2 km before farm Silverfontein (–CC), *Klak 753* (BOL); Nuwedam, Springbok (–CC), *Bruyns 9146* (BOL). 3018 (Kamiesberg): 1 km north of Rooidoringkloof turn-off (-AA), *Klak 866* (BOL); Southeast of Hôsabees in 'Pypmaker se Poort', road to Gamoep (–AB), *Hilton-Taylor 2239* (BOL). Precise locality unknown: Bushmanland, *SUG 8917* (BOL).

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5. SCHLECHTERANTHUS CONNATUS (L. Bolus) R. F. Powell, Taxon 65: 259. 2016. *Ruschia connata* L. Bolus, Notes Mesembryanthemum 1: 139. 1928. *Mesembryanthemum connatum* (L. Bolus) N. E. Br., Gard. Chron. 3: 32. 1930. *Octopoma connatum* (L. Bolus) L. Bolus, J. S. African Bot. 29: 49. 1963; Hartmann, Bradleya 16: 72. 1998; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 2: 189. 2001; Klak in Snijman, Pl. Gr. C.F.R. 2: 219. 2013. TYPE: SOUTH AFRICA. Northern Cape Province: Springbok (2917) between Doornpoort and Brakfontein [2917], Jul 1927, *Pillans 5794* (holotype: BOL!).

Sparse low-growing decumbent to upright shrub, to 100 mm tall. *Branches* to 7 mm wide; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, lanceolate in outline; 8.0–20.0 x 1.5–5 mm; leaf pairs fused for ¼ of length; mucro prominent; slightly velvety; green. *Flowers* solitary; on central primary axis to 55 mm long, at least 3 times longer than length of capsule, bracteoles present more than ½ way down axis; spines absent; filamentous staminodes few to many, forming an incomplete to complete series around the stamens. *Fruit* 8-locular; top prominently convex, base cup-shaped, resulting in capsule appearing subglobose; ca. 6.0 x 6.0 mm; closing rodlet conspicuous, protruding distally; valve wings absent. Figure 12.

Diagnostic characters—*Schlechteranthus connatus* may be confused with *S. abruptus* as they share a similar leaf shape and size, but is distinguished by the longer primary axis, which is at least 3 times longer than the capsule (Fig. 1C), and bracteoles present more than ½ the way down the axis (primary axis equal to length of capsule and bracteole mostly at base of capsule in *S. abruptus*). The capsule itself is also markedly globose in *S. connatus* (Figs. 1C, 6B), as a result of the subglobose base and convex top, whereas in *S. abruptus* the base of the capsule is funnel-shaped and the top not prominently

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rounded. *Schlechteranthus connatus* also lacks the prominently velvety leaf surface of *S. abruptus*, and is only slightly velvety.

Distribution and ecology—*Schlechteranthus connatus* is widespread in northern Namaqualand extending from the Richtersveld southwards into the Knersvlakte (Fig. 7E). It is generally found on rocky soil associated with gneiss or quartz and sometimes occurs in red loamy sand. The species flowers from September to October.

Additional specimens examined—South Africa. NORTHERN CAPE: 2816 (Oranjemund): Richtersveld, 5 km westwards along Holgat river from Eksteenfontein turn-off, along Kuboos-Lekkersing road (–DD), *Klak 1504* (BOL). 2817 (Vioolsdrif): 6 km south of Eksteenfontein (–CC), *Bruyns 9106* (BOL); Eksteenfontein, Pypfontein (–CC), *Bruyns 9132* (BOL); Richtersveld, 15 km east of Eksteenfontein, on road to Vioolsdrif (–CD), *Klak 1513* (BOL); Klipbokberge, Richtersveld (–CD), *Stellenbosch University Gardens 9289* (BOL); Doornriver, Richtersveld, Little Namaqualand (–CD), *Hort. Stellenbosch 8792* (BOL). 2917 (Springbok): 24 km north of Grasvlakte on road to Eksteenfontein (–AB), *Bruyns 9104* (BOL); 19 km south of Port Nolloth on road to Grootmis, Kwagganap River (–AC), *Klak 2397* (BOL); South-East of Naroegas (–AD), *Klak 1499* (BOL); between Bulletrap and the N7 (–BD), *Powell 10* (NBG); 2 km towards Nigramoep (–DA), *Klak 927* (BOL); Springbok, Drie Draai (–DD), *Bruyns 9152* (BOL). 3017 (Hondeklipbaai): south of Kran se vlei, Hondeklip Bay (–AD), *Klak 899* (BOL); Grootvleipass, near Kamieskroon (–BB), *Klak 1853* (BOL); ca. 1 km north of Kamieskroon hotel (–BB), *Klak 1871* (BOL); 6 miles south of Garies, Namaqualand (–DD), *Acocks 15102* (BOL); Garies district, farm Tweerivier (–DD), *Klak 2410* (BOL); Garies dist., farm Tweerivier, near Groenrivier (–DD), *Klak 2403* (BOL). 3018 (Kamiesberg): Kamiesberg, northern slopes of Weeskindpeak, 8 km southwest of Leliefontein (–AC), *Helme 3226* (BOL); Klipbok, Knersvlakte (–CB), *Bruyns 5372* (BOL); Garies dist., Farm Keurbos (–CB), *Klak 1263* (BOL); Rietkloof, Knersvlakte (–DC),

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591 *Matthews 19381, National Geographic-IPC Tour 119* (BOL); Garies, farm Stofkrall (–DD),
592 *Klak 1525* (BOL). 3019 (Loeriesfontein): Loeriesfontein, entrance to Donkiedam (–CC),
593 *Bruyns 10859* (BOL). WESTERN CAPE: 3118 (Vanrhynsdorp): Komkans Bitterfontein (–
594 AA), *Bruyns 12806* (BOL); Near Goerap, along road to Rietfontein (–AA), *Klak 2299*
595 (BOL); Knersvlakte, Kareeberg, on farm Kareeberg (–BA), *Klak 2284b* (BOL); Knersvlakte,
596 Vanrhynsdorp div., farm Arizona (–BC), *Klak 632* (BOL); Knersvlakte, Rooiberg, S of
597 Nuwerus (–BC), *Desmet 1805* (NBG) Farm Quaggaskop, Vanrhynsdorp (–BC), *Klak 1452*
598 (BOL); Zandkraal, Vanrhynsdorp (–DB), *Acocks 14859* (BOL). Precise locality unknown:
599 Between Garies and Kamieskroon, *L. Bolus 24220* (BOL).
600
601 6. SCHLECHTERANTHUS SPINESCENS (L. Bolus) R. F. Powell, Taxon 65: 259. 2016. *Ruschia*
602 *spinescens* L. Bolus, Notes Mesembryanthemum 2: 175. 1930. *Arenifera spinescens*
603 (L. Bolus) H. E. K. Hartmann, Bradleya 14: 38. 1996; Hartmann, Ill. Handb. Succ. Pl.
604 Aizoaceae 1: 69. 2001; Buys in Snijman, Pl. Gr. C.F.R. 2: 178. 2013. TYPE: SOUTH
605 AFRICA. Western Cape Province: Whitehill near Matjiesfontein, Laingsburg
606 [3320BC], Oct 1929, *Compton sub BOL 19081* (holotype: BOL!).
607
608 Sparse upright to spreading shrub, to 200 mm tall. *Branches* to 8 mm wide; upper
609 branches often whitish to grey; leaves crowded towards tips of branches, old leaves not
610 remaining on the branches. *Leaves* triquetrous, lanceolate in outline, 3.0–13.0 x 2.0–3.0 mm,
611 leaf pairs fused for $^1/_6$ to $^1/_5$ of length; mucro prominent; slightly velvety; deep green. *Flowers*
612 in simple cymose inflorescence; central primary axis to 15 mm long, equal proportionally to
613 capsule length, bracteoles absent; spines present, arranged in simple cyme, formed by old
614 peduncles where capsules have fallen off, held at obtuse angle, blunt, mostly elevated above
615 terminal leaf pair, but also present below terminal leaf pair; filamentous staminodes many,

forming a complete series around the stamens. *Fruit* 7 to 8-locular; top convex, base narrowly funnel-shaped, 2.5 x 2.0 mm; closing rodlet conspicuous; valve wings narrow, up to $^1/_3$ width of valve. Figure 13.

Diagnostic characters—*Schlechteranthus spinescens* is most similar to *S. pungens* as they have similar leaf shapes in outline (Fig. 2B, E) and overlap in distribution (Fig. 7F, G). However, *S. spinescens* can be easily recognised by the different arrangement of spines and spine formation. In *S. spinescens*, the spines are arranged in a simple cyme with blunt spines derived from old peduncles where the capsule have fallen off (Fig. 4A), while in *S. pungens* the spines are arranged in a compound cyme, with sharp spines derived from aborted axillary buds (Fig. 4C).

Distribution and ecology—This species occurs in the Western Cape and Namaqualand, and is the only species in the subgenus to extend into the Little Karoo towards Uniondale (Fig. 7F). Plants grow in a range of soils, including quartzite, shale and sand, but generally in shallow soils. This species flowers in October.

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Additional specimens examined—South Africa. NORTHERN CAPE: 3018 (Kamiesberg): Namaqualand, Varsche River Extension, Vanrhynsdorp, quartz lag gravel patch between Preekstoelkop and N7 (–BB), *Desmet and Ellis 1345* (NBG); 35 miles west of Loeriesfontein (–DD), *Lavis 162* (BOL). WESTERN CAPE: 3218 (Clanwilliam): On sand flat near Calvinia road, about 2 miles out of Clanwilliam (–BA), *Galpin s.n.* (BOL); Namaqualand, Wolwenes, Knersvlakte, Heuweltjie veld to west of N7 (–BC), *Desmet and Ellis 1498* (BOL); Rondegat, Clanwilliam (–BD), *Esterhuysen s.n.* (BOL). 3219 (Wuppertal): Syfering, west of Elandsvlei (–AD), *Klak 1370* (BOL); Cederberg, farm Ramkraal (–AD), *Klak 953* (BOL); Skitterykloof (–DC), *Klak 2424* (BOL). 3320 (Montagu): 6 km west of Matjiesfontein, farm Aasvoelbos (–AB), *Klak 1685* (BOL). 3323 (Willowmore): along road to Willowmore, Uniondale (–DA), *Fourcade 4370* (BOL).

