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*

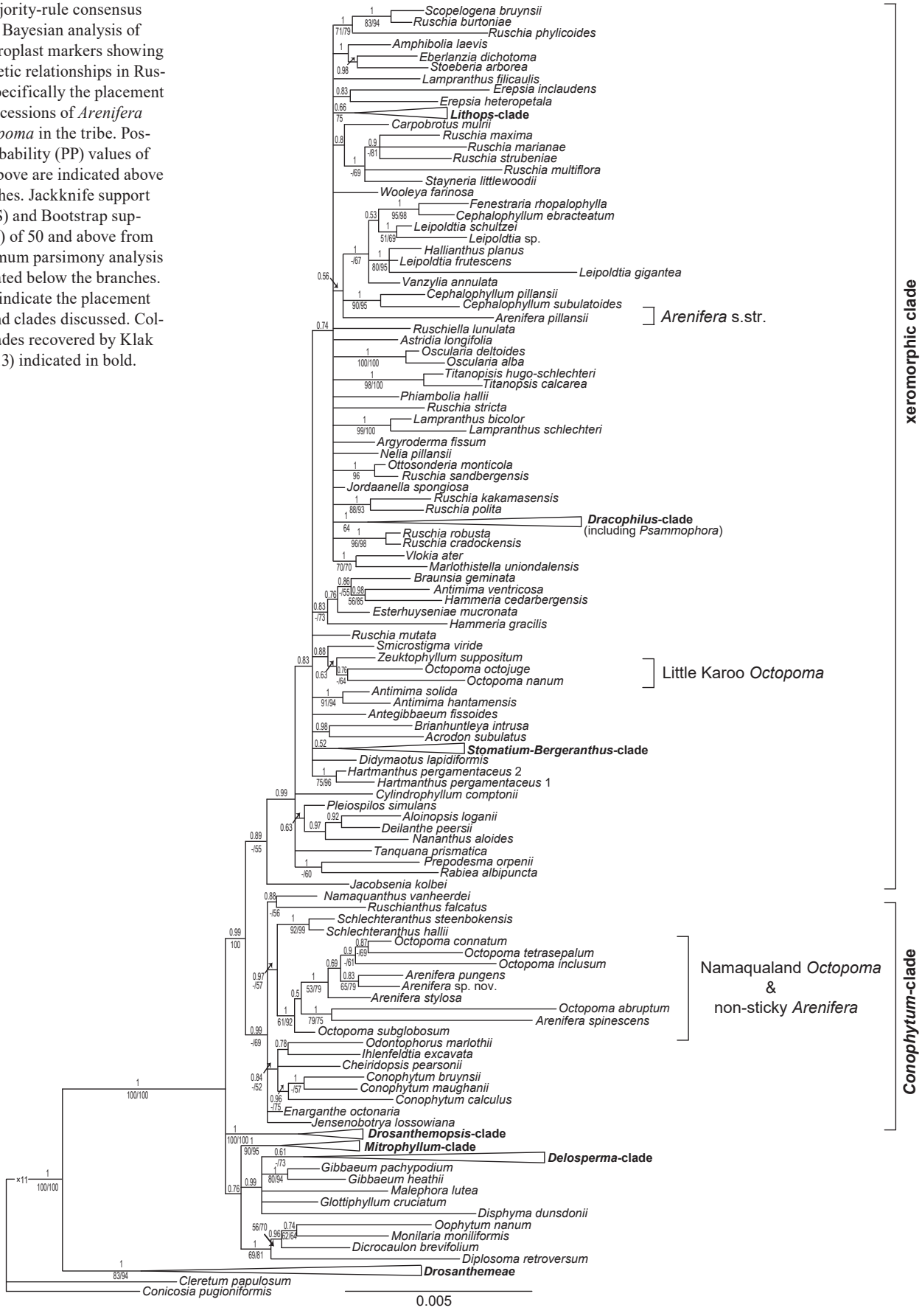

Fig. 1. Majority-rule consensus tree from Bayesian analysis of nine chloroplast markers showing phylogenetic relationships in Ruschieae, specifically the placement of new accessions of *Arenifera* and *Octopoma* in the tribe. Posterior probability (PP) values of 0.5 and above are indicated above the 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	–	–	+	+	+

