American Journal of Botany 93(12): 1828–1847. 2006.

A MOLECULAR PHYLOGENETIC STUDY OF SOUTHERN AFRICAN APIACEAE[1]

CAROLINA I. CALVIÑO,[2,3,5] PATRICIA M. TILNEY,[4] BEN-ERIK VAN WYK,[4] AND STEPHEN R. DOWNIE[2]

[2]Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA; [3]Instituto de Botánica Darwinion, Buenos Aires, Argentina; and [4]University of Johannesburg, Auckland Park, South Africa

It has been suggested that southern Africa is the origin of the predominantly herbaceous Apiaceae subfamily Apioideae and that the woody habit is plesiomorphic. We expand previous molecular phylogenetic analyses of the family by considering all but three of the approximately 38 genera native to southern Africa, including all genera whose members, save one, have a woody habit. Representatives of five other genera are included because they may be closely related to these southern African taxa. Chloroplast DNA *rps16* intron and/or nuclear rDNA ITS sequences for 154 accessions are analyzed using maximum parsimony, Bayesian, and maximum likelihood methods. Within Apioideae, two major clades hitherto unrecognized in the subfamily are inferred. The monogeneric *Lichtensteinia* clade is sister group to all other members of the subfamily, whereas the *Annesorhiza* clade (*Annesorhiza, Chamarea*, and *Itasina*) plus *Molopospermum* (and *Astydamia* in the ITS trees) are the successive sister group to all Apioideae except *Lichtensteinia*. Tribe Heteromorpheae is expanded to include *Pseudocarum*, "Oreofraga" ined., and five genera endemic to Madagascar. The southern African origin of subfamily Apioideae is corroborated (with subsequent migration northward into Eurasia along two dispersal routes), and the positions of the herbaceous *Lichtensteinia* and *Annesorhiza* clades within the subfamily suggest, surprisingly, that its ancestor was herbaceous, not woody.

Key words: Apiaceae; Apioideae; cpDNA *rps16* intron; phylogeny; rDNA ITS; Saniculoideae; southern Africa; woodiness.

Southern Africa stands out by its great biological distinctiveness. The area customarily treated for floristic purposes as southern Africa includes Botswana, Lesotho, Namibia, South Africa, and Swaziland. The African subcontinent is remarkable for both its high plant richness and endemism at all taxonomic levels (Goldblatt, 1978; Goldblatt and Manning, 2002). It is also the origin, as well as the center of radiation, of numerous, diverse lineages of flowering plants. Approximately 53% of the total flora of the Cape region of South Africa has a shrubby habit, an overwhelming proportion even when compared with other Mediterranean floras such as those of California or Chile (Goldblatt and Manning, 2002). While the prevalence of woody species on oceanic islands has long attracted the attention of evolutionary biologists, less is known about the origin of the herbaceous vs. woody habit in continental floras, especially in the floristically unique southern Africa. It is often assumed that herbaceous plants within a family are derived from woody ancestors; such assumptions, however, have generally relied on implicit hypotheses of character-state polarity. It is of evolutionary interest to ascertain if the prevalence of woodiness in the southern African flora is the result of phylogenetic heritage or adaptation.

[1] Manuscript received 19 March 2006; revision accepted 5 October 2006.

The authors thank D. Katz-Downie, F. Sun, C. Carr, B. Robertson, B. Patel, and J. Heilveil for assistance in the laboratory, the curators of herbaria cited in the text for access to specimens, K. Spalik for providing DNAs, and D. Katz-Downie, S. Martínez, F. Zuloaga, and two anonymous reviewers for helpful comments on the manuscript. This work was supported by grants to S. R. Downie from the National Science Foundation (DEB 0089452) and the National Center for Supercomputing Applications (DEB 030005N), utilizing the IBM pSeries 690 system at the University of Illinois at Urbana-Champaign (UIUC). Travel funds to C. I. Calviño were provided by UIUC's School of Integrative Biology Enhancement Fund and the Department of Plant Biology John R. Laughnan Award. This paper represents part of a Ph.D. dissertation (C.I.C.), for which funding from CONICET is gratefully acknowledged.