641 7. *SCHLECHTERANTHUS PUNGENS* (H. E. K. Hartmann) R. F. Powell, Taxon 65: 259. 2016.
642 *Arenifera pungens* H. E. K. Hartmann, Bradleya 14: 37. 1996; Hartmann, Ill. Handb.
643 Succ. Pl. Aizoaceae 1: 69. 2001; Buys in Snijman, Pl. Gr. C.F.R. 2: 178. 2013. TYPE:
644 SOUTH AFRICA. Western Cape Province: Vanrhynsdorp [3118], Mar 1988,
645 *Hartmann, Dehn, Gölling, Rust and Stüber 25739* (holotype: HBG, photo!).
646
647 Large upright to spreading shrub, often dome-shaped, to 400 mm tall. *Branches* to 4
648 mm wide; upper branches white; leaves crowded towards tips of branches, old leaves not
649 remaining on the branches. *Leaves* triquetrous, mostly oblong, sometimes ovoid in outline,
650 3.0–6.0 x 2.0–2.5 mm, leaf pairs fused for ¼ to ⅓ of length; mucro prominent; slightly
651 velvety; dark green. *Flowers* in compound cymose inflorescences; central primary axis to 13
652 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged
653 in compound cyme, secondary axes with spines arranged in compound cyme, tertiary axes
654 with spines arranged in simple cyme; spines derived from aborted axillary buds and less
655 frequently from old peduncles; spines branching at an angle <45°, sharp, elevated above
656 terminal leaf pair; filamentous staminodes few to many, forming an incomplete to complete
657 series around stamens. *Fruit* 7 to 8-locular; top convex, base funnel-shaped, 2.0–3.0 x 4.0–4.5
658 mm; closing rodlet conspicuous; valve wings very narrow, up to $^1/_3$ width of valve.
659 ***Diagnostic characters—****Schlechteranthus pungens* is most similar to *S. stylosus*, as
660 both species are large shrubs (up to 400mm tall) with spines derived from aborted axillary
661 buds (Fig. 4), but *S. pungens* differs with longer (3.0–6.0 mm) mostly oblong leaves (Fig. 2E)
662 in outline (shorter (1.5–2.0 mm) and ovoid (Fig. 2H) in *S. stylosus*). Although the spines are
663 derived in a similar manner to *S. stylosus*, the spines in *S. pungens* are arranged in a
664 compound cyme on the secondary axes (Fig. 4C), while in *S. stylosus* the spines are arranged
665 in a simple cyme on the secondary axes (Fig. 4B).

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Schlechteranthus pungens and *S. spinescens* share a similar leaf shape (Fig. 2B, E) and their distributions overlap (Fig. 7F, G). However, *S. pungens* is distinguished by the spines that are arranged in a compound cyme and are derived from aborted axillary buds (Fig. 4C) (spines arranged in simple cyme and derived from old peduncles where capsules have fallen off in *S. spinescens* (Fig. 4D)).

***Distribution and ecology*—**This species has a wide distribution, occurring from Hondeklipbaai southwards to Calvinia (Fig. 7G) and is found on low slopes, growing in very rocky soils. *Schlechteranthus pungens* flowers in September.

Additional specimens examined—South Africa. NORTHERN CAPE: 2817 (Vioolsdrif): Namaqualand District, Richtersveld, south-west of Helskloof Valley between hills where road splits (–CD), *Burgoyne 11105* (PRE). 2917 (Springbok): Steinkopf, west of Steinkopf (–BC), *Klak 925* (BOL); between Bulletrap and the N7 (–BD), *Powell 12* (NBG); Namaqualand District. 6 km on second Komaggas road W of Springbok (–CD), *Burgoyne 11235* (PRE). 2918 (Gamoep): 26 km east of Springbok, farm Smorenskadu (–CA), *Klak 2358* (BOL). 3017 (Hondeklipbaai): Namaqualand, Kookfontein, north-northeast of Soebatsfontein (–BA), *Desmet 400* (NBG). 3018 (Kamiesberg): 23 miles east of Kliprand (–DA), *Stayner sub KGB 1208/62* (BOL); 35 miles west of Loeriesfontein, Calvinia (–DD), *Stayner 1970* (BOL). 3019 (Loeriesfontein): Loeriesfontein, Donkiedam Farm (–CC), *Klak 1285* (BOL); Loeriesfontein Dist., Donkiedam Farm (–CC), *Klak 1291* (BOL). WESTERN CAPE: 3118 (Vanryhnsdorp): Bitterfontein, Komkans (–AA), *Bruyns 12804* (BOL); Nuwerus, on farm Aurora (–AB), *Bruyns 7583* (BOL); 2 km south of Nuwerus (–AB), *Bruyns 9093* (BOL); Namaqualand, Farm Moedverloor 208 (–AD), *Desmet and Ellis 1459* (NBG); Namaqualand, Droesand, Rooipan, Knersvlakte (–BC), *Desmet and Ellis 1508* (NBG); Zout Rivier Bridge (–BC), *Matthews sub NBG 282/38* (BOL); Knersvlakte, farm Arizona, behind old farmhouse shed (–BC), *Klak 1450* (BOL). 3119 (Calvinia): Blaukrantz

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pass (–DB), *Pearson 3909* (BOL); South-western corner of Elandsberg (–DC), *Bruyns 9180* (BOL). 3219 (Wuppertal): Bossiesberg, Calvinia (–BA), *Bruyns 7568* (BOL).

8. *SCHLECHTERANTHUS STYLOSUS* (L. Bolus) R. F. Powell, Taxon: 259. 2016. *Ruschia stylosa* L. Bolus, Notes Mesembryanthemum 1: 144. 1928. *Mesembryanthemum stylosum* (L. Bolus) N. E. Br., Gard. Chron. 3: 32. 1930. *Eberlanzia stylosa* (L. Bolus) L. Bolus, Notes Mesembryanthemum 3: 387. 1958. *Arenifera stylosa* (L. Bolus) H. E. K. Hartmann, Bradleya 14: 38. 1996; Hartmann, Ill. Handb. Succ. Pl. Aizoaceae 1: 70. 2001; Buys in Snijman, Pl. Gr. C.F.R. 2: 178. 2013. TYPE: SOUTH AFRICA. Northern Cape Province: Hills NE of Arris Drift [2816DA], Jul 1927, *Pillans 5742* (holotype: BOL!).

Ruschia armata L. Bolus, Notes Mesembryanthemum 1: 145. 1928. *Mesembryanthemum armatum* N. E. Br., Gard. Chron. 3: 32. 1930. *Eberlanzia armata* (L. Bolus) L. Bolus, Notes Mesembryanthemum 3: 387. 1958. TYPE: SOUTH AFRICA. Northern Cape Province: between Brakfontein and Oograbies Mt., Little Namaqualand [2917AA], Oct 1926, *Pillans 5789* (holotype: BOL!).

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Dense spreading shrub, often dome-shaped, to 400 mm tall. *Branches* to 7 mm wide; upper branches brown; leaves crowded towards tips of branches, old leaves not remaining on the branches. *Leaves* triquetrous, ovoid in outline, 1.5–2.0 mm x 2.0–2.5 mm, leaf pairs fused for ⅓ to ½ of length; mucro prominent; slightly velvety; yellow-green to deep green. *Flowers* in compound cymose inflorescences; central primary axis to 8 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged in compound cyme, secondary axes with spines arranged in simple cyme; spines derived from aborted axillary buds and less frequently from old peduncles; spines branching at ± 45°, sharp, elevated above terminal leaf

pair; filamentous staminodes few to absent, forming an incomplete series around the stamens. *Fruit* 7 to 9-locular; top convex, base funnel-shaped, ca. 4.0 x 3.0 mm; closing rodlet conspicuous; valve wings absent. Figure 14.

Diagnostic characters—*Schlechteranthus stylosus* may be confused with *S. parvus* as they have a similar leaf size with a high degree of fusion (Fig. 2H, I) and the same spine arrangement (Fig. 4B). However, *S. stylosus* can be distinguished by the few filamentous staminodes which do not form a complete series around the stamens (many filamentous staminodes which form a complete series in *S. parvus*). The leaves are also ovoid in outline in *S. stylosus* (subglobose in *S. parvus*) with a very prominent mucro (mucro obscure in *S. parvus*). *Schlechteranthus stylosus* is also typically a larger shrub (up to 400 mm in height) compared to *S. parvus* (up to 150 mm in height).

Schlechteranthus stylosus may be confused with *S. pungens*, as both species are large shrubs with spines derived from aborted axillary buds (Fig. 4), but *S. stylosus* is distinguished by the ovoid leaves in outline (mostly oblong in *S. pungens*) and spines arranged in a simple cyme on the secondary axes (Fig. 4B) (spines arranged in compound cyme on secondary axes in *S. pungens* (Fig. 4C)).

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Distribution and ecology—*Schlechteranthus stylosus* is distributed widely in northern Namaqualand, occurring from the Richtersveld to just south of Springbok (Fig. 7H). The species is found solely on rocky quartzite soils, generally on flats, but sometimes found on the lower slopes of hills. This species flowers from August to September.

Additional specimens examined—South Africa. NORTHERN CAPE: 2816 (Oranjemund): Arris drift (–DA), *Hall sub NBG 116/53* (BOL); Sendelings Drift (–DB), *Hall s.n.* (BOL). 2817 (Vioolsdrif): On pass up the Vandersterberg towards Koeskopfontein (–AC), *Powell 75* (NBG); Grasdrif, Richtersveld (–AD), *Williamson 3267* (BOL); Richtersveld between Helskloof se Hek and Eksteenfontein (–CA), *Williamson 3304* (BOL); Gelykberg,

Richtersveld (–CA), *Hall s.n.* (BOL); Lekkersing, 5 km north of Uitspanpoort (–CC), *Bruyns 9123* (BOL); Cape Prov. Nama'land, Richtersveld, Rooiberg middlemost (–CD), *NBG 423/61* (BOL); Rooiberg, Richtersveld (–CD), *NBG 432/62* (BOL); ± 15 km north of Kosies (–DC), *Bruyns 9252*. 2917 (Springbok): About 12 km W of Bulletrap (–BC), *Klak 1255* (BOL); Springbok area on road to Komaggas; ± 5 km from Namaqua National Park gate (–CD), *Burgoyne 11799* (PRE).

