

New tribal delimitations for the early diverging lineages of Apiaceae subfamily Apioideae

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Abstract Phylogenetic analyses of the cpDNA *trnQ-trnK* 5' exon region for 27 genera and 42 species of Saniculoideae and early diverging lineages of Apioideae were carried out to assess or confirm the tribal placements of the following anomalous genera: *Annesorhiza*, *Astydamia*, *Chamarea*, *Choritaenia*, *Ezoscadium*, *Itasina*, *Lichtensteinia*, *Marlothiella*, *Molopospermum* and *Phlyctidocarpa*. To accommodate these unique early diverging members of the Apiaceae and to reflect their relationships, a new tribal classification system has become necessary. Many of the early diverging genera (herein referred to as the protoapioids) can readily be distinguished from the euapioids (the remaining apioids) by the presence of scattered druse crystals in the mesocarp. The major morphological discontinuity within the family, however, lies between the combined protoapioids and euapioids (representing an expanded Apioideae s.l., including the Saniculoideae) and the subfamilies Azorelloideae and Mackinlayoideae. The broadened subfamily Apioideae is diagnostically different from the other subfamilies in the absence of rhomboidal crystals, the presence of druse crystals scattered throughout the mesocarp (subsequently lost in the euapioids), and the non-woody endocarp. No such diagnostic characters are available to support the traditional or recently expanded concept of Saniculoideae. The broadened concept of Apioideae is also supported by the sporadic presence of true wings. This character can be variously interpreted from a phylogenetic point of view, but nevertheless has considerable diagnostic value. A new tribal classification system for the protoapioids is proposed on the basis of molecular, morphological and anatomical evidence. This new system is intended to be practical and non-hierarchical to allow for future realignments amongst the tribes, as more evidence becomes available. It makes provision for hitherto poorly known African taxa and comprises the following eight tribes, five of which are newly described: Annesorhizeae, Choritaenieae, Heteromorpheae, Lichtensteinieae, Marlothielleae, Phlyctidocarpeae, Saniculeae and Steganotaenieae.

Keywords Annesorhizeae; Choritaenieae; euapioids; Lichtensteinieae; Marlothielleae; Phlyctidocarpeae; protoapioids; *trnQ-trnK* 5' exon

■ INTRODUCTION

The Apiaceae are a large and taxonomically complex family comprising 463 genera and ca. 3500 species (Plunkett & al., in press), with a nearly cosmopolitan distribution. The classification system of Drude (1897–98) made little provision for the comparatively small sub-Saharan African and Madagascar contingent (80 genera and 354 species), many of which were unknown or poorly studied at the time. As suggested by Burt (1991) and later by molecular systematic studies (Downie & al., 1996, 1998, 2000; Plunkett & al., 1996a,b; Downie & Katz-Downie, 1999; Calviño & al., 2006; Calviño & Downie, 2007; Magee & al., 2008a,b) the African genera are crucial to an understanding of the evolutionary history of the family and its full range of morphological diversity. Many African taxa have indeed been found to occupy early diverging positions within Apiaceae, suggesting a southern African origin for both Apioideae (Calviño & al., 2006) and Saniculoideae (Calviño &

Downie, 2007). In recent years, tribal delimitations within the largest and most complicated subfamily, the Apioideae, have been the focus of intensive phylogenetic research (see Downie & al., 2001, in press). The most recent treatment of the “core” apioid lineages by Downie & al. (in press), based largely on nrDNA ITS sequence data, recognized 14 tribes and 12 major clades. In this treatment the earliest diverging lineages of the subfamily were excluded due to their highly divergent ITS sequences and consequent alignment difficulties. Although there has been much progress on the generic placement of several anomalous taxa within these early diverging lineages (Calviño & al., 2006; Calviño & Downie, 2007; Magee & al., 2008a) the correct placement of several critical African taxa has remained uncertain. This paper is aimed at resolving the early diverging lineages of the Apioideae, assessing their relationship to their sister subfamily Saniculoideae, and proposing a new tribal classification for these lineages using anatomical, morphological and DNA sequence data.

■ MATERIAL AND METHODS

Morphological data. — Populations of several taxa were studied and sampled *in situ* in South Africa and Namibia over a period of several years. This material was supplemented by a study of all relevant taxa from the following herbaria: BM, BOL, JRAU, K, LE, MO, NBG (including SAM and STE), P, PRE, S, THUNB-UPS and WIND.

Fruit from herbarium specimens and formaldehyde-acetic acid-alcohol (FAA) preserved material were used in the anatomical study. Herbarium material was first rehydrated and then placed in FAA for a minimum of 24 h. This material was subsequently treated according to a modification of the method of Feder & O'Brien (1968) for embedding in glycol methacrylate (GMA). Transverse sections, about 3 μ m thick, were made using a Porter-Blum ultramicrotome. The sections were examined for the presence of crystals using a light microscope, prior to staining using the periodic acid Schiff/toluidine blue (PAS/TB) method of Feder & O'Brien (1968). To study the three-dimensional structure of the vittae, mature fruit were softened by soaking in boiling water for 24 h. The exocarp was then peeled off while keeping the fruit submerged to prevent desiccation. All illustrations were made by the first author with the aid of a camera lucida attachment on a Zeiss compound microscope or a Wild M3Z stereomicroscope.

Taxon sampling. — The cpDNA *trnQ-trnK* region for 46 accessions, representing 27 genera and 42 species of Saniculoideae and early diverging lineages of Apioideae and two accessions of the South African azorelloid genus *Hermas* L. were examined. Sources of material, together with their corresponding taxonomic authorities and GenBank accession numbers, are listed in Appendix 1. Data from the *trnQ-trnK* region for 27 of these 46 accessions were available from previous studies (Calviño & Downie, 2007; Calviño & al., 2008a,b, in prep.; Downie & al., 2008; Tilney & al., 2009). Sequence data from the *rps16* intron region were available for a further ten accessions (Calviño & al., 2006; Downie & Katz-Downie, 1999; Magee & al., 2008a). *Rps16* intron data for the remaining nine accessions and data from the *trnQ-rps16* and *rps16-trnK* intergenic spacers for 15 and 17 accessions, respectively, were obtained specifically for this study. The monotypic southern African genera *Choritaenia* Benth., *Marlothiella* H. Wolff and *Phlyctidocarpa* Cannon & Theobald were included as previous authors had suggested their placements within the early diverging lineages of the Apioideae-Saniculoideae clade (Calviño & al., 2006; Calviño & Downie, 2007; Liu & al., 2003, 2007a, 2007b; Nicolas & Plunkett, 2009; Tilney & al., 2009). The genus *Hermas* (Azorelloideae) was used to root the trees, based on results from previous molecular phylogenetic analyses (Calviño & al., 2006).

Molecular data. — Total DNA was extracted from herbarium or silica material using either the 2 \times CTAB method of Doyle & Doyle (1987) or the DNeasy Plant Mini Kit (Qiagen). For amplification of the chloroplast gene *rps16* and its flanking intergenic spacer regions (i.e., *trnQ-rps16* 5' exon and *rps16* 3' exon-*trnK* 5' exon) we used the primers of Downie & Katz-Downie (1999) and Lee & Downie (2006), respectively. Successfully amplified PCR products were purified using either

the QIAquick PCR purification kit (Qiagen), according to the manufacturer's instructions, or the ExoSAP protocol of Werle & al. (1994) using 5 units of Exonuclease I and 0.5 units of Shrimp Alkaline Phosphatase. Sequencing reactions were carried out using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) and sequenced on either an ABI (Applied Biosystems) 3130 XL or 3730 XL sequencer.

Phylogenetic analyses. — Complementary DNA strands were assembled and edited using Sequencher v.3.1.2 (Gene Codes Corporation) and aligned initially using the default pairwise and multiple alignment parameters in the computer program Clustal X (gap opening cost = 15.00, gap extension cost = 6.66, DNA transition weight = 0.50; Jeanmougin & al. 1998). This alignment was then checked and adjusted manually where necessary, with gaps positioned so as to minimise nucleotide mismatches. Unambiguous gaps were scored as presence/absence characters using the simple indel coding method of Simmons & Ochoterena (2000).

For analyses of the entire cpDNA *trnQ-trnK* region, the portion of the matrix representing the *trnQ-rps16* 5' exon region was scored as missing in three accessions (*Polemanna grossulariifolia*, *Chamarea snijmaniae*, *Steganotaenia commiphoroides*) and the portion representing the *rps16* 3' exon-*trnK* region was scored as missing in one accession (*Polemanniopsis* sp. 1) because of difficulties in amplifying these regions in these taxa. In addition, portions of the *trnQ-rps16* 5' exon region for one accession of *Choritaenia capense* (1618 bp) and of the *rps16* 3' exon-*trnK* region for one accession of *Phlyctidocarpa flava* (580 bp) were also unobtainable despite our best efforts. In *Molopospermum peloponnesiacum* we were unable to amplify both intergenic spacer regions. Overall, missing data represented approximately 10% of the entire matrix.

Phylogenetic analyses were conducted initially using the parsimony (MP) algorithm of PAUP* v.4.0b10 (Swofford, 2002). Character transformations were treated as unordered and equally weighted (Fitch parsimony; Fitch, 1971). Tree searches were performed using a heuristic search with 1000 random sequence additions, tree bisection-reconnection (TBR) branch swapping, and the mulpars option in effect. Bootstrap percentage values (BP; Felsenstein, 1985) were determined from 1000 bootstrap replicates, holding 10 trees per replicate, with TBR and mulpars selected. Only values greater than or equal to 50% are reported, and the following scale was applied to evaluate support percentages: \leq 74%, weak; 75%–84%, moderate; and 85%–100%, strong. After model selection using Modeltest v.3.06 under the corrected Akaike information criterion (Akaike, 1974; Posada & Crandall, 1998), Bayesian inference (BI; Yang & Rannala, 1997) was implemented using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). We employed the K81uf+G model for the nucleotide data and the 'standard' model (using default parameters) for the indel data (Lewis, 2001). The analysis was performed for 2,000,000 generations of Monte Carlo Markov chains (MCMC) with trees saved every 100 generations. The analysis was judged to have reached stationarity when the standard deviation between the split frequencies stabilised below 0.009. The initial one-fourth of the trees were discarded as the

'burn-in' phase. A majority rule consensus tree was produced from the remaining trees in order to show the posterior probabilities (PP). The following scale was used to evaluate the PP's: 0.5–0.84, weak; 0.85–0.94, moderate; 0.95–1.0, strong.