[5] Author for correspondence (ccalvino@life.uiuc.edu)

The immense phylogenetic importance of those plants native to southern Africa is exemplified by the family Apiaceae. Burtt (1991), Van Wyk (2001), and Van Wyk and Tilney (2004) emphasized the significance of these plants in terms of their unique morphological diversity and the difficulties in finding obvious, close relatives for many of them. Some of these genera have been postulated to be links between subfamilies Apioideae and Saniculoideae (Burtt, 1991); others may have evolved independently from these subfamilies (Van Wyk, 2001). The many unusual and defining features of these African plants include an arborescent or shrubby habit, deciduous leaves with dentate-aristate margins, and heteromorphic mericarps (Liu et al., 2003; Liu, 2004; Van Wyk and Tilney, 2004). Early molecular phylogenetic investigations revealed that two of these genera, *Heteromorpha* Cham. & Schltdl. and *Anginon* Raf., arborescent and shrubby plants endemic to sub-Saharan and southern Africa, either constitute a clade sister group to all other taxa of subfamily Apioideae or comprise successively basal-branching lineages within the subfamily (Downie et al., 1996, 1998; Plunkett et al., 1996a, b). The phylogenetic placements of these taxa suggested that southern Africa is the likely origin of the largely north temperate subfamily Apioideae and that the woody habit is plesiomorphic within the subfamily. Following these studies, Downie and Katz-Downie (1999) showed that the African endemic genera *Anginon*, *Dracosciadium* Hilliard & B. L. Burtt, *Glia* Sond., *Heteromorpha*, and *Polemannia* Eckl. & Zeyh. unite as a well-supported monophyletic group (named the *Heteromorpha* clade and, subsequently, tribe Heteromorpheae; Downie et al., 1998, 2000b). They further revealed that the woody apioid African endemic genera, *Polemanniopsis* B. L. Burtt and *Steganotaenia* Hochst., comprise a clade sister group to subfamily Saniculoideae, suggesting that this subfamily may have also evolved from woody ancestors. In the neighbor-joining and maximum likelihood trees inferred from phylogenetic analyses of chloroplast DNA (cpDNA) *rps16* intron sequences, tribe Heteromorpheae is sister group to

all other Apioideae (Downie and Katz-Downie, 1999). The strict consensus tree inferred from maximum parsimony analysis of these data, however, suggests that either tribe Heteromorpheae or the species *Annesorhiza altiscapa* Schltr. ex H. Wolff (or both) may be a sister group to a clade comprising the other members of subfamily Apioideae. *Annesorhiza* Cham. & Schltdl. is a genus of perennial herbs endemic to southern Africa, thus its position as a possible sister group to all other Apioideae is intriguing for it suggests a herbaceous ancestry for the subfamily, contrary to what has been generally assumed.

The importance of southern African Apiaceae in the early evolutionary history of the family is now well established, but knowledge of these umbellifers is limited by inadequate sampling, especially of the herbaceous African endemics. The relationships of the herbaceous taxa to the woody African genera and to the more northern herbaceous members of the family are not clear, and explicit hypotheses that include a broad representation of these woody and herbaceous African genera are lacking. Therefore, increasing the sampling of both herbaceous and woody southern African umbellifers (and including several non-African genera considered as belonging to the earliest diverging lineages of Apioideae) is critical in illuminating the direction of evolution of their unusual characters (many of which are hypothesized to be plesiomorphic within the family; Van Wyk and Tilney, 2004) and the historical biogeography of subfamily Apioideae.

The major objectives of this study are to (1) ascertain the phylogenetic placements of native southern African Apiaceae based on cpDNA *rps16* intron and/or nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences, with emphasis on those genera whose members have a woody habit; (2) corroborate a southern African origin of subfamily Apioideae, as suggested by previous molecular systematic investigations, and reconstruct its subsequent biogeographic history; and (3) test the prevailing hypothesis that the woody habit is plesiomorphic in the subfamily. Progress on reclassifying the subfamily Apioideae, as well as having a better understanding of its origin and early diversification, can only be achieved upon consideration of these extraordinary African plants.