9. **Schlechteranthus parvus** R. F. Powell & Klak, sp. nov. TYPE: SOUTH AFRICA. Northern Cape Province: Komaggas, alongside road between Koingnaas and Komaggas [3017AA], 15 May 2014, *Powell, Klak and Magee 41* (holotype: NBG!; isotype: BOL!).

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Small erect to spreading dome-shaped shrublet to 150 mm tall. *Branches* to 4 mm thick; upper branches brown; leaves crowded towards tips of branches, old not remaining on the branches. *Leaves* triquetrous, globose to subglobose in outline, 2.0–4.0 x 1.5–2.0 mm, leaf pairs fused for ¾ of the length; mucro obscure; slightly velvety; yellow-green. *Flowers* in compound cymose inflorescences; central primary axis to 4 mm long, equal proportionally to capsule length, bracteoles absent; spines present, arranged in compound cyme, secondary axes with spines arranged in simple cyme; spines derived from aborted axillary buds and less frequently from old peduncles; spines branching at ± 45°, sharp, elevated above terminal leaf pair; filamentous staminodes many, forming a complete series around the stamens. *Fruit* 7 to 8-locular; top convex, base funnel-shaped, ± 3.9 x 4.7 mm; closing rodlet conspicuous; valve wings absent. Figure 5.

***Etymology*—**The species epithet refers to the small nature of the shrub and its leaves.

765 ***Diagnostic characters—****Schlechteranthus parvus* is the smallest among the spiny
766 species in subgenus *Microphyllus* and only reaches 150 mm in height. It is most similar to *S.*
767 *stylosus* as they share small leaves with a high degree of fusion (Fig. 2H, I) and the same
768 spine arrangement, with spines derived from aborted axillary buds (Fig. 4B). However, *S.*
769 *stylosus* is a much larger shrub and reaches 400 mm in height.

770 The new species is distinguished by its many filamentous staminodes, which form a
771 complete series around the stamens (Fig. 5A) (filamentous staminodes in *S. stylosus* are few
772 or absent, forming an incomplete series around the stamens). The leaf shape can also be used
773 to distinguish the species, with *S. parvus* possessing leaves subglobose in outline without a
774 prominent mucro (leaves ovoid with a very prominent mucro in *S. stylosus*). *Schlechteranthus*
775 *parvus* shares a globose leaf shape with *S. subglobsus* (Fig. 2D, I) but can be distinguished by
776 the highly fused leaves, the obscure mucro (leaf pairs fused for $^{1}/_{3}$ of length and mucro very
777 prominent in *S. subglobosus*), and also by the flowers arranged in a compound cymose
778 inflorescence (flowers solitary in *S. subglobosus*).

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779 ***Distribution and ecology—****Schlechteranthus parvus* occurs along the western coast of
780 Namaqualand (Fig. 7I), associated with quartz patches. Although the species has not been
781 collected in flower, the species flowered in September in the greenhouse at Kirstenbosch.

782 ***Additional specimens examined—***South Africa. NORTHERN CAPE: 2917
783 (Springbok): 19 km south of Port Nolloth on road to Grootmis, Kwaggakop River, east of
784 road on low hillocks (–AC), *Klak 2387* (BOL); west of Wolfberg mine, along road from
785 Kleinsee to Springbok (–CB), *Klak 2400* (BOL). **3017 (Hondeklipbaai):** Komaggas,
786 alongside road between Koingnaas and Komaggas (–AA), *Klak 2362* (BOL).

787

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798

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858 TABLE 1. Character matrix used for the morphological cladistic analyses for

859 *Schlechteranthus*. Description of characters and character states are included in Appendix 1.

Species	1	2	3	4	5	6	7	8
S. abruptus	1	1	0	0	0	0	0	0
S. connatus	1	1	0	0	0	0	1	0
S. hallii	0	0	0	0	0	0	0	0
S. inclusus	1	1	0	0	0	0	0	0
S. parvus	1	1	1	1	1	1	0	0
S. pungens	1	1	1	1	1	1	0	1
S. spinescens	1	1	1	1	1	0	0	0
S. steenbokensis	0	0	0	0	0	0	0	0
S. stylosus	1	1	1	1	1	1	0	0
S. subglobosus	1	1	0	0	0	0	1	0
S. tetrasepalus	1	1	0	0	0	0	1	0

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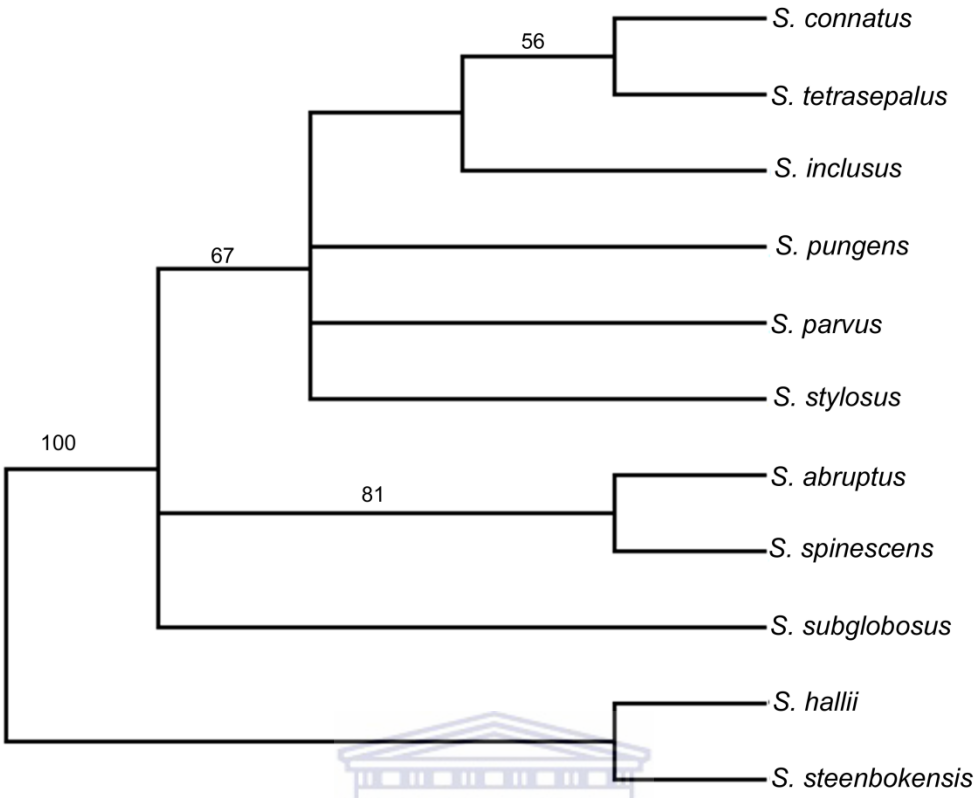

862

863 APPENDIX 1. Maximum parsimony estimate based on nine plastid gene regions showing
864 the phylogenetic relationships of subgenus *Microphyllus.* Bootstrap support values greater than
865 50 are indicated above the branches.

866

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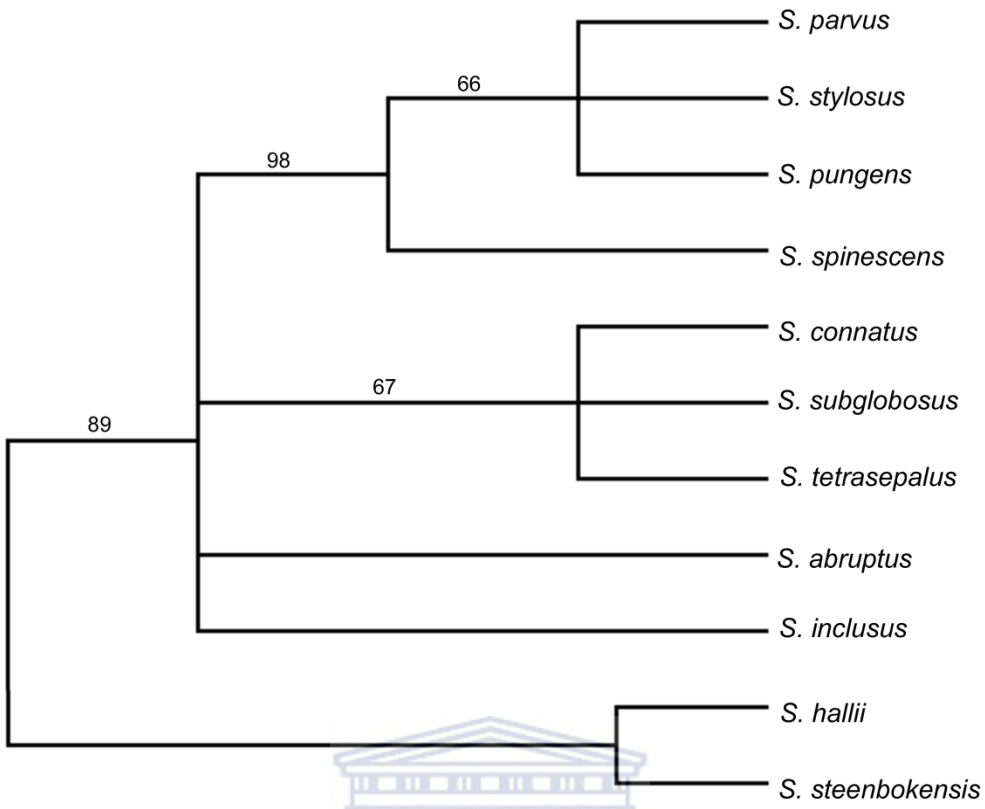

APPENDIX 2. Maximum parsimony estimate based on eight coded morphological characters for species subgenus *Microphyllus.* Bootstrap support values greater than 50 are indicated above the branches.

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APPENDIX 3. Morphological characters and character states coded in the morphological cladistics analysis for species of subgenus *Microphyllus;* **1**-locule number: 10–12 locules=0; 7–9 locules=1. **2**-closing bodies: large, blocking ¾ of locule=0; small, blocking $^{1}/_{3}$ of locule=1. **3**-inflorescence: solitary=0; cymose=1. **4**-bracteole: present=0; absent=1. **5**-peduncles becoming spinescent: absent=0; present=1. **6**-spines derived from aborted buds: absent=0; present=1. **7**- primary axis conspicuously lengthened, at least 1½ longer than the length of the capsule: absent=0; present=1. **8**-spines arranged in compound cyme on secondary axis: absent=0; present=1.