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*)

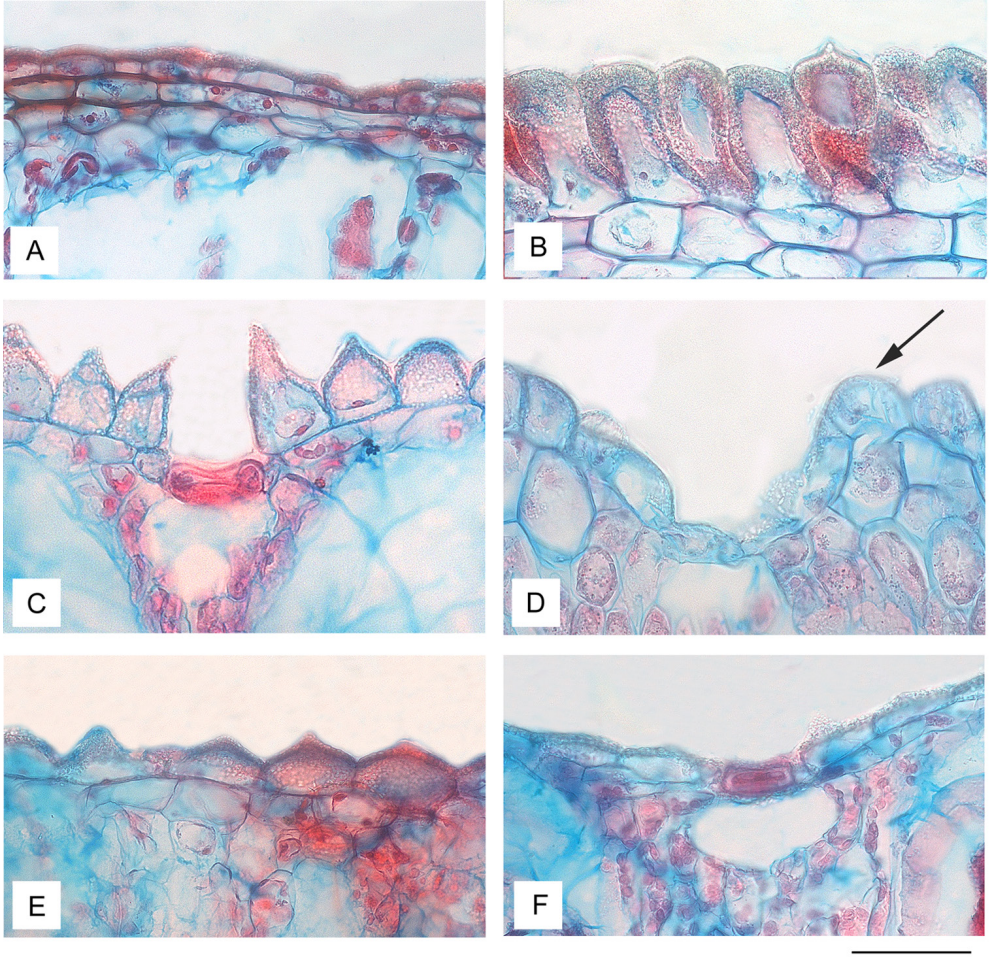

Fig. 2. Transverse sections through the leaves of *Arenifera*, *Octopoma* and *Schlechteranthus* species showing characters of taxonomic importance. **A,** Hypodermis found in southern *Octopoma* species, *O. nanum*; **B,** Prominently thickened and anticlinally elongated oblong epidermal cells in *Schlechteranthus* 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.