MATERIALS AND METHODS

Taxa and outgroup selection—A total of 154 accessions was examined for cpDNA *rps16* intron and/or nuclear rDNA ITS sequence variation. In the phylogenetic analysis of *rps16* intron sequences, 130 accessions were considered, which included 60 genera and 116 species of Apiaceae and two genera (three species) of Araliaceae. DNA sequences from 54 of these accessions were obtained specifically for this study (Table 1); data for the remaining 76 accessions were obtained during a previous study of woody southern African taxa (Downie and Katz-Downie, 1999). In the ITS phylogenetic analysis, 84 accessions of Apiaceae (representing 32 genera and 66 species) were considered and of these, 58 are new (Table 1). Voucher data and GenBank accession numbers for the 26 accessions examined previously for ITS sequence variation are available elsewhere: *Komarovia* Korovin and *Physospermum* Cusson (Downie et al., 1998); *Aulacospermum* Ledeb., *Eleutherospermum* K. Koch, *Erigenia* Nutt., *Hansenia* Turcz., *Parasilaus* Leute, and *Pleurospermum* Hoffm. (Katz-Downie et al., 1999); *Molopospermum* W. D. J. Koch (Downie et al., 2000a); "Oreofraga" ined., *Physospermopsis* H. Wolff, and *Pleurospermum* (Downie et al., 2000c); *Cyclorhiza* M. L. Sheh & R. H. Shan, *Notopterygium* H. Boissieu, *Tongoloa* H. Wolff, and *Trachydium* Lindl. (Valiejo-Roman et al., 2002a); *Anginon* and *Bupleurum* L. (Neves and Watson, 2004); and *Haplosphaera* Hand.-Mazz. and *Sinolimprichtia* H. Wolff (Valiejo-Roman et al., 2006). Sixty accessions were common to both cpDNA and ITS data sets.

Burtt (1991) and Van Wyk (2000) recognized 36–38 genera of Apiaceae as native to southern Africa, of which 19 are endemic to southern Africa and five are endemic to sub-Saharan Africa. We included 27 of these genera in this study, including 16 of the endemics. With the exception of the monotypic genus *Marlothiella* H. Wolff, which was excluded from our study because of inadequate material for DNA extraction, we considered all native African genera whose members exhibit a woody habit. Several of these genera represent shrubs or small trees (*Anginon, Heteromorpha, Polemannia, Polemanniopsis,* and *Steganotaenia*), while others comprise both subshrubs (or shrubs) and herbaceous members (*Deverra* DC., *Diplolophium* Turcz., *Peucedanum* L., and *Stenosemis* Sond.). We examined 10 of 12 species of *Anginon* (Allison and Van Wyk, 1997), five of seven species of *Heteromorpha* (Winter and Van Wyk, 1996), all three species of *Polemannia* (Van Wyk, 2000), the monotypic *Polemanniopsis*, and one of two species of *Steganotaenia* (Van Wyk, 2000). We also included eight endemic herbaceous genera: *Annesorhiza*, *Chamarea* Eckl. & Zeyh., *Choritaenia* Benth., *Dasispermum* Raf., *Dracosciadium*, *Hermas* L., *Itasina* Raf., and *Lichtensteinia* Cham. & Schltdl. (Burtt, 1991). The genus *Hermas* is placed in Apiaceae subfamily Hydrocotyloideae (as that subfamily is traditionally circumscribed), but shares several features typical of subfamily Saniculoideae (B. J. de Villiers et al., University of Johannesburg, unpublished data). Eight genera native to southern Africa do not occur within basal Apioideae and, thus, were omitted from the study. Their phylogenetic placements are as follows: *Alepidea* F. Delaroche and *Arctopus* L. (subfamily Saniculoideae; Plunkett and Lowry, 2001; C. I. Calviño and S. R. Downie, unpublished data); *Agrocharis* Hochst. (Scandiceae; Downie et al., 2000b); *Berula* W. D. J. Koch (Oenantheae; Hardway et al., 2004); *Pimpinella* L. (Pimpinelleae; Downie et al., 2000a, 2001); *Sonderina* H. Wolff (Tordylieae; S. R. Downie, unpublished data); *Stoibrax* Raf. (*Apium* clade; K. Spalik, University of Warsaw, unpublished data); and *Capnophyllum* Gaertn. (apioid superclade; Downie et al., 1998, 2001). In summary, of the approximately 38 genera of Apiaceae native to southern Africa, the phylogenetic placements of all but three have now been considered, either in this study or elsewhere. These exceptions include *Marlothiella*, *Ezosciadium* B. L. Burtt., and *Phlyctidocarpa* Cannon & W. L. Theob. Each of these genera is monotypic, with both *Marlothiella* and *Phlyctidocarpa* occurring in Namibia, and *Ezosciadium* restricted to the Eastern Cape of South Africa.