882 FIGURE LEGENDS

883 FIG. 1. (A) Internal structure of capsules in subgenus *Microphyllus,* with arrows indicating
884 closing bodies (CB) and covering membranes (CM). (B) Spinescent inflorescence of *S.*
885 *pungens* held above terminal leaf pair. (C) Capsules of *S. connatus* on long central axis,
886 arrow indicating the position of the bracteole. (D) Magenta flower of *S. stylosus* with the
887 stamens arranged in a cone in the centre of the flower, as is diagnostic of the genus
888 *Schlechteranthus*. (E) Evenly spaced leaves along the stem in *S. subglobosus*. (F) Cup-shaped
889 bracteole in *S. inclusus,* placed at the base of the capsule. (G) Old leaves that remain on stem
890 in *S. inclusus.* (H) Compact habit of *S. inlusus* found growing on a gneiss rocky outcrop
891 along a river course. Photographs: R.F.Powell.
892
893
894 FIG. 2. Leaves of subgenus *Microphyllus,* illustrating the variation in leaf size and degree of
895 fusion of the leaf pairs. (A) *Schlechteranthus connatus, Powell 10* (NBG). (B) *S. spinescens,*
896 *Klak 2424* (BOL). (C) *S. abruptus, Powell 5* (NBG). (D) *S. subglobosus, Powell 33* (NBG).
897 (E) *S. pungens, Powell 28* (NBG). (F) *S. inclusus, Powell 35* (NBG). (G) *S. tetrasepalus,*
898 *Klak 2411* (BOL). (H) *S. stylosus, Powell 75* (NBG). (I) *S. parvus, Powell 41*(NBG). Scale =
899 1 cm.
900
901
902 FIG. 3. (A) Consensus tree from the Bayesian inference analysis from molecular plastid data
903 (following Powell *et al.,* 2016), tree length=156, Consistency index (CI)=0.94, Retention
904 index(RI)=0.76. (B) Consensus tree from the Bayesian inference analysis from coded
905 morphological characters (Appendix 1) for species of subgenus *Microphyllus* and two species
906 of subgenus *Schlechteranthus* (outgroup taxa); tree length = 8, Consistency index (CI)=1.00,

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Retention index (RI)=1.00. Posterior probabilities (PP) and bootstrap (BS) values are indicated above the branches. ■ = synapomorphy without reversal, ● = autapomorphy, || = convergence, X = loss. Character identification numbers are indicated below the symbols.

FIG. 4. Inflorescence structure and spine arrangement in the spiny species, *Schlechteranthus parvus, S. pungens, S. spinescens* and *S. stylosus*. (A) Inflorescence arranged in a simple cyme on the primary axis (pa), with blunt spines (bs) derived from old peduncles (p) where capsules (c) have fallen off, as found in *S. spinescens*. (B) Inflorescence arranged in compound cyme on primary axis and in simple cyme on secondary axes (indicated by dashed lines) with sharp spines (ss) derived from aborted buds (ab) on the two outer axes (as in *S. parvus* and *S. stylosus*), blunt spines also sometimes present where capsules have fallen off. (C) Inflorescence arranged in compound cyme on primary axis, compound cyme on the secondary axes and in a simple cyme on the tertiary axes (ta) (indicated by dotted lines) as found in *S. pungens*, with sharp spines (ss) derived from aborted buds (ab) and blunt spines also sometimes present where capsules have fallen off.

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FIG. 5. A new species of *Schlechteranthus, S. parvus*. (A) Flower of *S. parvus* with many filamentous staminodes (FS), forming a complete series around the stamens. (B) Old flower of *S. parvus*, with spines developing from aborted apical buds. (C) Spine arrangement in compound cymose in *S. parvus.* (D) Branch of *S. parvus* cut at base to illustrate small size and spines. (E) *Schlechteranthus parvus* in habitat, illustrating the small size of the species and display of dense spines.

FIG. 6. Capsule shape and structure in subgenus *Microphyllus*. (A) Capsule with flattened apex and funnel-shaped base, as found in *S. pungens*. (B) *Schlechteranthus connatus* with convex top and subglobose base. (C) Internal capsule structure found in subgenus *Microphyllus* (*S. connatus*), arrows indicating the closing bodies (cb) and covering membranes (cm). Scale = 1 cm.

FIG. 7. Known geographical distributions of the species in subgenus *Microphyllus*. (A) *Schlechteranthus inclusus*. (B) *S. abruptus*. (C) *S. tetrasepalus*. (D) *S. subglobosus*. (E) *S. connatus.* (F) *S. spinescens*. (G) *S. pungens*. (H) *S. stylosus*. (I) *S. parvus.*

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FIG. 8. Distribution and number of species per Quarter Degree Square (QDS) (Edwards and Leistner 1971) in the Greater Cape Floristic Region, South Africa for (A) the genus *Schlechteranthus*; (B) subgenus *Schlechteranthus;* (C) subgenus *Microphyllus*

FIG. 9. Drawing of type material for *S. inclusus* (*Pillans sub BOL 17758* (BOL)). (A) Habit and leaf arrangement of *S. inclusus.* (B) Terminal opposite leaf pair with flower in bud. (C) Flower with stamens and filamentous staminodes collected in a cone in the centre of flower, as is diagnostic for the genus. (D) Flower with arrangement of stamens loosely arranged in a cone, typically found near the end of the flowering season. (E) Flower with all staminodes removed showing the nectaries arranged in a ring. (F) Open capsule with small closing bodies and complete covering membranes. (G) Longitudinal section of flower showing the

arrangement of the style, stamens and petaloid staminodes. (H) Floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A–D = 10 mm; E, G = 3.3 mm; F, H = 2.5 mm. Artist B. Carter.

FIG. 10. Drawing of type material for *S. abruptus* (*Bolus sub NBG 1504/33* (BOL)). (A) Closed capsule of *S. abruptus.* (B) Open flower with stamens and filamentous staminodes collected in a cone. (C) Flowering branch of *S. abruptus*. (D) Habit and leaf arrangement of *S. abruptus*. (E) Longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes. (F) Enlarged top part of leaf illustrating the velvety leaf surface. (G) Floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. (H) Open capsule with small closing bodies and complete covering membranes. (I) Flower with all staminodes removed showing the nectaries arranged in a ring. Scale: A–D = 10 mm; E–I = 2.5 mm. Artist B. Carter.

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FIG. 11. Drawing of type material for *S. subglobosus* (*Pillans 5844* (BOL)). (A) Habit and leaf arrangement of *S. subglobusus*. (B) Flower with all staminodes removed showing the nectaries arranged in a ring. (C) Longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes. (D) Enlarged leaf of *S. subglobosus* illustrating the dome-shaped epidermal cells. (E) Floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A = 10 mm; B–C = 2 mm; D–E = 2.5 mm. Artist B. Carter.

FIG. 12. Drawing of type material for *S. connatus* (*Pillans 5794* (BOL)). (A) Habit and leaf arrangement of *S. subglobusus*. (B) Floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. (C) Longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes. (D) Flower with all staminodes removed showing the nectaries arranged in a ring. (E) Open capsule with small closing bodies and complete covering membranes. Scale: A = 10 mm; B–D = 3.3 mm; E = 2.5 mm. Artist B. Carter.

FIG. 13. Drawing of type material for *S. spinescens* (*Compton sub BOL 19081* (BOL)). (A) Habit and leaf arrangement of *S. spinescens*. (B) Enlarged flower in bud. (C) Enlarged leaf, showing the fused opposite leaf pairs. (D) Open capsule with small closing bodies and complete covering membranes. (E) Flower with all staminodes removed showing the nectaries arranged in a ring. (F) Longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes. (G) Floral components illustrating the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A = 10 mm; B–C = 5 mm; D = 2.5 mm; E = 3.3 mm; F–G = 2 mm. Artist B. Carter.

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FIG. 14. Drawing of type material for *S. stylosus* (*Pillans 5789* (BOL)). (A) Flower with all staminodes removed showing the nectaries arranged in a ring. (B) Habit and leaf arrangement of *S. stylosus*. (C) Enlarged leaf and components of calyx. (D) Spine development from aborted axillary buds and cymose inflorescens. (E) New growth with early stages of development of spines and capsules. (F) Longitudinal section of flower showing the arrangement of the style, stamens and petaloid staminodes. (G) Floral components illustrating

1007 the style, stamens, filamentous staminodes and petaloid staminodes. Scale: A = 2.5 mm; B=

1008 10 mm; C = 5 mm; D–E = 10 mm; F–G = 3.3 mm. Artist M. Page.

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Figure2

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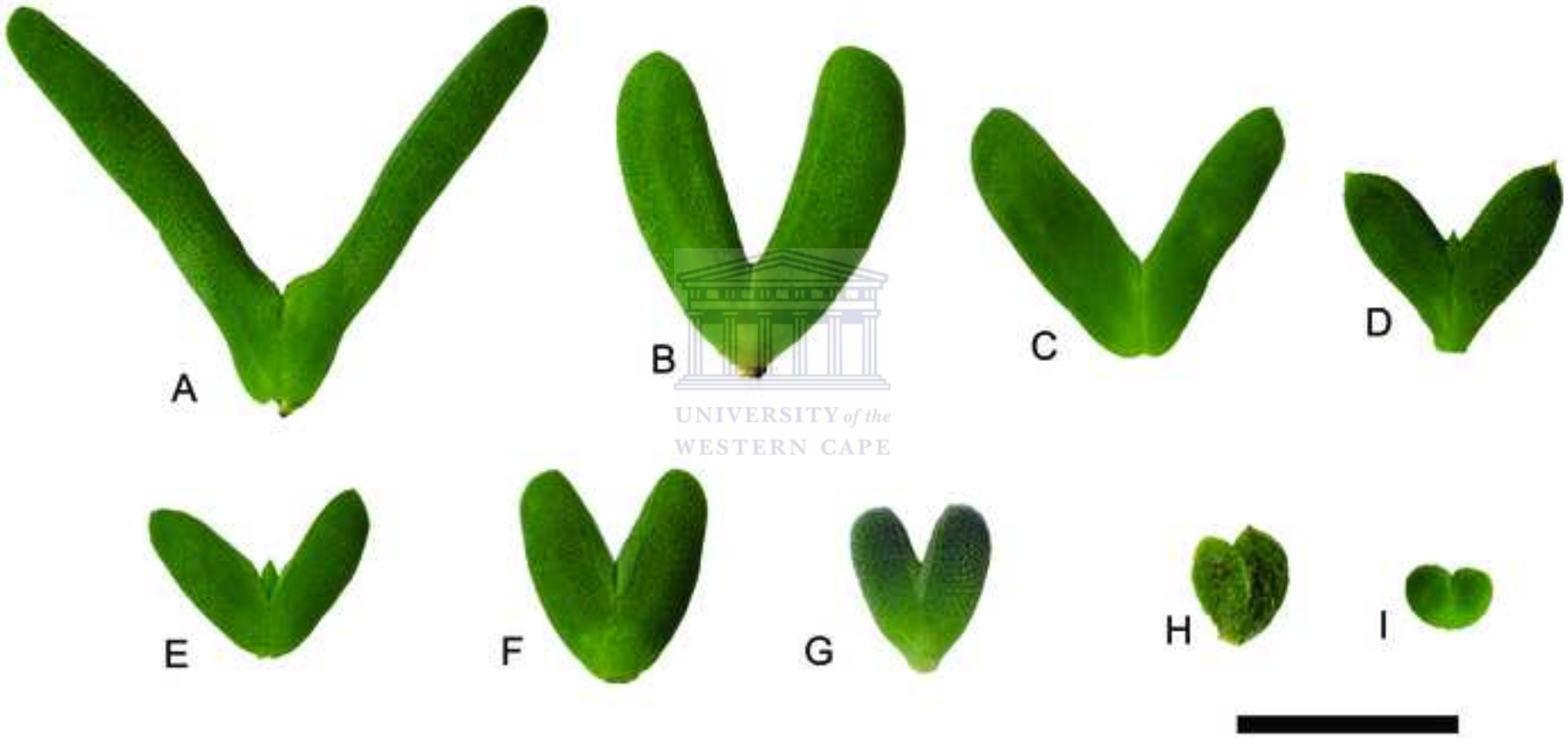