Fourteen morphological and anatomical characters considered important for defining early diverging lineages within the Apioideae-Saniculoideae clade were scored for the 46 taxa included in the molecular analyses (Appendix 2; Table S1 in the Electronic Supplement to this article). Character data were taken from field observations of the South African taxa, herbarium specimens and literature (Winter & al., 1993; Van Wyk & al. 1999; Tilney & Van Wyk, 2001; Vessio, 2001; Liu & al., 2003, 2007a,b, 2009; Liu, 2004; Magee & al., 2008a,b; Tilney & al., 2009; Yembaturova & al., in press). These morphological data were then reconstructed on the MP trees using Mesquite v.2.5 (Maddison & Maddison, 2008).

■ RESULTS

Phylogenetic analyses. — The matrix, representing the entire *trnQ-trnK* region, consisted of 5072 unambiguously aligned positions and 587 binary scored indels, and resulted in 2045 variable and 1285 parsimony informative characters. MP analyses of these data yielded two minimal length trees, each of 2113 steps (ensemble consistency indices [CI; Kluge & Farris, 1969] of 0.75 and 0.66, with and without uninformative characters, respectively; ensemble retention index [RI; Farris, 1989] of 0.86). The MP strict consensus tree was identical in topology to that inferred from the BI analysis (Fig. 1). In both analyses, the same clades and relationships as reported previously (Calviño & al., 2006; Calviño & Downie, 2007; Magee & al., 2008a) were retrieved. The subfamily Saniculoideae sensu Calviño & Downie is monophyletic (PP 0.99, BP 74), with tribes Saniculeae (i.e., Saniculoideae s.str.) and Steganotaenieae strongly supported (PP 1.0, 1.0, BP 100, 99, respectively). *Phlyctidocarpa* is sister group to *Polemanniopsis* and *Steganotaenia* (tribe Steganotaenieae) with strong or weak support depending on the analysis (PP 0.97, BP 71). Subfamily Apioideae sensu Calviño & Downie is also supported as monophyletic (PP 1.0, BP 76) and is sister group to subfamily Saniculoideae sensu Calviño & Downie (PP 1.0, MP 100). Within the apioide clade a strongly supported subclade comprising *Choritaenia*, *Lichtensteinia* Cham. & Schldl. and *Marlothiella* (PP 1.0, MP 100) is sister group to all remaining Apioideae lineages. The accessions of *Bupleurum* L. (tribe Bupleureae), *Physospermum* Cusson (tribe Pleurospermeae) and *Sium* L. (tribe Oenantheae), representing the upper lineages of the subfamily, are a sister group to a strongly supported tribe Heteromorphae (PP 1.0, BP 100). Successively sister to this group is a strongly supported *Annesorhiza* Cham. & Schldl. clade (PP 1.0, BP 89), comprising *Annesorhiza*, *Astydamia* DC., *Chamarea* Eckl. & Zeyh., *Ezosciadium* B.L. Burt, *Itasina* Raf. and *Molopospermum* W.D.J. Koch.

Reconstruction of morphological and anatomical characters. — Parsimony-based reconstructions of 14 morphological and anatomical characters (Appendix 2) considered

important for defining early diverging lineages within the Apioideae-Saniculoideae clade are each summarised onto one of the two minimal length trees inferred from MP analysis of *trnQ-trnK* nucleotide substitution and scored indel data (Fig. 2). True wings/ribs on the fruit (character 7, state 1; Fig. 2G; Fig. 3E, H–K), parenchymatous or lignified endocarp (character 10, state 1; Fig. 2J; Fig. 3C–D, F–K), absence of rhomboidal crystals (character 13, state 1; Fig. 2M), and the presence of druse crystals scattered throughout the mesocarp (character 14, state 1; Fig. 2N) were reconstructed as synapomorphies for the Apioideae-Saniculoideae clade. The base of the trees was reconstructed as ambiguous for each of the aforementioned characters. However, if a further outgroup of either Azorelloideae or Mackinlayoideae had been included, this node would certainly be reconstructed for the plesiomorphic state as in *Hermas*. As shown by Calviño & al. (2008a), Saniculoideae s.str. (i.e., tribe Saniculeae sensu Calviño & Downie) is supported by the presence of simple umbels (character 3, state 1; Fig. 2C), sessile or subsessile fructiferous flowers (character 4, state 1; Fig. 2D), large involucre bracts forming a prominent pseudanthium (character 5, state 1; Fig. 2E), and fruit with surface outgrowths (character 6, state 1; Fig. 2F). No characters were found to support the clades comprising either Saniculoideae sensu Calviño & Downie (*Phlyctidocarpa*–*Petagnaena*) or Apioideae sensu Calviño & Downie (*Sium*–*Lichtensteinia*). The presence of regular vittae (character 11, state 1; Fig. 2K; Fig. 3I–K) was reconstructed as synapomorphies for the lineages *Sium*–*Molopospermum* and two species of *Alepidea* La Roche (Fig. 3D), and as an apomorphy for *Phlyctidocarpa* (Fig. 3G). Large rib oil ducts (character 12, state 1; Fig. 2L; Fig. 3C–D, F–H), traditionally a character for Saniculoideae s.str., was ambiguously reconstructed as either a synapomorphy for the Apioideae–Saniculoideae clade with secondary reversals in the upper lineages (from the *Annesorhiza* clade upwards), *Arctopus*, *Sanicula europaea*, and *Petagnaena* or as a convergent character state in Saniculoideae and in *Lichtensteinia* and *Marlothiella*. The woody habit (character 1, state 1; Fig. 2A) was reconstructed as convergent for both *Marlothiella* and the tribe Steganotaenieae, and was ambiguous for the ancestor to tribe Heteromorphae. Although *Dracosciadium* Hilliard & B.L. Burt is a rhizomatous herb, character 1 was coded as polymorphic to reflect the missing Madagascan taxa with which it is allied (Calviño & al., in prep.). As this genus forms a clade with the woody Madagascan genera, it is likely that with more accessions the woody habit may be reconstructed as a synapomorphy for the Heteromorphae, with the herbaceous habit of *Dracosciadium* reconstructed as a reversal. Bifurcate ribs (character 8, state 1; Fig. 2H), surface vesicles (character 6, state 1; Fig. 2F), and regular vittae (character 11, state 1; Fig. 2K) seem to have evolved independently in *Phlyctidocarpa* (Fig. 3G) and other lineages. The proteranthous leaves (character 2, state 1; Fig. 2B) in *Lichtensteinia* and Steganotaenieae were reconstructed as synapomorphies for each lineage. Steganotaenieae was also supported by the presence of heteromeric carp fruit (character 9, state 1; Fig. 2I), where only the sepaline ribs are well-developed (Fig. 3H). This character was also found in the genus *Heteromorpha* Cham. & Schldl. (Fig. 3K).

The number of parsimony informative indels and reconstructed morphological apomorphies are indicated on a BI phylogram (Fig. 4), above and below branches, respectively. The branch leading to the Apioideae-Saniculoideae clade was relatively long and supported by 31 parsimony informative indels (although until more outgroup taxa from the Azorelloideae are included, we cannot be certain how many of these

indels are synapomorphic for *Hermas*) and four morphological synapomorphies. In contrast, the branches leading to Saniculoideae sensu Calviño & Downie (*Phlyctidocarpa*–*Petagnaea*) and Apioideae sensu Calviño & Downie (*Sium*–*Lichtensteinia*) were both relatively short, supported by only three and two parsimony informative indels, respectively (Fig. 4), and without any reconstructed morphological synapomorphies.

Fig. 1. Bayesian inference (BI) tree of *trnQ-trnK* sequence data with the previous subfamilial circumscriptions indicated alongside. *Phlyctidocarpa flava* was not included in the study of Calviño & Downie, but presumed to be included within subfamily Saniculoideae based on preliminary evidence (Calviño & Downie, 2009). Posterior probability (PP) values are presented above the branches. Bootstrap percentage (BP) values (>50%) are presented below the branches.