Representatives of five other genera were also considered, as prior studies or unpublished phylogenetic analyses suggested their close relationships to tribes Pleurospermeae or Heteromorpheae. These taxa are *Molopospermum peloponnesiacum* (L.) W. D. J. Koch (Downie et al., 2000a), "Oreofraga morrisiana" ined. (Downie et al., 2000c), *Chamaesium paradoxum* H. Wolff (Spalik and Downie, 2006), *Pseudocarum* C. Norman spp. (Van Wyk et al., 1999; Van Wyk, 2001), and *Astydamia latifolia* (L. f.) Baillon (K. Spalik, University of Warsaw, unpublished data). *Molopospermum* is a monotypic genus, with a limited distribution in montane and subalpine zones of the Pyrenees, Massif Central, and southern Alps; "Oreofraga morrisiana" (M. F. Watson and E. L. Barclay, Royal Botanic Garden Edinburgh, unpublished data) is a yet to be described species from Socotra; *Chamaesium* H. Wolff is a genus of small, montane, perennial herbs distributed from the east Himalayas to southwest China; *Pseudocarum* is a suffrutescent climber native to tropical east Africa and Madagascar; and *Astydamia* DC. is endemic to the Canary Islands. We included additional representatives of tribe Pleurospermeae, as well as *Erigenia* and members of the *Komarovia* clade, because these taxa comprise lineages adjacent to tribes Heteromorpheae and Bupleureae in previous phylogenetic analyses (Downie et al., 2001; Valiejo-Roman et al., 2002a).

All trees resulting from analyses of *rps16* intron sequences were rooted with *Aralia* L. and *Hydrocotyle* L. of the family Araliaceae (Chandler and Plunkett, 2004). As additional outgroups, we included members of Apiaceae subfamilies Azorelloideae and Mackinlayoideae (Chandler and Plunkett, 2004; Plunkett et al., 2004), primarily to help place the genus *Hermas*, which has been traditionally treated as a hydrocotyloid. Rooting of the ITS trees was not as straightforward because of high sequence divergence across the range of taxa considered. Among the various coding and noncoding loci utilized most commonly for Apiaceae phylogenetic study, the ITS region is the most rapidly evolving (Downie et al., 2001). At deep-level analyses within the family, however, its high rate of nucleotide substitution and many small insertion–deletion events (indels) make the alignments particularly problematic, confounding hypotheses of positional homology (Downie and Katz-Downie, 1996; Downie et al., 1998, 2000c, 2001). Additionally, at this level of comparison, optimization alignment of ITS sequence data with respect to gap and mismatch weighting can result in different phylogenetic estimates (Petersen

TABLE 1. New accessions of Apiaceae from which cpDNA *rps16* intron and/or nuclear rDNA ITS sequences were obtained, with corresponding DNA accession and GenBank reference numbers and voucher information. Herbarium acronyms are according to Holmgren et al. (1990). References to previously published *rps16* intron and ITS sequences are cited in the text. Accessions *Chamarea gracillima* 2597 and *C. longipedicellata* each had ITS sequence heterogeneity and were not included in the phylogenetic analysis, nor were they submitted to GenBank.