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Figure3

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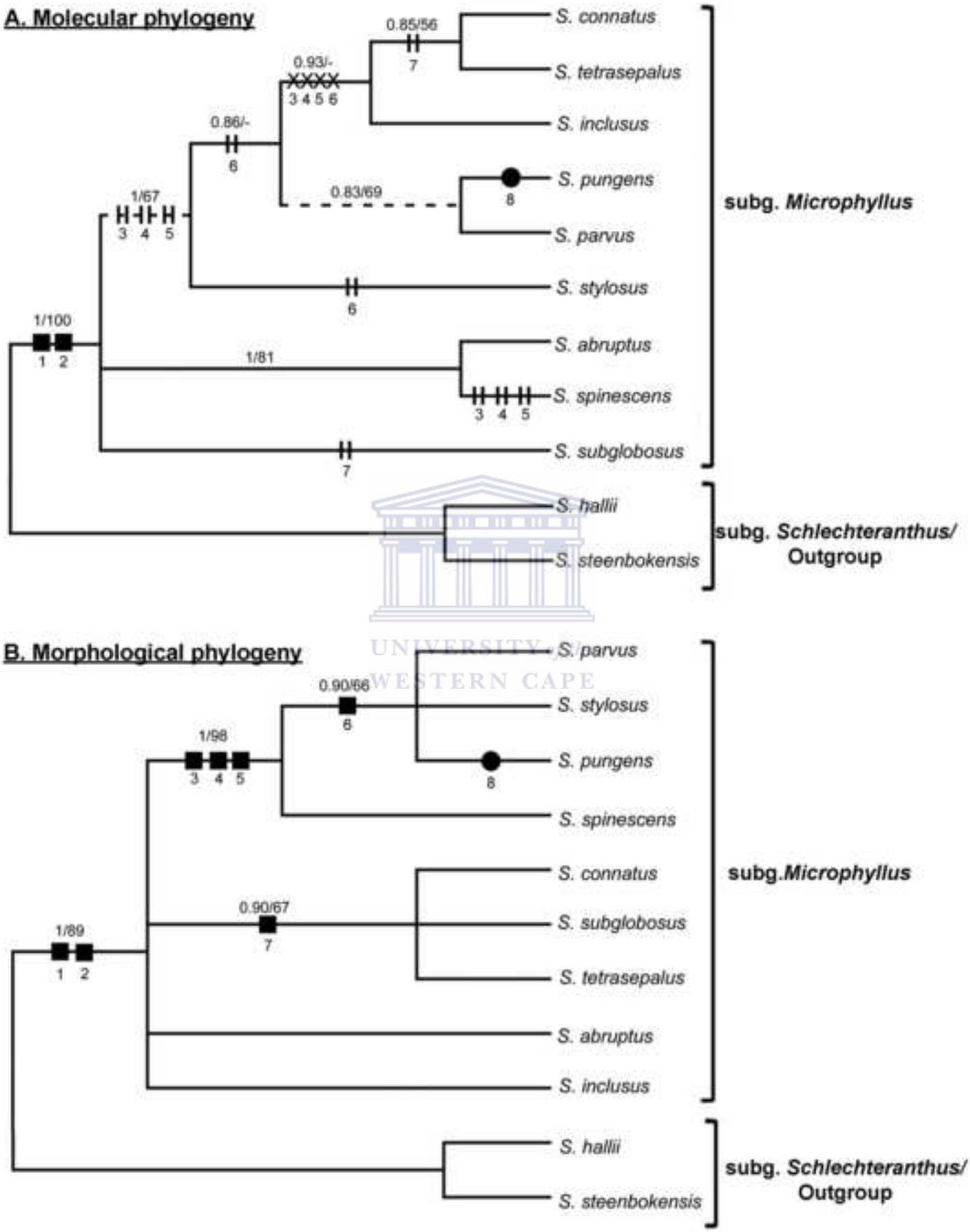

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Figure4

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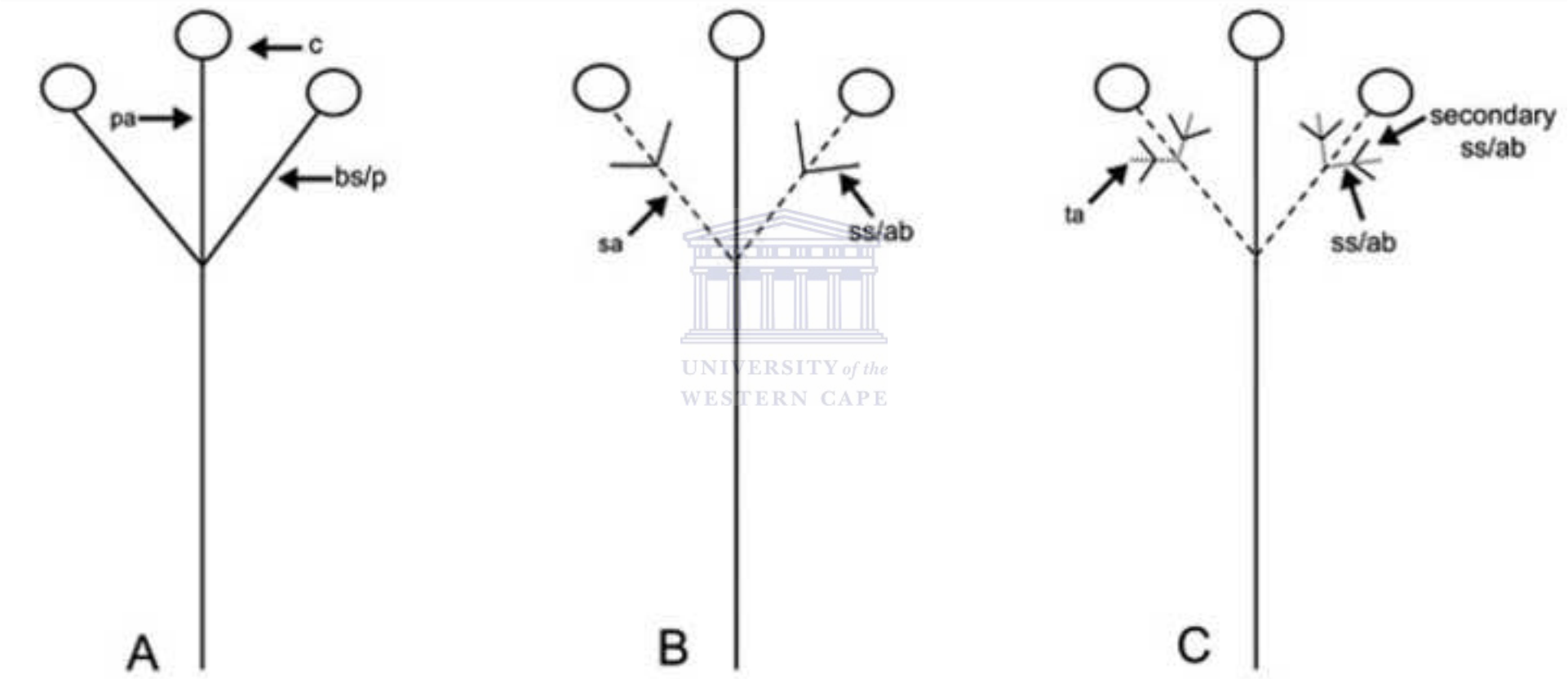