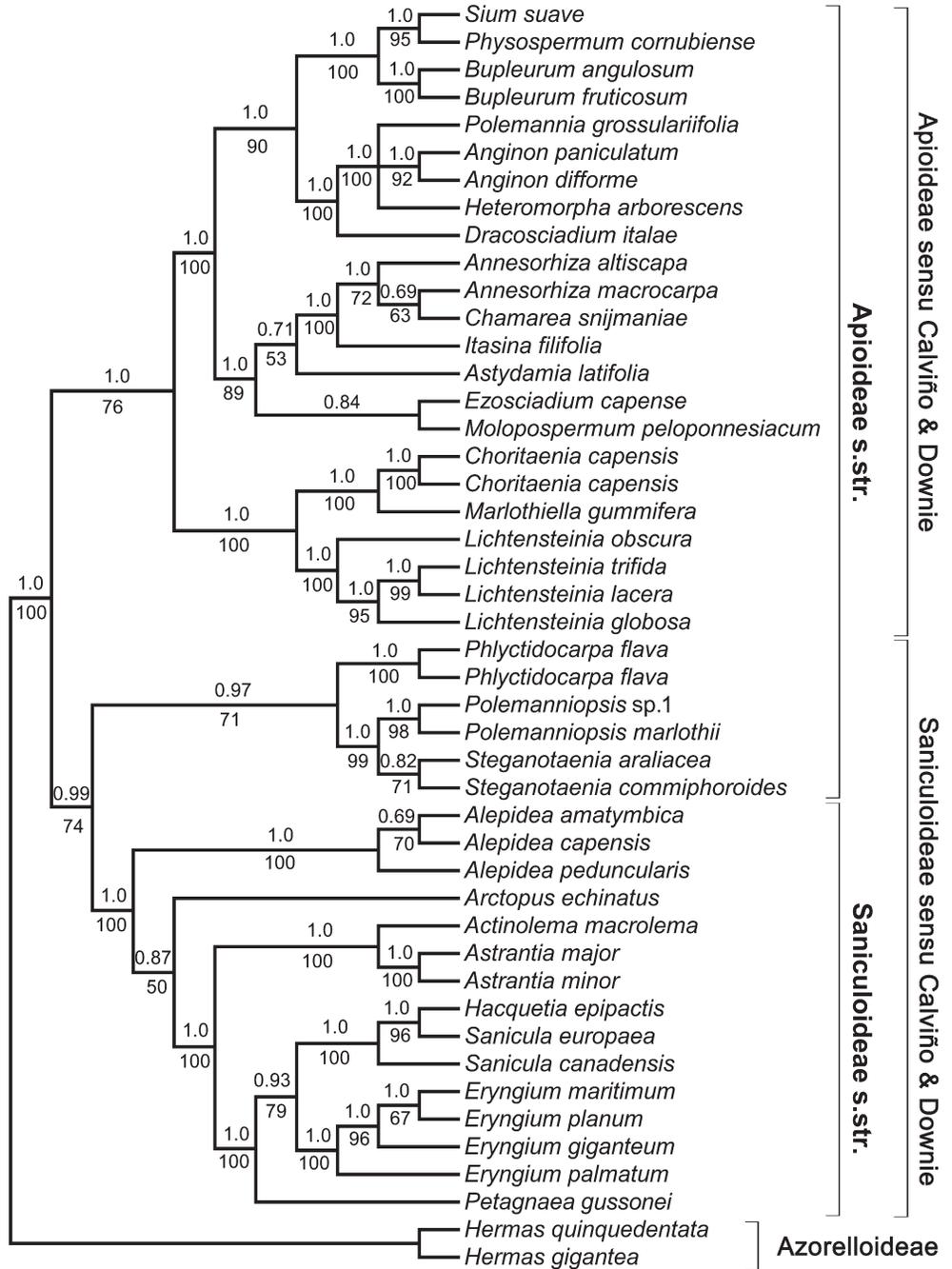
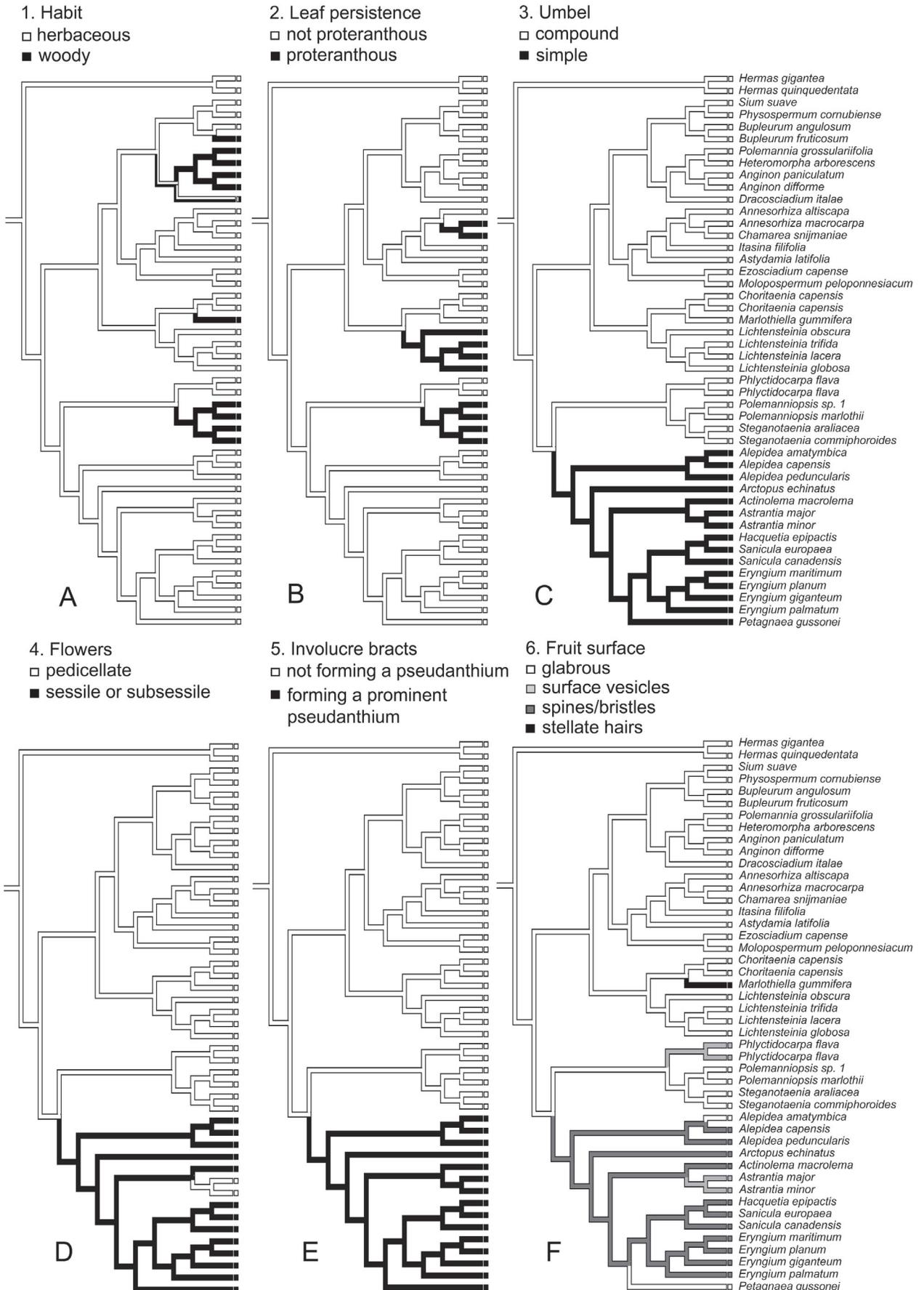
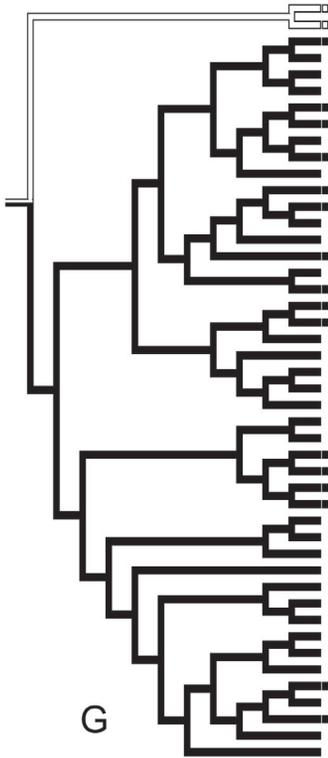


Fig. 2. A–N, Reconstruction of morphological characters 1 to 14 from Appendix 2, when optimized over one of the two minimal length trees inferred from MP analysis of the *trnQ-trnK* sequence data. The proposed new tribal and subfamilial classification is indicated alongside Fig. 2N.