Taxon	DNA accession no.	Voucher information	GenBank no.
Anginon difformе (L.) B. L. Burtt	2455	South Africa, Kirstenbosch Botanic Garden (coll. no. 1914/36), 14 January 2003, *Downie 2455* (ILL)	cpDNA: AY838391 ITS: DQ368813
Anginon difformе	2582	South Africa, Zuurberg National Park, Lot 16 on Circular Route, 25 June 1985, *B-E & M van Wyk 35* (JRAU)	cpDNA: AY838392 ITS: DQ368814
Anginon fruticosum I. Allison & B-E. van Wyk	2583	South Africa, Hex River Pass, roadside, 15 November 1989, *Van Wyk 2911* (JRAU)	cpDNA: AY838393 ITS: DQ368815
Anginon fruticosum	2610	South Africa, top of Hex River Pass, 15 November 1989, *Allison 86* (JRAU)	cpDNA: AY838394 ITS: DQ368816
Anginon intermedium I. Allison & B-E. van Wyk	2585	South Africa, Richtersveld, summit of the Ploegberg complex, 20 September 1989, *Viviers 2112* (JRAU)	cpDNA: AY838395 ITS: DQ368817
Anginon jaarsveldii B. L. Burtt	2605	South Africa, top of Pella Mountain, 22 November 1991, *Allison 157* (JRAU)	cpDNA: AY838396 ITS: DQ368818
Anginon paniculatum (Thunb.) B. L. Burtt	2458	South Africa, roadside between Citrusdal and Clanwilliam, 15 January 2003, *Downie 2458* (ILL)	cpDNA: AY838397 ITS: DQ368819
Anginon pumilum I. Allison & B-E. van Wyk	2587	South Africa, Bredasdorp, Windhoek Provincial Nature Reserve, July/August 1968, *van der Merwe 843* (PRE)	cpDNA: AY838398 ITS: DQ368820
Anginon pumilum	2606	South Africa, De Hoop Nature Reserve, near entrance gate, 23 November 1991, *Allison 158a* (JRAU)	cpDNA: AY838399 ITS: DQ368821
Anginon rugosum (Thunb.) Raf.	513	South Africa, Western Cape, *Batten 1018* (UC), L. Constance pers. coll. no. C-2399	ITS: DQ368822
Anginon rugosum	2607	South Africa, 2.4 km from Reed Valley to Paterson, 1 December 1991, *Allison 210* (JRAU)	cpDNA: AY838400 ITS: DQ368823
Anginon swellendamense (Eckl. & Zeyh.) B. L. Burtt	2463	South Africa, Karoo National Botanic Garden, Worcester, 16 January 2003, *Downie 2463* (ILL)	cpDNA: AY838401 ITS: DQ368824
Anginon swellendamense	2513	South Africa, Uniondale, 7.5 km NNW of Uniondale at De Rust turnoff, 21 September 1991, *Van Wyk 3255* (JRAU)	cpDNA: AY838402 ITS: DQ368825
Anginon tenuior I. Allison & B-E. van Wyk	2584	South Africa, Montagu, near upper southern side of Ouberg Pass, Waboomsberg, 5 August 1989, *Viviers & Vlok 449* (JRAU)	cpDNA: AY838403 ITS: DQ368826
Anginon verticillatum (Sond.) B. L. Burtt	1375	South Africa, summit of the Ploegberg complex, 20 September 1989, *Viviers 2111* (E)	ITS: DQ368827
Anginon verticillatum	2608	South Africa, top of Numeesberg, 24 November 1991, *Allison 170* (JRAU)	cpDNA: AY838404 ITS: DQ368828
Annesorhiza altiscapa Schltr. ex H. Wolff	1371	South Africa, Nieuwoudtville, Glenlyon Farm, 15 August 1993, *Batter AB1192* (E)	ITS: DQ368829
Annesorhiza altiscapa	2586	South Africa, Nieuwoudtville Nature Reserve, Klipkoppies, 28 September 1979, *Van Wyk 174* (JRAU)	cpDNA: AY838405 ITS: DQ368830
Annesorhiza fibrosa B-E. van Wyk	2504	South Africa, Nieuwoudtville, Oorlogskloof, at Middelberg, 11 September 1994, *Van Wyk 3597* (JRAU)	cpDNA: AY838406 ITS: DQ368831
Annesorhiza filicaulis Eckl. & Zeyh.	2591	South Africa, Cedarberg, Dwarsrivier, in Boskloof, on path between Kliphuis and Dwarsrivier, 2 March 2001, *Van Wyk et al. 4066b* (JRAU)	cpDNA: AY838407 ITS: DQ368832
Annesorhiza latifolia Adamson	2590	South Africa, Springbok, near entrance to Klipdam, drainage line below granite dome, 19 November 1996, *Van Wyk* 3674b (JRAU)	cpDNA: AY838408 ITS: DQ368833
Annesorhiza macrocarpa Eckl. & Zeyh.	2454	South Africa, Noordhoek Village Commonage, near beach, 13 January 2003, *Downie 2454* (ILL)	cpDNA: AY838409 ITS: DQ368834
Annesorhiza macrocarpa	2796	South Africa, 5.8 km from Robertson on McGregor road, 9 October 1993, *Van Wyk et al. 3483* (JRAU)	cpDNA: AY838410 ITS: DQ368835
Astydamia latifolia (L. f) Baillon	2238	Canary Islands, Tenerife, cult. Botanical Conservatory Mulhouse, France (coll. no. 98156), 1 July 2002, *Hildenbrand s.n.* (ILL)	ITS: DQ368836
Chamaesciadium acaule C. A. Mey.	105	Republic of Georgia, Mtiuleti, Khevi, Kazbegi area, 3 km SW of Kazbegi, 2 July 1999, *Merello et al. 2429* (MO)	cpDNA: AY838411
Chamaesium paradoxum H. Wolff	3085	China, Qinghai, Dari (Darlag) Xian, Huleanma, Jianshe Xiang, S side of the Huang He and SW of confluence with the Dari He, 11 August 1993, *Ho et al. 1130* (MO)	ITS: DQ782335
Chamarea capensis (Thunb.) Eckl. & Zeyh.	2596	South Africa, Clanwilliam, hillside above motel, *Metelerkamp 555* (BOL)	ITS: DQ368837
Chamarea gracillima (H. Wolff) B. L. Burtt	2597	South Africa, Worcester, stony slopes in Elands Kloof, *Esterhuysen 15728* (BOL)	
Chamarea gracillima	2798	South Africa, Simonstown, Batsata Cove, Cape of Good Hope Nature Reserve, 10 February 1972, *Taylor 8068* (NBG)	cpDNA: AY838412 ITS: DQ368838
Chamarea longipedicellata B. L. Burtt	2592	South Africa, Sutherland, Farm Soek-Op, 102 km from Ceres/Calvinia road to Middelpos, 20 August 1983, *Snijman 658* (PRE)	cpDNA: AY838413
Chamarea snijmaniae B. L. Burtt	2593	South Africa, Nieuwoudtville, Glen Lyon Farm, 12 August 1983, *MacGregor s.n.* (NBG 135580)	cpDNA: AY838414 ITS: DQ368839