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Figure5

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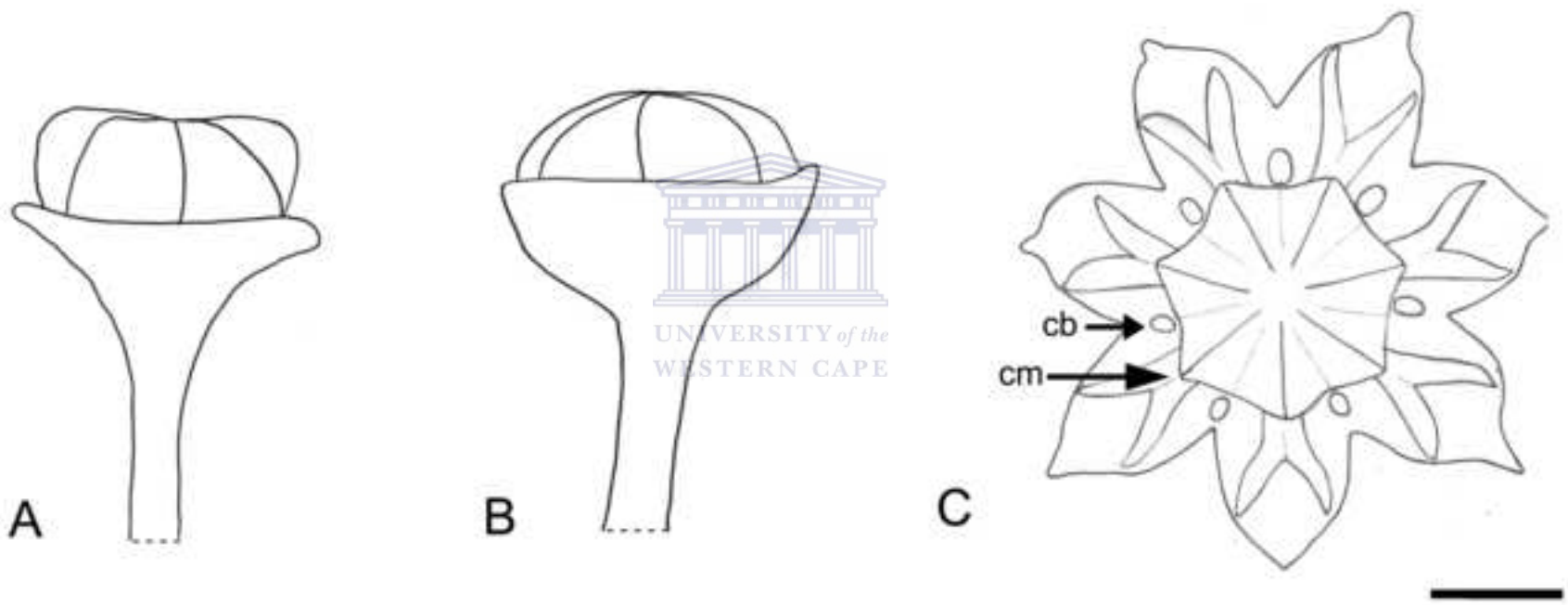

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Figure7

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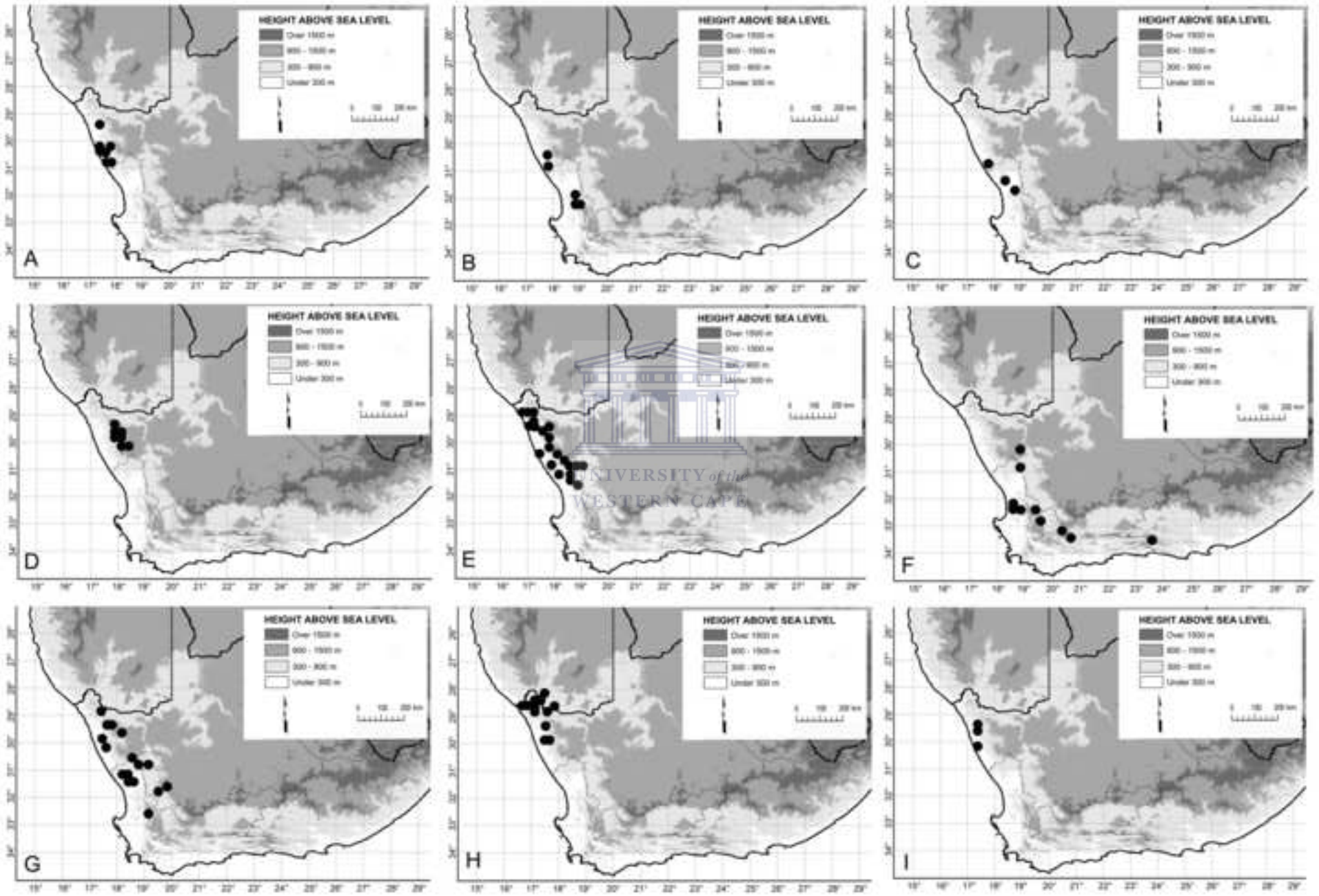

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Figure8

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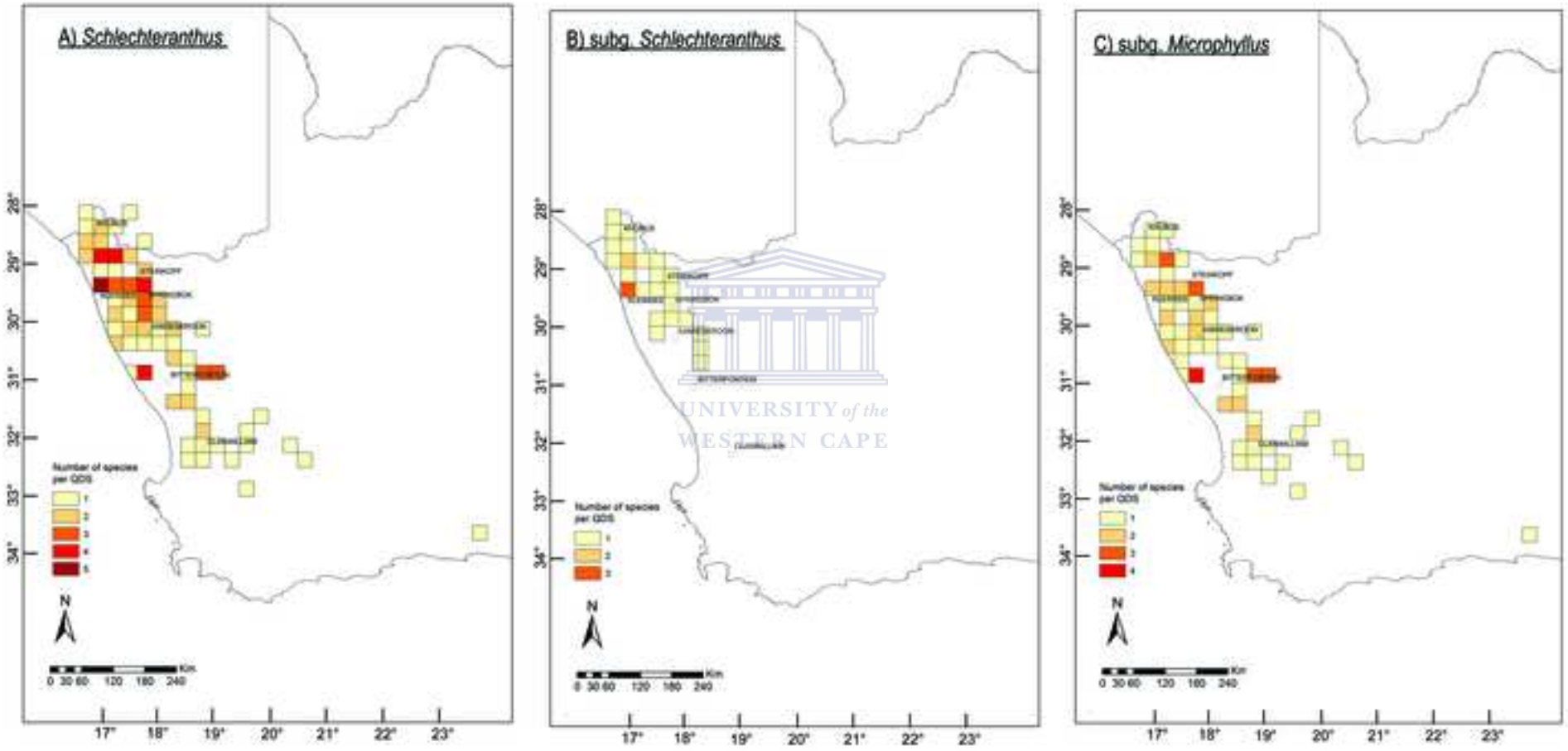