7. Fruit wing/rib development

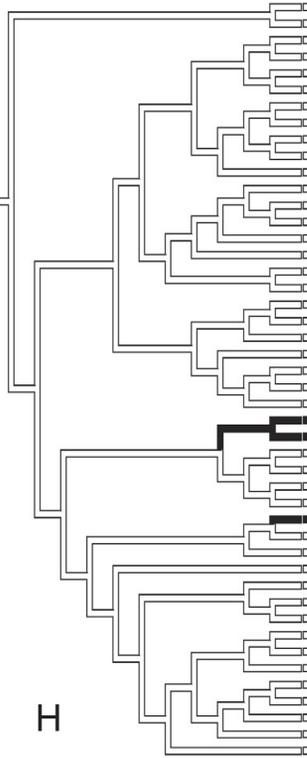
- pseudo-wings/ribs
- true wings/ribs



G

8. Fruit ribs shape

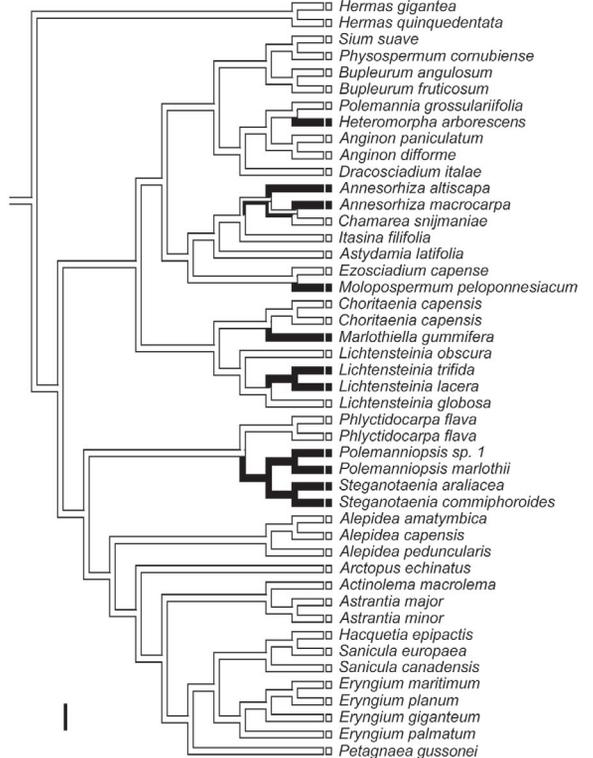
- simple
- bifurcate



H

9. Fruit symmetry

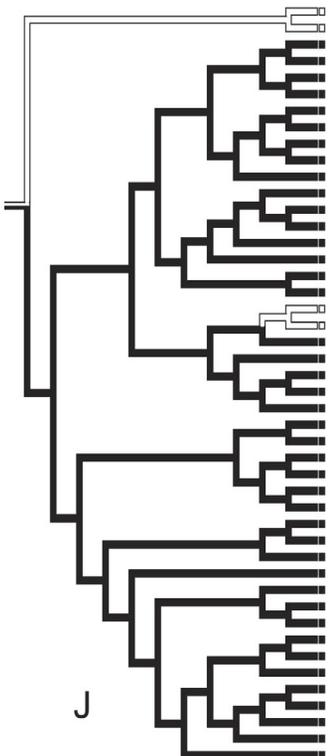
- homomericarpic
- heteromericarpic



I

10. Endocarp

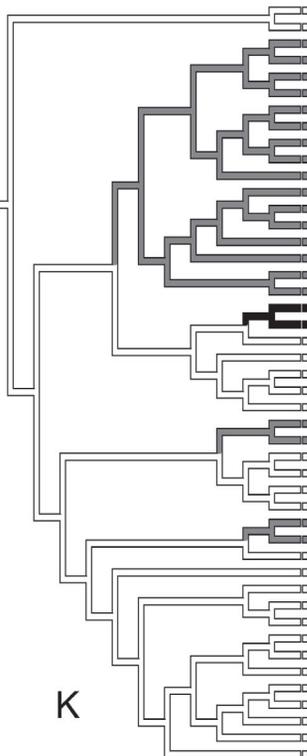
- woody
- parenchymatous/lignified



J

11. Regular vittae

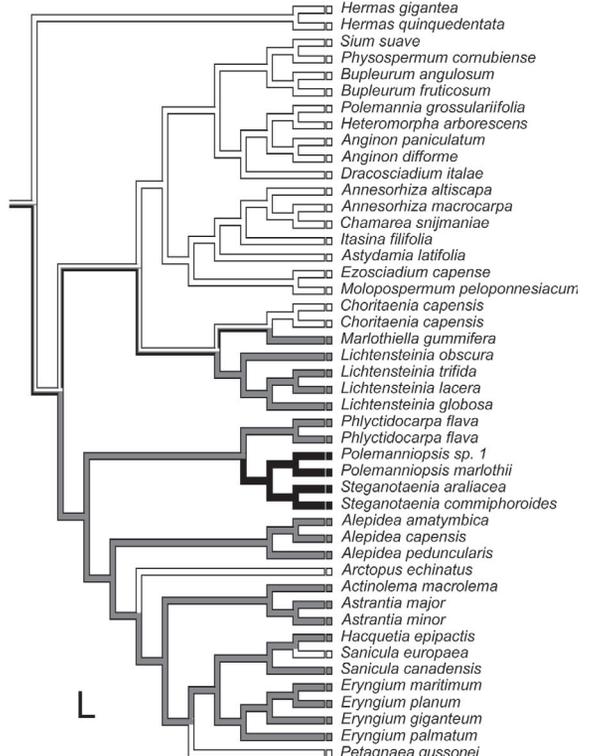
- absent
- present
- oil vesicles



K

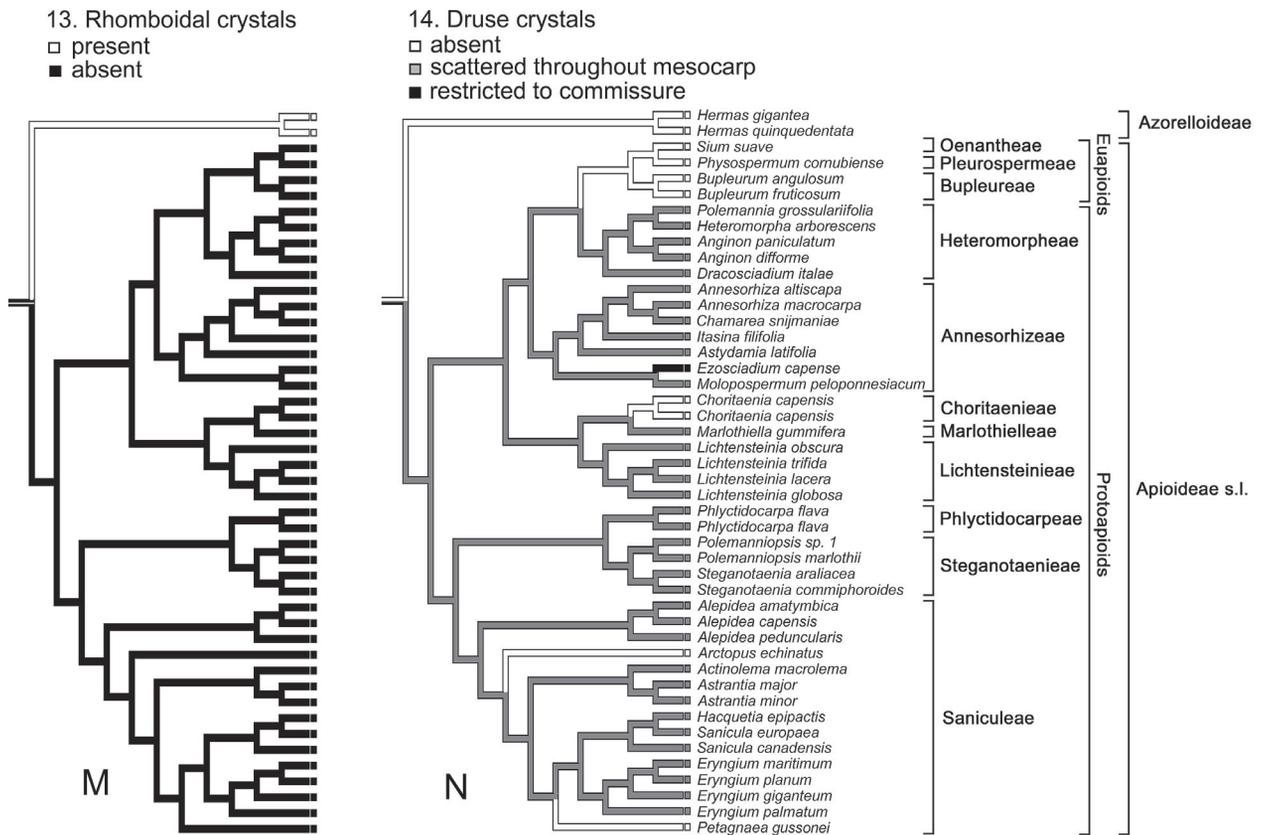
12. Rib oil ducts

- small
- large
- forming cavities



L

- Hermas gigantea*
- Hermas quinqueidentata*
- Sium suave*
- Physospermum comubiense*
- Bupleurum angulosum*
- Bupleurum fruticosum*
- Polemannia grossularifolia*
- Heteromorpha arborescens*
- Anginon paniculatum*
- Anginon difforme*
- Dracosciadium italaе*
- Annesorhiza altiscapa*
- Annesorhiza macrocarpa*
- Chamarea snijmaniae*
- Itasina filifolia*
- Astydamia latifolia*
- Ezosciadium capense*
- Molopospermum peloponnesiacum*
- Choritaenia capensis*
- Choritaenia capensis*
- Marlothiella gummifera*
- Lichtensteinia obscura*
- Lichtensteinia trifida*
- Lichtensteinia lacera*
- Lichtensteinia globosa*
- Phlyctidocarpa flava*
- Phlyctidocarpa flava*
- Polemanniopsis sp. 1*
- Polemanniopsis marlothii*
- Steganotaenia araliacea*
- Steganotaenia commiphoroides*
- Alepidea amatymbica*
- Alepidea capensis*
- Alepidea peduncularis*
- Arctopus echinatus*
- Actinolema macrolema*
- Astrantia major*
- Astrantia minor*
- Hacquetia epipactis*
- Sanicula europaea*
- Sanicula canadensis*
- Eryngium maritimum*
- Eryngium planum*
- Eryngium giganteum*
- Eryngium palmatum*
- Petagnaеa gussonei*



DISCUSSION

“Euapioids” and “protoapioids”. — The early diverging lineages (a paraphyletic assemblage herein referred to as the protoapioids) of the Apioideae-Saniculoideae clade are readily separated from the remaining apioid lineages (the euapioids) by the presence of scattered druse crystals of calcium oxalate in the mesocarp of the fruit (character 14; Fig. 2N). Optimization of this character by Calviño & al. (2008a) suggested that the scattered druse crystals in the mesocarp were either absent or ambiguously reconstructed in the ancestor of the Apioideae-Saniculoideae clade. This was, however, the result of the incorrect scoring of this character as absent in some of the protoapioid taxa (viz., *Anginin Raf.*, *Polemanniopsis*, *Steganotaenia* and several species of *Lichtensteinia*) based on the reports of Liu & al. (2003, 2006) and Liu (2004). Druse crystals are often dissolved and therefore easily overlooked in PAS/TB-stained anatomical sections. Further confounding this problem, they may be only sparsely distributed throughout the fruit mesocarp, so that many sections need to be studied. After careful examination of both stained and unstained (where necessary) fruit sections, we are now able to confirm the presence of scattered druse crystals in almost all protoapioid taxa. Druse crystals were absent only in *Arctopus L.*, *Choritaenia* and *Petagnaea*, and restricted to the commissure in *Ezoscidium*. These results confirm the suggestion by Liu & al. (2006) that the presence of scattered druse crystals in the mesocarp is a synapomorphy for the Apioideae-Saniculoideae clade.