TABLE 1. Continued.

Taxon	DNA accession no.	Voucher information	GenBank no.
Chamarea sp. nov. (=*Annesorhiza elsiae* Vessio, Tilney & B-E. van Wyk)	2594	South Africa, Worcester, western slopes at base of Audensberg Ridge Peak above the Brandwacht valley, 4 February 1962, *Esterhuysen 29462* (BOL)	cpDNA: AY838415 ITS: DQ368840
Chamarea sp. nov. aff. *gracillima* (Burtt, 1991, p. 263)	2799	South Africa, Humansdorp district, Papiesfontein, 20 July 1979, *Cowling 681* (GRA)	cpDNA: AY838416 ITS: DQ368841
Choritaenia capensis Benth.	2409	South Africa, Transvaal, Klerksdorp, Wolwerand, 4 September 1972, *Hanekom 1834* (MO)	ITS: DQ368842
Dasispermum suffruticosum (Berg.) B. L. Burtt	2451	South Africa, Betty's Bay, near penguin colony, 12 January 2003, *Downie 2451* (ILL)	cpDNA: AY838417
Deverra burchellii (DC.) Eckl. & Zeyh.	2630	South Africa, Polokwane, near airfield, 7 January 2003, *Van Wyk & Tilney 4114* (JRAU)	cpDNA: AY838418
Deverra denudata (Viv.) Pfisterer & Podlech subsp. *aphylla* (Cham. & Schltdl.) Pfisterer & Podlech	2507	South Africa, Beaufort West, on top of Nuweveldsberge plateaux, between Farms Klavervlei and Rockdale, on Fraserburg road, 9 March 1990, *Vlok 2270* (JRAU)	cpDNA: AY838419
Diplolophium somaliense Verdc.	1305	Africa, *MT 9176* (E)	ITS: DQ368843
Dracosciadium italae Hilliard & B. L. Burtt	1376	South Africa, Natal, Ngotshe District, Itala Nature Reserve, Lovwsburg Escarpment, 21 January 1983, *Porter 620* (E)	ITS: DQ368844
Dracosciadium saniculifolium Hilliard & B. L. Burtt	1377	South Africa, Natal, Bergville District, Royal Natal National Park, Witsieshoek, *Stewart & Manning 2241* (E)	ITS: DQ368845
Glia prolifera (Burm. f.) B. L. Burtt	1308	South Africa, Cape Province, Fernkloof Nature Reserve, 4 February 1992, *Barker 96/A* (E), cult. RBGE (coll. no. 19923034)	ITS: DQ368846
Glia prolifera	2511	South Africa, *Winter 77* (JRAU)	ITS: DQ368847
Hermas gigantea L. f.	2600	South Africa, Attakwasberg, Vreysberg, E of Gouwrits River, 16 December 1988, *McDonald 1769* (NBG)	cpDNA: AY838420
Hermas quercifolia Eckl. & Zeyh.	2604	South Africa, Zachariashoek Catchment, Klein Drakenstein Mountains, Upper Kasteelkloof, 9 January 1969, *Kruger 892* (NBG)	cpDNA: AY838421
Hermas quinquedentata L. f.	2601	South Africa, Boland Trail, Sir Lowry's Pass, 23 January 1983, *Burman 1080* (BOL)	cpDNA: AY838422
Hermas sp. nov.	2602	South Africa, Piketberg, Levant Peak, 13 September 1980, *Esterhuysen 35492* (BOL)	cpDNA: AY838423
Heteromorpha arborescens var. *abyssinica* (A. Rich.) H. Wolff	804	South Africa, Natal, Alfred District, Zuurberg, *Hilliard & Burtt 10191* (UC), cult. University of California, Berkeley (coll. no. 86.1400), L. Constance pers. coll. no. C-2038	ITS: DQ368848