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Figure9

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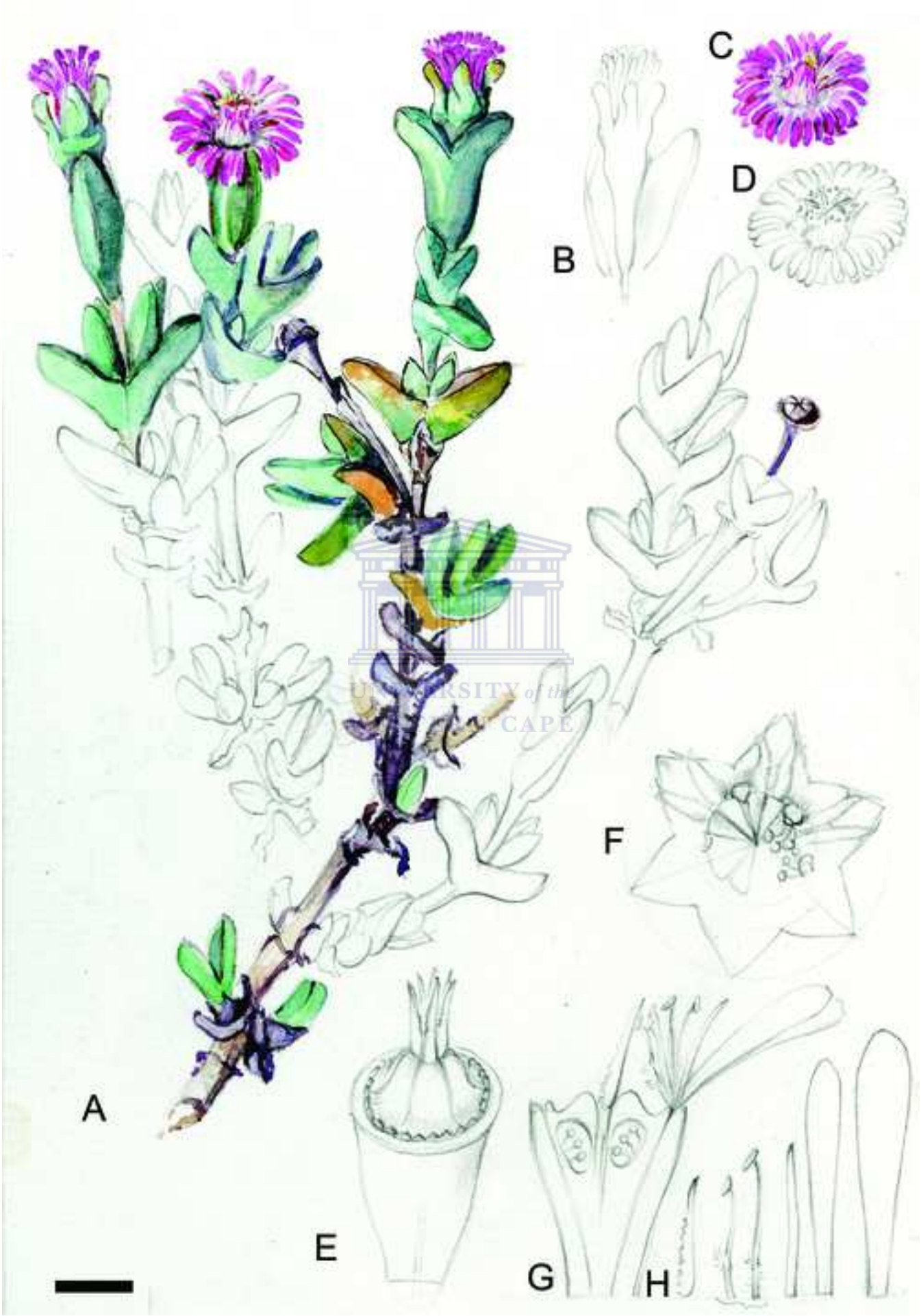

Figure10

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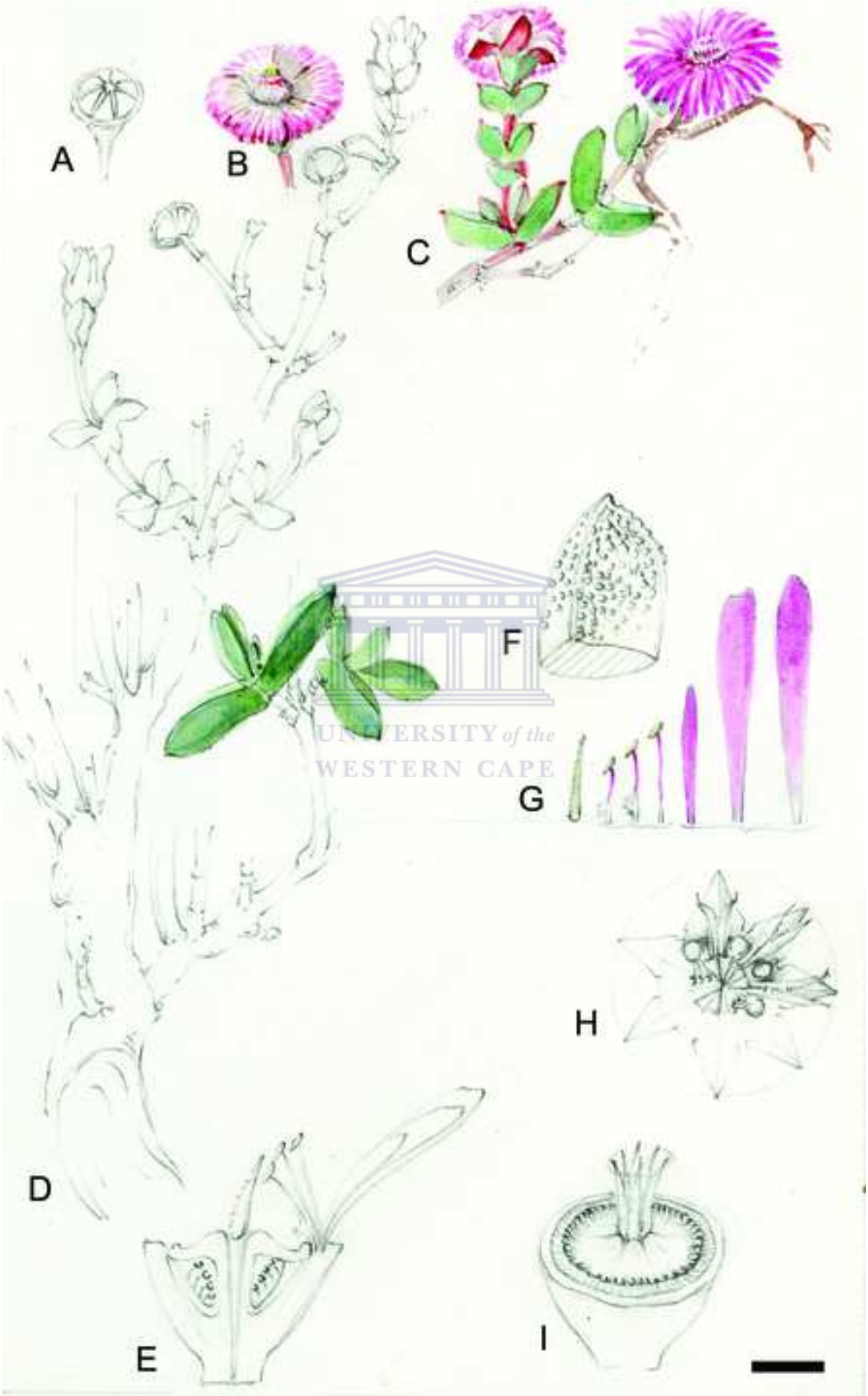

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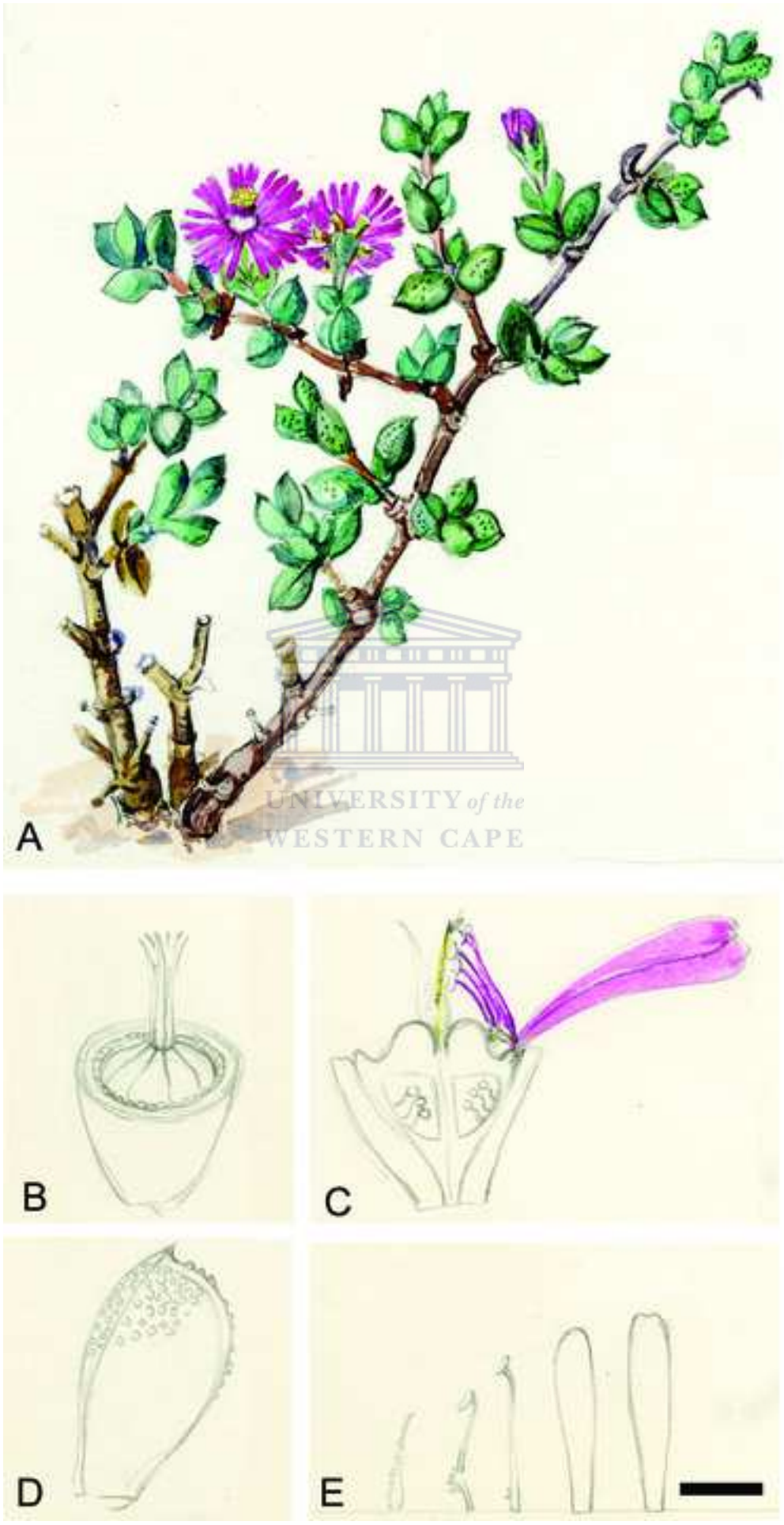