***Phlyctidocarpa*.** — In both the BI and MP trees the two accessions of *Phlyctidocarpa flava* comprised a strongly supported monophyletic group (PP 1.0, BP 100), weakly to strongly supported as a sister group to the tribe Steganotaeniaceae (PP 0.97, BP 71). This monotypic Namibian endemic genus had previously been placed within Drude’s (1897–98) subfamily Apioideae, tribe Ammineae, by Theobald & Cannon (1973), based on the presence of crystals in the commissure and at the base of the fruit ribs, as well as the arrangement of the vittae. These authors acknowledged the similarity of the scattered druse crystals with members of the Saniculoideae s.str. but the significance of this similarity remained unclear to them. It has subsequently become evident that the presence of scattered druse crystals in the fruit is not restricted to the Saniculoideae s.str. (Liu & al., 2006; character 14; Fig. 2N), but that this character extends also into the early diverging lineages of Apioideae s.str. Calviño & Downie (2007) suggested the placement of *Phlyctidocarpa* within Saniculoideae based on a preliminary molecular phylogenetic study but the genus was not yet formally included in their broadened circumscription of Saniculoideae. The isolated position of *Phlyctidocarpa* within the clade comprising both Steganotaeniaceae and Saniculeae, as found in this study, has subsequently also been confirmed by Nicolas and Plunkett (pers. comm.) using *trnT-trnD* and *rpl16* intron data. However, the results from these authors place *Phlyctidocarpa* successively sister to Steganotaeniaceae and Saniculeae, as opposed to the direct sister relationship with Steganotaeniaceae recovered in our analyses.

Phlyctidocarpa is unusual in that, like many of the other protoapioid lineages, it combines characters traditionally used to define both Apioideae s.str. and Saniculoideae s.str. The fruit (Fig. 3G) have unusual surface blisters on the ribs and large rib oil ducts (as in most Saniculoideae s.str.), together with prominent regular vittae (as in most Apioideae s.str.). Although the surface blisters are superficially similar to those found in the saniculoid genus *Astrantia* L. (Fig. 3C), they differ in that the ribs on which they are borne are bifurcate in transverse section (Fig. 3G) due to a longitudinal division or groove. As a result, both the vallecular vittae and rib oil ducts appear, in transverse section, to be located in the vallecules between the ribs. Somewhat bifurcate ribs are also found in *Alepidea woodii* and *A. cordifolia* (Yembaturova & al., in press).

Choritaenia, Lichtensteinia and Marlothiella. — Previous analyses of molecular sequence data (Calviño & al., 2006; Calviño & Downie, 2007) placed the South African endemic genus *Lichtensteinia* as the earliest diverging lineage within Apioideae sensu Calviño & Downie. Recently, in an assessment of the subfamily Azorelloideae, Nicolas & Plunkett (2009) using molecular data showed that the anomalous genus *Choritaenia* was not related to the other genera of the Azorelloideae but rather formed a clade together with *Lichtensteinia*, that was weakly supported as sister group to Saniculoideae sensu Calviño & Downie. In the present study, both the MP and BI trees recover a strongly supported clade (PP 1.0, BP 100) comprising *Choritaenia, Lichtensteinia* and *Marlothiella*, with this clade as sister group to the remaining

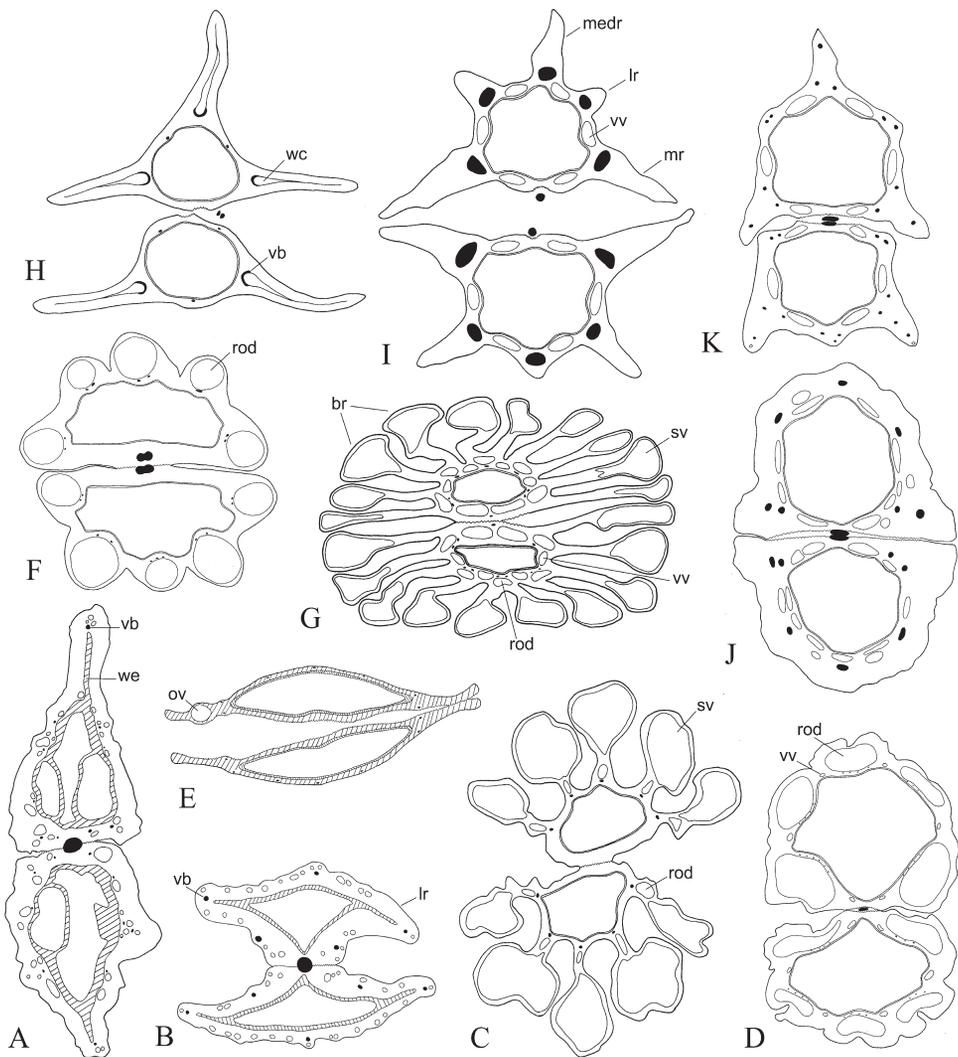


Fig. 3. Transverse sections through fruits of selected members of Apiaceae and Mydocarpaceae. **A**, *Myodocarpus involucratus*; **B**, *Hermas villosa*; **C**, *Astrantia major*; **D**, *Alepidea cordifolia*; **E**, *Choritaenia capensis*; **F**, *Lichtensteinia trifida*; **G**, *Phlyctidocarpa flava*; **H**, *Polemanniopsis marlothii*; **I**, *Annesorrhiza macrocarpa*; **J**, *Anginon difforme*; **K**, *Heteromorpha arborescens*. Vouchers: A, Lowry & Oskolski 4639 (MO); B, Anon s.n. (JRAU); C, Pauca 165b (PE); D, Van Wyk 4232 (JRAU); E, Hanekom 1834 (PRE); F, Winter & Tilney 4164 (JRAU); G, Gress & al. 6075 (PRE); H, Taylor 11269 (PRE); I, Rourke 1700 (NBG); J, Van Wyk 2944 (JRAU); K, Greenway 12558 (PRE). br, bifurcate rib; lr, lateral rib; medr, median rib; mr, marginal rib; ov, oil vesicle; rod, rib oil duct; sv, surface vesicle; vb, vascular bundle; vv, vallecular vitta; wc, wing cavity; we, woody endocarp. Scale: A–E, I–K = 0.8 mm; F = 0.5 mm; G = 1.0 mm; H = 0.4 mm.

members of Apiaceae sensu Calviño & Downie. *Choritaenia* is an annual herb endemic to the dry interior of southern Africa (Liu & al., 2007a) and clearly represents a highly adapted lineage. Traditionally placed within the Hydrocotyloideae (now largely included in Azorelloideae and Mackinlayoideae) due to the presence of a woody endocarp, *Choritaenia* differs from the other members of Azorelloideae in the conspicuous oil vesicles located in its wings (an autapomorphy for the genus), absence of rhomboidal druse crystals, marginal wings composed entirely of the mesocarp, and further lignification of the mesocarp (Fig. 3E). Oil vesicles, although rare in Apiaceae, have also been recorded in *Bilacunaria* Pimenov & V.N. Tikhom. and *Smyrniopsis* Boiss. (Tamaschan, 1945; pers. obs.); however, in these taxa they are

dispersed in the mesocarp of the fruits. Amongst Apiaceae included in this investigation, wings may develop in one of two ways (Calviño & al., 2008a). In the first, wings are due to the compression or folding of the carpel and are as such composed of both the mesocarp and endocarp with the vascular bundle located at the margin (Fig. 3A–B). This type (type 3 of Calviño & al., 2008a, hereafter referred to as pseudo-wings) seems to be the plesiomorphic state, present in wing-fruited members of Azorelloideae, Mackinlayoideae, and even in the closely related families Araliaceae and Myodocarpaceae. In the second wing configuration type, the wings are due to an expansion of the mesocarp and are as such composed only of mesocarp, with the vascular bundle usually located at its base (Fig. 3E–J). This type (type 2 of Calviño & al., 2008a,