Heteromorpha arborescens (Spreng.) Cham. & Schltdl. var. *arborescens*	2631	South Africa, Near Alicedale, Eastern Cape, 15 February 2003, *Van Wyk 4122* (JRAU)	cpDNA: AY838424 ITS: DQ368849
Heteromorpha arborescens var. *arborescens*	42	South Africa, cult. UIUC from seeds obtained from Real Jardín Botánico, Spain, *Downie 42* (ILL)	ITS: DQ368850
Heteromorpha involucrata Conrath	1944	South Africa, Transvaal, Barberton District, near Sheba City, 11 January 1985, *Balkwill & Cadman 2625* (E)	ITS: DQ368851
Heteromorpha involucrata	1328	Tanzania, Mbeya District, Mshewe Rapids, 9 February 1990, *Lovett et al. 4142* (E)	ITS: DQ368852
Heteromorpha involucrata	1335	Tanzania, Mbeya District, Punguluma Hills above Mshewe and Muvwa villages, 12 February 1990, *Lovett et al. 4158* (E)	ITS: DQ368853
Heteromorpha papillosa C. C. Towns.	2589	Namibia, Farm Regenstein: WIN 32. Südhang von Berg nördlich Grossherzog Friedrichberg, 24 April 1972, *Giess 11763* (PRE)	cpDNA: AY838425 ITS: DQ368854
Heteromorpha pubescens Burtt Davy	1369	South Africa, Transvaal, Letaba 2 District, Lekgalameetse Nature Reserve, 10 April 1990, *Balkwill et al. 5602* (E)	ITS: DQ368855
Heteromorpha stenophylla Welw. ex Schinz var. *transvaalensis* (Schltr. & H. Wolff) P. J. D. Winter	1370	South Africa, Transvaal, Barberton District, Songimvelo Game Reserve, 5 December 1991, *Balkwill et al. 6665* (E)	ITS: DQ368856
Itasina filifolia (Thunb.) Raf.	2453	South Africa, Cape Point, 13 January 2003, *Downie 2453* (ILL)	cpDNA: AY838426 ITS: DQ368857
Lichtensteinia lacera Cham. & Schltdl.	2464	South Africa, Silvermine lookout point, 13 January 2003, *Downie 2464* (ILL)	cpDNA: AY838427
Lichtensteinia obscura (Spreng.) Koso-Pol.	2457	South Africa, Olifants River, between Citrusdal and Clanwilliam, 15 January 2003, *Downie 2457* (ILL), *Van Wyk 4104* (JRAU)	cpDNA: AY838428 ITS: DQ368858
Lichtensteinia sp. nov.	2462	South Africa, Elandskloof Pass, upper part of valley, near Middelberg, S of Clanwilliam, 16 January 2003, *Downie 2462* (ILL), *Van Wyk 4107* (JRAU)	cpDNA: AY838429 ITS: DQ368859
Lichtensteinia trifida Cham. & Schltdl.	2460	South Africa, Middelberg, Piekenierskloof Pass (picnic site), 15 January 2003, *Downie 2460* (ILL)	cpDNA: AY838430 ITS: DQ368860
Lichtensteinia trifida	2461	South Africa, Elandskloof Pass, upper part of valley, near Middelberg, S of Clanwilliam, 16 January 2003, *Downie 2461* (ILL), *Van Wyk 4106* (JRAU)	cpDNA: AY838431 ITS: DQ368861
Molopospermum peloponnesiacum (L.) W. D. J. Koch	1358	France, Mt. Lewis Pyrenees, 20 June 1987, *Argent ML2* (E)	cpDNA: AY838432