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Figure12

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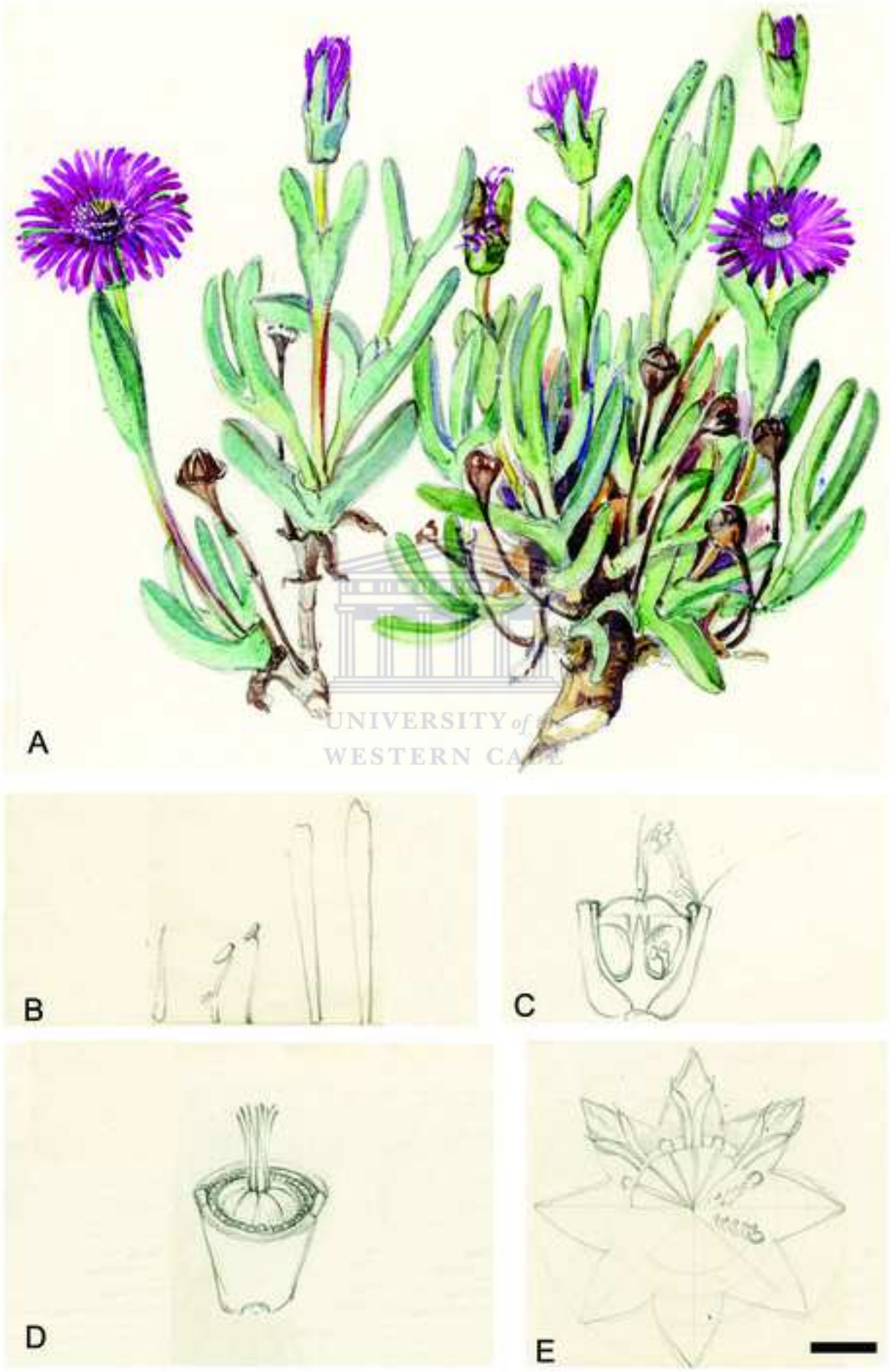

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Figure13

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Figure14

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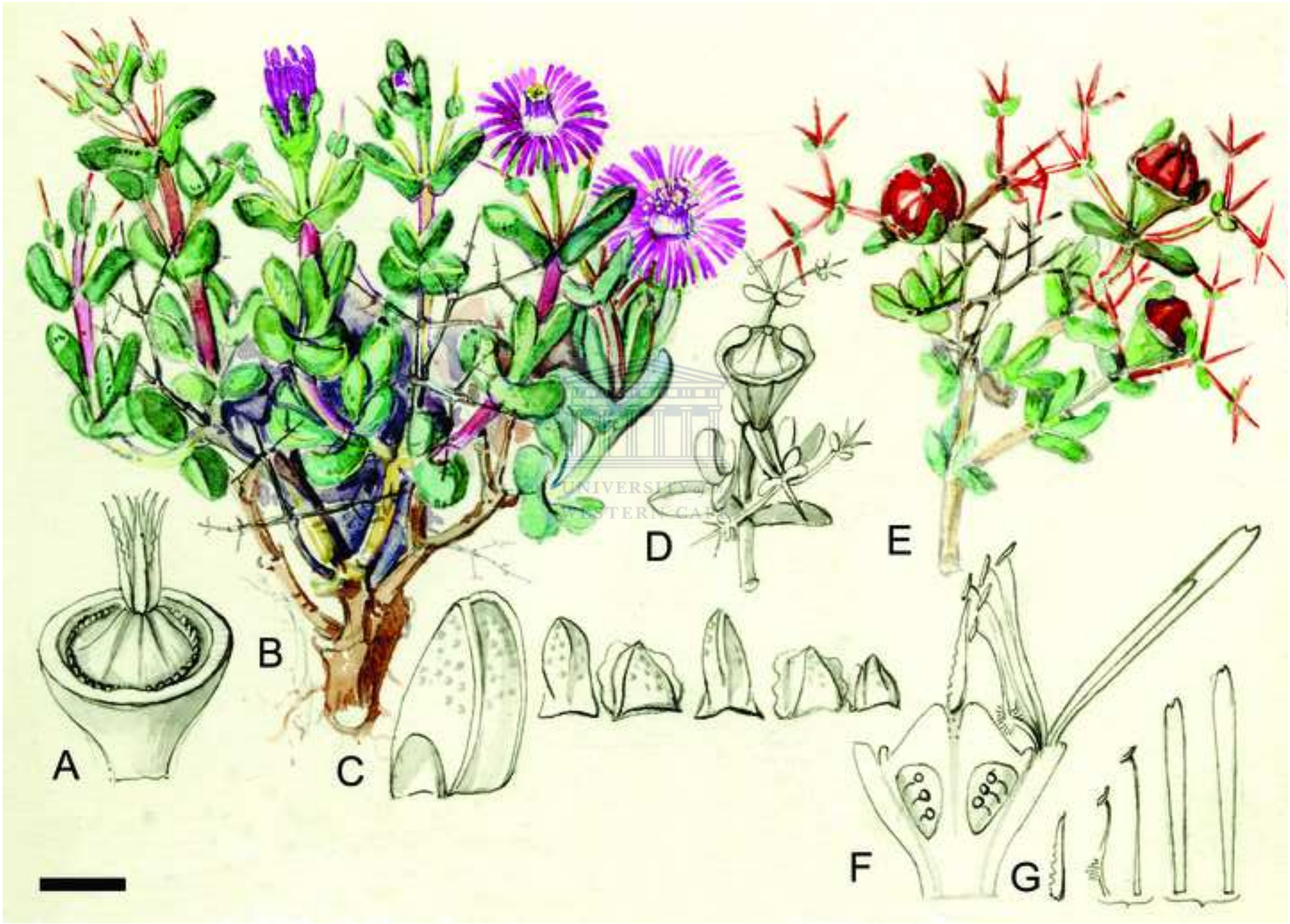
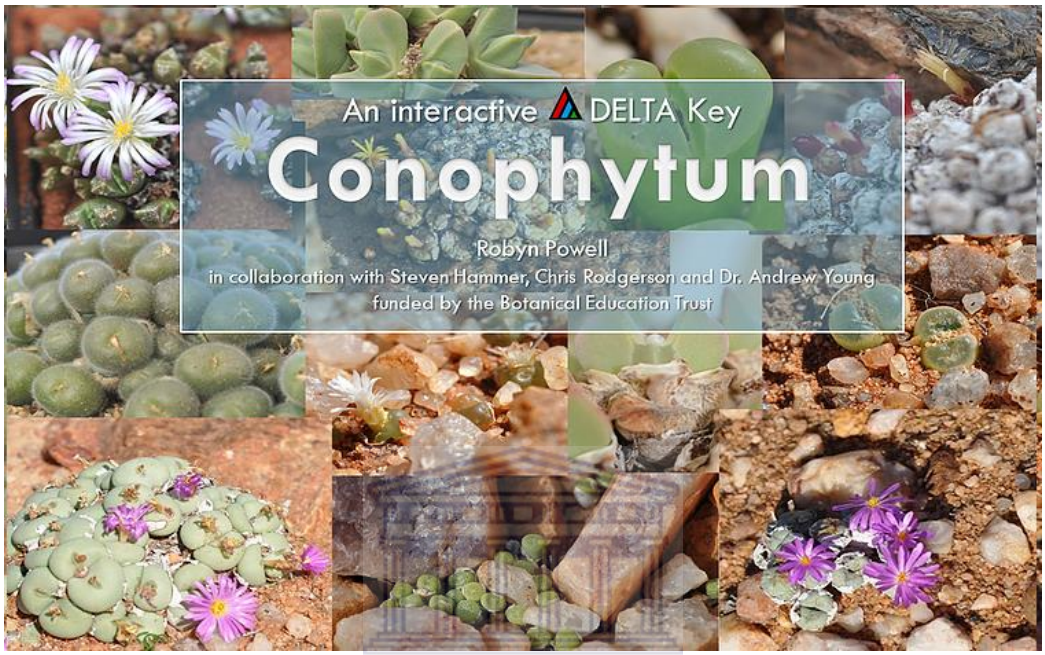


APPENDIX E4. **Powell RF, Hammer S, Rodgerson C, Young A. 2015.** *Conophytum*: an interactive DELTA Key.

The digital DELTA key, the DELTA software and instructions are available online at: http://rpowell2.wixsite.com/conophytum-delta-key



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