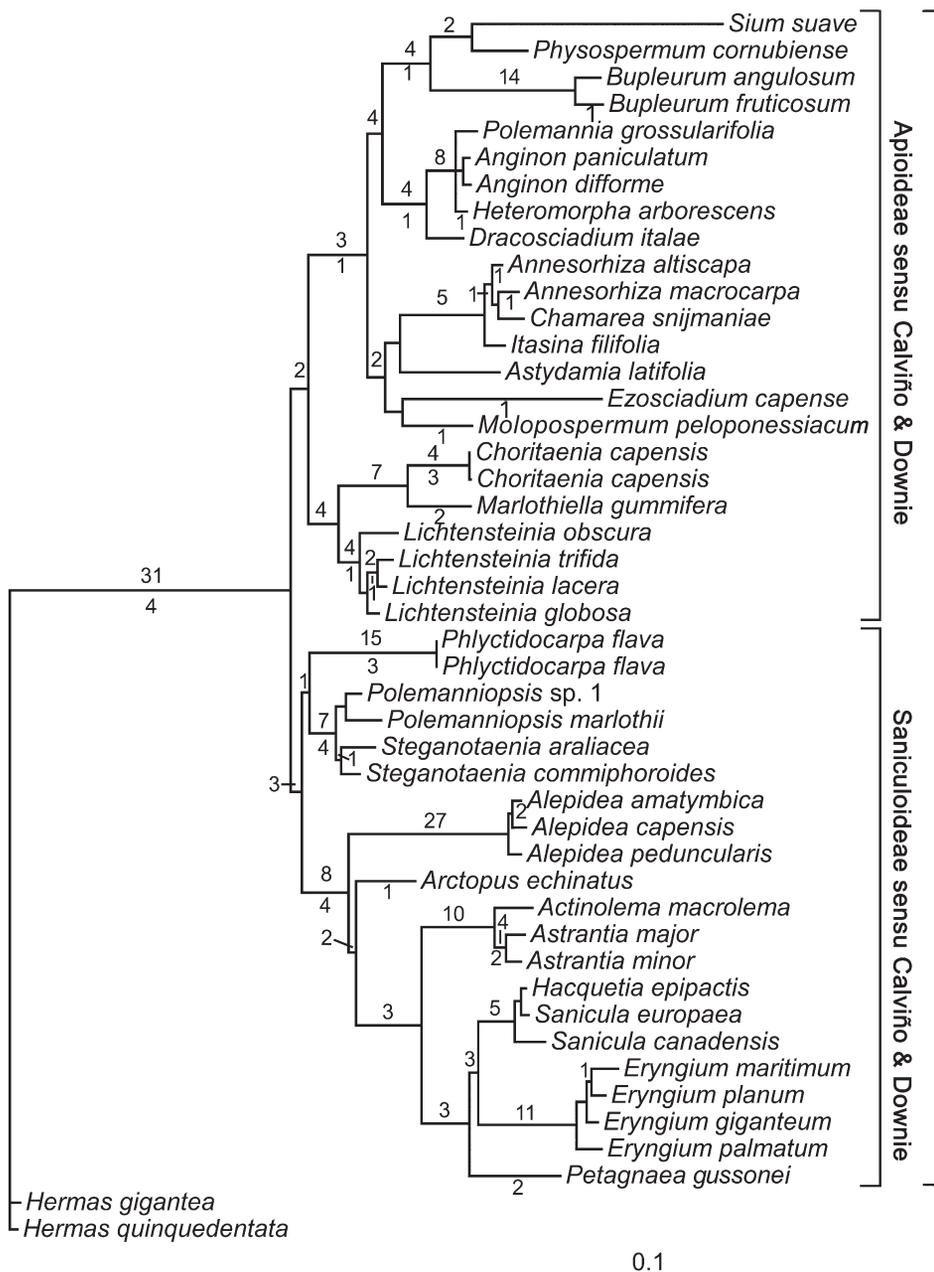


Fig. 4. Bayesian inference phylogram of *trnQ-trnK* sequence data. Numbers above the branches indicate the distributions of parsimony informative indels and those below the branches the number of morphological apomorphies as constructed from character optimization. The scale indicates the number of expected substitutions per site.

hereafter referred to as true wings) is restricted to winged or prominently ribbed members of the Apioideae-Saniculoideae clade and was reconstructed in this study as a synapomorphy for this group (character 7, Fig. 2G). As this character was not directly applicable to non-winged members of the Apioideae-Saniculoideae clade, it was scored in the analysis as missing for these taxa (Table S1 in the Electronic Supplement). Calviño & al. (2008a) also described a third wing type composed entirely of exocarp in some lineages of *Eryngium* L., but this state was not observed in any of the fruits investigated in this study.

Tilney & al. (2009) suggested that the Namibian endemic genus *Marlothiella* may be the closest relative of *Lichtensteinia*, due to the shared presence of concentric rings of cells around the large rib oil ducts and the marked differences that may be present in the size of the rib ducts (Fig. 3F) so that the fruit may appear heteromericarpic in transverse section. Indeed, our analyses of the *trnQ-trnK* region support a close relationship between these two otherwise very different genera. *Marlothiella* was strongly supported (PP 1.0, BP 100) as a sister group to *Choritaenia*, with *Lichtensteinia* sister group to this clade. Liu & al. (2004) suggested that both *Lichtensteinia* and *Marlothiella* be placed within an expanded Saniculoideae based on the presence of large rib oil ducts and the absence of regular vittae (Fig. 3F). Calviño & al. (2008a) considered the presence of rib oil ducts in the fruit as a plesiomorphy in Saniculoideae due to their presence in both Azorelloideae and Mackinlayoideae. Moreover, they considered the loss (or reduction) of rib oil ducts to be a synapomorphy for the upper lineages of Apioideae (from the *Annesorhiza* clade upwards). Large rib oil ducts (traditionally a character for Saniculoideae s.str.) when reconstructed onto the MP trees (character 12; Fig. 2L) could most parsimoniously be considered as either a synapomorphy for the Apioideae-Saniculoideae clade with a secondary reversal in the upper lineages of Apioideae (upwards from the *Annesorhiza* clade), or the result of convergence in the *Phlyctidocarpa-Petagnaena* and *Marlothiella-Lichtensteinia* clades. Since relatively large rib oil ducts also occur in some members of the Azorelloideae, there are various ways of interpreting the evolution of the character (see Calviño & al., 2008a).

Annesorhiza clade. — Initially, the *Annesorhiza* clade was recognised by Calviño & al. (2006) as comprising *Annesorhiza*, *Chamarea* and *Itasina*. Although *Astydamia* and *Molopospermum* were weakly recovered as being closely related, the authors excluded them from the *Annesorhiza* group until their placements had been confirmed with further data. Magee & al. (2008a) subsequently showed that the annual, South African endemic genus *Ezoscadium* was also related to this clade. In both the MP and BI analyses, all accessions of these six genera were strongly supported to comprise a monophyletic group (PP 1.0, BP 89), successively sister to the tribe Heteromorphae and subsequent lineages of the Apioideae. The *Annesorhiza* clade comprises herbaceous members largely typical of the euapioid tribes with regular vittae and compound leaves, but differs most prominently in the presence of scattered druse crystals (except in *Ezoscadium*, where they are restricted to the commissural area; Magee & al., 2008a), the thick and

highly lignified vascular bundles in the fruit (Fig. 3I), and the proteranthous or deciduous leaves.

Paraphyly of Apioideae. — The subfamily Apioideae s.str. as circumscribed by Drude (1897–98) is clearly not monophyletic when the African taxa are considered (Downie & Katz-Downie, 1999; Downie & al., 2001; Liu & al., 2003, 2006; Calviño & al., 2006; Calviño & Downie, 2007), and the characters traditionally used to segregate this subfamily are reconstructed to be either plesiomorphic (e.g., compound umbels) or synapomorphic and restricted to the upper lineages of the subfamily (e.g., inconspicuous rib oil ducts, presence of regular vittae, absence of scattered druse crystals); Calviño & al. (2008a and this study). Calviño & Downie (2007), based on phylogenetic studies, accommodated many of the conflicting African taxa in Apioideae or Saniculoideae resulting in two recircumscribed monophyletic subfamilies. However, as shown by Calviño & al. (2008a), the subfamilies Apioideae sensu Calviño & Downie and Saniculoideae sensu Calviño & Downie are each not supported by any morphological or anatomical synapomorphies studied to date. The nodes supporting Apioideae sensu Calviño & Downie (*Sium-Lichtensteinia*) and Saniculoideae sensu Calviño & Downie (*Phlyctidocarpa-Petagnaena*) are both strongly to moderately supported with a low rate of nucleotide substitutions per site in the BI trees (fig. 6 in Calviño & Downie, 2007; Fig. 4 in this study). Calviño & al. (2008a) proposed that *Steganotaeniaeae* and *Saniculeae* (sister tribes of subfamily Saniculoideae sensu Calviño & Downie) have evolved independently for a long time or have accumulated many independent morphological changes, thus masking any shared morphological feature that was once present in their common ancestor. Synapomorphies of the latter, however, persist at the molecular level. This hypothesis explains the lack of evident morphological or anatomical synapomorphies in Saniculoideae sensu Calviño & Downie. The same reasoning can be applied to subfamily Apioideae sensu Calviño and Downie. Despite the lack of morphological or anatomical synapomorphies for each of these subfamilies, Calviño & Downie (2007) decided to maintain the two as distinct, monophyletic taxa. The spirit for their classification was basically threefold: (1) to maintain the identity of two of the major lineages of Apiaceae that commonly originated in South Africa but later independently dispersed to, and diversified in, other parts of the world; (2) to be able to readily reflect a sister group relationship between subfamilies Apioideae and Saniculoideae; and (3) to prevent the loss of long-accepted taxonomic concepts through the re-accommodation of problematic taxa to adequately reflect phylogeny. The controversy around this classification lies in the absence of any morphological or anatomical synapomorphies that define each subfamily. In order to define subfamilies in Apiaceae based on morphological or anatomical synapomorphies, an alternative would be to incorporate the two tribes of Saniculoideae sensu Calviño & Downie within a more widely delimited subfamily Apioideae (Apioideae s.l. as defined herein).

The Saniculoideae s.str. have traditionally been defined by the presence of simple spinescent or setiferous leaves, simple umbels, showy involucre bracts forming a pseudanthium,

sessile or subsessile fructiferous flowers, fruit with exocarp outgrowths, distinct rib oil ducts, scattered druse crystals and the absence of regular vittae. In contrast, Apiaceae have been circumscribed by the usually compound leaves, prominent compound umbels, fruit with inconspicuous rib oil ducts and regular vittae. Many of the protoapioid genera, however, share characters with both subfamilies. *Lichtensteinia*, *Marlothiella*, *Polemanniopsis* and *Steganotaenia*, have prominent compound umbels typical of Apiaceae but saniculoid-like fruit with large rib oil ducts and without regular vittae (Fig. 3F, H). The fruit of *Phlyctidocarpa* (Fig. 3G) share the blistered exocarp and large prominent rib oil ducts with members of Saniculoideae, together with a compound umbel and regular vittae as in Apiaceae. It is interesting to note the recent discovery of regular vittae in some species of the most early diverging saniculoid genus *Alepidea* (Yembaturova & al., in press; Fig. 3D) which further narrows the taxonomic distance between the two subfamilies. Due to the presence of equally isolated and morphologically divergent lineages within the protoapioids, the rank of subfamily for Saniculoideae no longer seems to be appropriate. Drude's (1897–98) classification system reflects incomplete knowledge of several other lineages (mostly African) that are morphologically as distinct as the Saniculoideae. The only logical way to delimit a morphologically congruent Apiaceae would be to treat the Saniculoideae s.str. as a tribe (the Saniculeae) within a more widely delimited subfamily Apiaceae. The union of Apiaceae and Saniculoideae into a single subfamily was also proposed by Koso-Poljansky (1916) who included both subfamilies within his Ligusticoideae, based on the parenchymatous endocarps. Optimization of morphological and anatomical data onto one of the two minimal length trees obtained from the MP analyses of the *trnQ*-*trnK* region (Fig. 2) shows that such an expanded Apiaceae s.l. is well supported by four synapomorphies (Fig. 4), namely the presence of true wings/ribs (wings or prominent ribs consisting only of mesocarp and exocarp; Fig. 2G), the parenchymatous or sometimes lignified endocarp (becoming secondarily woody in *Choritaenia*; Fig. 2J), the absence of rhomboidal crystals (Fig. 2M), and the presence of druse crystals of calcium oxalate scattered throughout the mesocarp (subsequently lost in the euapioids; Fig. 2N).

Tribal delimitations. — While morphological and anatomical characters support the lineage comprising the Apiaceae s.l., as well as many of the terminal clades within the protoapioids (herein recognized as tribes), there are no available morphological or anatomical characters to support the hierarchical relationships among the tribes (i.e., the clades comprising Lichtensteinieae–Oenantheae, Lichtensteinieae–Choritaenieae, Steganotaenieae–Phlyctidocarpeae or Saniculeae–Phlyctidocarpeae). Therefore, in order to reflect the available morphological, anatomical and molecular data, the protoapioids are here segregated into eight small and morphologically isolated tribes (each well-supported by several morphological and/or anatomical characters and/or molecular sequence data), which probably represent relicts from a once more numerous African apioid flora. A taxonomic key to the subfamilies of Apiaceae and tribes of the protoapioids is provided below.

■ TAXONOMIC TREATMENT

Key to the subfamilies of Apiaceae and tribes of the protoapioids (Apiaceae s.l.):

- 1 Endocarp woody, adjacent mesocarp usually parenchymatous; rhomboidal crystals usually present, druse crystals absent; true wings absent; wing-like structures (pseudowings) or prominent ribs comprising endocarp and mesocarp with vascular tissue at the tip **2**
 - 1 Endocarp parenchymatous or sometimes becoming lignified, if somewhat woody then with adjacent mesocarp lignified; rhomboidal crystals absent; druse crystals present or absent; true wings often present; wings or prominent ribs comprising only mesocarp, with vascular tissue almost always at the base **Apiaceae s.l., 3**
 - 2 Fruits laterally compressed; mericarps not separating at maturity; carpophore absent. **Mackinlayoideae**
 - 2 Fruits isodiametric or dorsally compressed; mericarps separating at maturity; carpophore present **Azorelloideae**
 - 3 Fruit with oil vesicles in the wings. **Choritaenieae**
 - 3 Fruit without oil vesicles in the wings **4**
 - 4 Inflorescence a pseudanthium; regular vittae absent, if rarely present then with larger rib oil ducts and amphiseaminal druse crystals in the fruit **Saniculeae**
 - 4 Inflorescence umbellate, if rarely a pseudanthium then with regular vittae, poorly developed rib oil ducts, and without amphiseaminal druse crystals in the fruit. **5**
 - 5 Druse crystals of calcium oxalate absent or commissural only; without tanniferous epidermal cells. **euapioids**
 - 5 Druse crystals of calcium oxalate amphiseaminal, if reduced to commissure only, then with tanniferous epidermal cells and only partly bifid carpophore. **6**
 - 6 Regular vittae absent **7**
 - 6 Regular vittae present. **9**
 - 7 Fruit winged; wing cavities present; woody shrubs or trees. **Steganotaenieae**
 - 7 Fruit not winged; large rib oil ducts surrounded by a concentric ring of cells; herbs or small woody shrublets. **8**
 - 8 Rhizomatous herbs; leaves large, coriaceous. **Lichtensteinieae**
 - 8 Woody shrublets; leaves small, succulent. **Marlothielleae**
 - 9 Fruit surface blistered; ribs bifurcate; vittae and rib oil ducts equal in size. **Phlyctidocarpeae**
 - 9 Fruit surface smooth; ribs not bifurcate; vittae larger than rib oil ducts **10**
 - 10 Perennial or annual herbs; leaves proteranthous or deciduous. **Annesorhizeae**
 - 10 Woody trees, shrubs, suffrutices or lianas; if rarely herbaceous then leaves persistent **Heteromorpheae**
1. **Annesorhizeae** Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, **trib. nov.** – Type: *Annesorhiza* Cham. & Schldl.
Heteromorphis similis umbello multiplexo, fructu crust-

allis drusaceis omnino in mesocarpio dispersis et vittis regularibus, sed habitu herbaceo, foliis proteranthis vel deciduis et fructu fasciculis vascularibus valde lignosis differt.

Annesorhizeae is similar to the Heteromorpheae in that the species have compound umbels, fruit with druse crystals scattered throughout the mesocarp and regular vittae. However, they differ from members of the Heteromorpheae in the herbaceous habit (rarely annual in *Ezoscadium*), the proteranthis or deciduous leaves and fruit with thick and strongly lignified vascular bundles. The tribe includes *Annesorhiza*, *Astydamia*, *Chamarea*, *Ezoscadium*, *Itasina* and *Molopospermum*. Although most of the genera are southern African endemics, *Molopospermum* is a European genus and *Astydamia* is restricted to North Africa and the Canary Islands.

2. Choritaeniae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, **trib. nov.** – Type: *Choritaenia* Benth.

Tribus multis Apioidearum umbello multiplexo alisque marginalibus veris similis, sed unicus vesiculis olei in alis marginalibus, carpophoro hygroskopico, endocarpio lignoso cum lignificatione adiuncta mesocarpium.

Choritaeniae shares the compound umbels and true marginal wings with many other tribes of the Apioideae, but is unique in that the fruit have oil vesicles in the marginal wings, a hygrosopic carpophore as well as a woody endocarp with further lignification of the mesocarp. It also differs from most other protoapioids in the annual and ephemeral habit and the absence of druse crystals scattered throughout the mesocarp of the fruit. The tribe is monogeneric and comprises the southern African genus *Choritaenia*.

3. Lichtensteiniae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, **trib. nov.** – Type: *Lichtensteinia* Cham. & Schldl.

Marlothiellae similis umbellis multiplexis, ductis olei maximis costalibus annulis concentricis cellularum circumcinctis, crystallis drusaceis omnino in mesocarpio dispersis, sed habitu herbaceo deciduo, foliis non succulentis saepe proteranthis marginibus dentatis setaceis, fructu sine pilis stellatis valde differt.

Lichtensteiniae is similar to Marlothiellae in the compound umbels, very large rib oil ducts surrounded by concentric ring of cells and druse crystals scattered throughout mesocarp, but differs markedly in the herbaceous deciduous habit, non-succulent leaves (often proteranthis, with dentate, setaceous margins) and fruit without stellate hairs. It is also similar to the tribe Annesorhizeae in the herbaceous and deciduous habit but differs markedly in the large ribs oil ducts and the absence of vittae in the fruit. The tribe is monogeneric and comprises the South African genus *Lichtensteinia*.

4. Marlothiellae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, **trib. nov.** – Type: *Marlothiella* H. Wolff.

Lichtensteiniae similis umbellis multiplexis, crystallis drusaceis omnino in mesocarpio dispersis et ductis olei maximis

costalibus annulis concentricis cellularum circumcinctis, sed habitu lignoso, foliis succulentis et fructu pilis stellatis differt.

Marlothiellae is similar to Lichtensteiniae in the presence of compound umbels, druse crystals scattered throughout the mesocarp, and the very large rib oil ducts surrounded by concentric rings of cells, but differs in the woody habit, succulent fleshy leaves and fruit with stellate hairs. Although it shares the woody habit and heteromeric fruit with members from both Heteromorpheae and Steganotaeniae, it differs from the former in the large rib oil ducts and the absence of regular vittae in the fruit and from the latter in the isodiametric fruit, ribs that do not develop into wings and that are without wing cavities. The tribe is monogeneric and comprises the Namibian endemic genus *Marlothiella*.

5. Phlyctidocarpeae Magee, C.I. Calviño, M. Liu, S.R. Downie, P.M. Tilney & B.-E. van Wyk, **trib. nov.** – Type: *Phlyctidocarpa* Cannon & Theobald.

Annesorhizeae similis habitu herbaceo, vittis regularibus et crystallis drusaceis omnino in mesocarpio dispersis, sed ductis olei costalibus magnis, costis bifurcatis vesiculis superficialibus magnis et fasciculis duobus ventralibus non evolutis differt.

Phlyctidocarpeae is similar to Annesorhizeae in the herbaceous habit, regular vittae and druse crystals scattered throughout the mesocarp, but differs in the large rib oil ducts, bifurcate ribs with large surface vesicles and the two weakly developed ventral bundles. Although it shares the large rib oil ducts, weakly developed ventral bundles and surface vesicles with members of the tribe Saniculeae it differs markedly in the absence of a pseudanthium, the pedunculate compound umbels, the presence of regular vittae and the bifurcate ribs. *Phlyctidocarpa* has previously been associated with superficially similar members of the tribe Apieae and other euapioid tribes but differs in the presence of druse crystals scattered throughout the mesocarp of the fruit as well as large rib oil ducts, bifurcate ribs with large surface vesicles and weakly developed ventral bundles. The tribe is monogeneric and comprises the Namibian endemic genus *Phlyctidocarpa*.

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Appendix 1. Accessions of Apiaceae from which the cpDNA *trnQ-trnK* sequences were obtained, with corresponding voucher information, taxonomic authorities, and GenBank reference numbers.

Actinolema macrolema Boiss., *Coode & Jones 2425* (E); *trnQ-rps16* DQ832386^c, *rps16* DQ832337^c, *rps16-trnK* DQ832473^c. *Alepidea amatymbica* Eckl. & Zeyh., *Mohle 480* (MO); *trnQ-rps16* DQ832387^c, *rps16* DQ832338^c, *rps16-trnK* DQ832474^c. *Alepidea capensis* (Berg.) R.A. Dyer, *Hilliard & Burt 16645* (E); *trnQ-rps16* DQ832389^c, *rps16* DQ832340^c, *rps16-trnK* DQ832476^c. *Alepidea peduncularis* Steud. ex A. Rich., *Gereau & Kayombo 4094* (MO); *trnQ-rps16* DQ832386^c, *rps16* DQ832337^c, *rps16-trnK* DQ832473^c. *Anginon difforme* (L.) B.L. Burtt, *Magee 130* (JRAU); *trnQ-rps16* FM986461^h, *rps16* FM986476^h, *rps16-trnK* FM986485^h. *Anginon paniculatum* (Thunb.) B.L. Burtt, *Downie 2458* (ILL); *trnQ-rps16* DQ832400^c, *rps16* AY838397^b, *rps16-trnK* DQ832487^c. *Annesorhiza altiscapa* H. Wolff, *Magee 97* (JRAU); *trnQ-rps16* FM986462^h, *rps16* FM986477^h, *rps16-trnK* FM986486^h. *Annesorhiza macrocarpa* Eckl. & Zeyh., *Downie 2454* (ILL); *trnQ-rps16* DQ832400^c, *rps16* AY838397^b, *rps16-trnK* DQ832487^c. *Arctopus echinatus* L., *Ornduff 7380* (UC); *trnQ-rps16* DQ832402^c, *rps16* DQ832351^c, *rps16-trnK* DQ832489^c. *Astrantia major* L., *Schilling 2937* (E); *trnQ-trnK* DQ832443^c. *Astrantia minor* L., *Vautier, Mermoud & Bersier s.n.* (ILL); *trnQ-trnK* DQ832444^c. *Astydamia latifolia* (L. f.) Kuntze, *Hildenbrand s.n.* (ILL); *trnQ-rps16* FM986463^h, *rps16-trnK* FM986487^h. *Bupleurum angulosum* L., *Younger 2565* (E); *trnQ-rps16* FM986464^h, *rps16* AF110568^a, *rps16-trnK* FM986488^h. *Bupleurum fruticosum* L., *McBeath 2592* (E); *trnQ-rps16* FM986465^h, *rps16* AF110569^a, *rps16-trnK* FM986489^h. *Chamarea snijmaniae* B.L. Burtt, *MacGregor s.n.* (NBG); *rps16* AY838414^b, *rps16-trnK* FM986490^h. *Choritaenia capensis* Benth.—*Zeitman 271* (PRE); *trnQ-rps16* FM986467^h, *rps16* FM986479^h, *rps16-trnK* FM986492^h. *Choritaenia capensis* Benth.—*Van Wyk 545* (PRU); *trnQ-rps16* FM986466^h, *rps16* FM986478^h, *rps16-trnK* FM986491^h. *Dracosciadium itale* Hilliard & B.L. Burtt, *Ngwenya & Singh 1279* (PRE); EU434657^b, EU434652^b. *Eryngium giganteum* M. Bieb., *Mc Neal 472* (UC); *trnQ-rps16* EU070440^d, *rps16* EU070502^d, *rps16-trnK* EU070564^d. *Eryngium maritimum* L., *Medina & al. MPI656* (TEX); *trnQ-rps16* EU070454^d, *rps16* EU070516^d, *rps16-trnK* EU070578^d. *Eryngium palmatum* Pančić & Vis., *Constance C-99* (UC); *trnQ-rps16* DQ832416^c, *rps16* DQ832365^c, *rps16-trnK* DQ832504^c. *Eryngium planum* L., *Downie 191* (ILL); *trnQ-trnK* DQ832456^c. *Ezoscadiium capense* (Eckl. & Zeyh.) B.L. Burtt, *Goldblatt & Porter 12578* (NBG); *trnQ-rps16* FM986468^h, *rps16* AM982518^f, *rps16-trnK* FM986493^h. *Hacquetia epipactis* DC., *M.F. & S.G. Gardner 2590* (E); *trnQ-rps16* DQ832423^c, *rps16* DQ832372^c, *rps16-trnK* DQ832511^c. *Hermas gigantea* L., *McDonald 1769* (NBG); *trnQ-rps16* DQ832425^c, *rps16* AY838420^b, *rps16-trnK* DQ832513^c. *Hermas quinquedentata* L. f., *Burman 1080* (BOL); *trnQ-rps16* DQ832426^c, *rps16* AY838422^b, *rps16-trnK* DQ832514^c. *Heteromorpha arborecens* Cham. & Schltdl.—*Van Wyk 4122* (JRAU); *trnQ-rps16* FM986469^h, *rps16* AY838424^b, *rps16-trnK* FM986494^h. *Itasina filifolia* (Thunb.) Raf., *Downie 2453* (ILL); *trnQ-rps16* FM986470^h, *rps16* AY838426^b, *rps16-trnK* FM986495^h. *Lichtensteinia globosa* B.-E. van Wyk & Tilney, *Downie 2462* (ILL); *trnQ-rps16* DQ832429^c, *rps16* AY838429^b, *rps16-trnK* DQ832517^c. *Lichtensteinia lacera* Cham. & Schltdl., *Downie 2464* (ILL); *trnQ-rps16* DQ832427^c, *rps16* AY838427^b, *rps16-trnK* DQ832515^c. *Lichtensteinia obscura* (Spreng.) Koso-pol.—*Downie 2457* (ILL); *trnQ-rps16* DQ832428^c, *rps16* AY838428^b, *rps16-trnK* DQ832516^c. *Lichtensteinia trifida* Cham. & Schltdl., *Downie 2460* (ILL); *trnQ-rps16* DQ832430^c, *rps16* AY838430^b, *rps16-trnK* DQ832518^c. *Marlothiella gummifera* H. Wolff, *Manheimer 2987* (JRAU); *trnQ-rps16* FM986471^h, *rps16* FM986480^h, *rps16-trnK* FM986496^h. *Molopospermum peloponnesiacum* (L.) W.D.J. Koch, *Argent ML2* (E); *rps16* AY838432^b. — *Petagnaea gussonei* (Spreng.) Rauschert, *Donila 2005* (PAL); *trnQ-trnK* DQ832466^c. *Phlyctidocarpa flava* Cannon & Theobald, *Merxmüller & Giess 30626* (WIND); *trnQ-rps16* FM986473^h, *rps16* FM986482^h, *rps16-trnK* FM986498^h. *Phlyctidocarpa flava* Cannon & Theobald, *Manheimer 2889* (JRAU); *trnQ-rps16* FM986472^h, *rps16* FM986481^h, *rps16-trnK* FM986497^h. *Physospermum cornubiense* (L.) DC., *Pimenov & al. s.n.* (MW); *trnQ-rps16* FM986474^h, *rps16* AF110556^a, *rps16-trnK* FM986499^h. *Polemanna grossulariifolia* Eckl. & Zeyh.—*De Castro 274* (JRAU); *rps16* AY838439^b, *rps16-trnK* FM986500^h. *Polemanniopsis marlothii* (H. Wolff) B.L. Burtt, *Downie 2459* (ILL); *trnQ-rps16* DQ832432^c, *rps16* DQ832374^c, *rps16-trnK* DQ832520^c. *Polemanniopsis* sp. 1, *Manheimer 2769* (JRAU); *trnQ-rps16* FM986475^h, *rps16* FM986483^h. — *Sanicula canadensis* L., *Downie 737* (ILL); *trnQ-trnK* DQ832467^c. *Sanicula europaea* L., *Catalán 1896* (JACA); *trnQ-trnK* DQ832468^c. *Sium suave* Walter, *Downie 12* (ILL); *trnQ-trnK* EF185274^c. *Steganotaenia araliacea* Hochst.—*Downie 2456* (ILL); *trnQ-rps16* DQ832442^c, *rps16* DQ832384^c, *rps16-trnK* DQ832530^c. *Steganotaenia commiphoroides* Thulin, *Friis & al. 4889* (K); *rps16* FM986484^h, *rps16-trnK* FM986501^h.

^aDownie & Katz-Downie (1999); ^bCalviño & al. (2006); ^cCalviño & Downie (2007); ^dCalviño & al. (2008b); ^eDownie & al. (2008); ^fMagee & al. (2008a); Tilney & al. (2009); ^hpresent study.

Appendix 2. Morphological and anatomical characters and states examined during this investigation and optimized onto the molecular trees.

1. Habit (herbaceous = 0; woody = 1); 2. Leaf persistence (not proteranthous = 0; proteranthous = 1); 3. Umbel (compound = 0; simple = 1); 4. Flowers (pedicellate = 0; sessile or subsessile = 1); 5. Involucre bracts (not forming a pseudanthium = 0; forming a prominent pseudanthium = 1); 6. Fruit surface (glabrous = 0; surface vesicles = 1; spines/bristles = 2; stellate hairs = 3); 7. Fruit wing/rib development (pseudo-wings/ribs = 0; true wings/ribs = 1); 8. Fruit ribs shape (simple = 0; bifurcate = 1); 9. Fruit symmetry (homomericarpic = 0; heteromericarpic = 1); 10. Endocarp (woody = 0; parenchymatous/lignified = 1); 11. Regular vittae (absent = 0; present = 1; oil vesicles = 2); 12. Rib oil ducts (small = 0; large = 1; forming cavities = 2); 13. Rhomboidal crystals (present = 0; absent = 1); 14. Druse crystals (absent = 0; scattered throughout mesocarp = 1; restricted to commissure = 2).