TABLE 1. Continued.

Taxon	DNA accession no.	Voucher information	GenBank no.
Parasilaus asiaticus (Korovin) Pimenov	1039	Tadjikistan, Nikolayevsky Spusk, cult. Moscow State University Botanical Garden, Russia, *Pimenov et al. s.n.* (MW)	cpDNA: AY838433
Peucedanum ferulaceum (Thunb.) Eckl. & Zeyh.	2500	South Africa, Knysna, Perdekop, between Plettenburg Bay and Avontuur, 11 November 1997, *Van Wyk 3881a* (JRAU)	cpDNA: AY838434
Peucedanum galbanum (L.) Drude	2452	South Africa, Noordhoek Pass, 13 January 2003, *Downie 2452* (ILL), *Van Wyk 4099* (JRAU)	cpDNA: AY838435
Peucedanum pearsonii Adamson	2499	South Africa, Khamiesberg, near top, 10 November 1988, *Van Wyk s.n.* (JRAU)	cpDNA: AY838436
Peucedanum pungens E. Mey.	2508	South Africa, Caledon, about 12 km N of Botrivier, Farm Welgemoed 113, about 2 km NW of Aasvoëlkop, 26 October 2000, *Helme 1865* (JRAU)	cpDNA: AY838437
Peucedanum strictum (Spreng.) B. L. Burtt	2609	South Africa, Du Toit's Kloof, 4 September 1994, *Winter & Van Wyk 171* (JRAU)	cpDNA: AY838438
Polemannia grossulariifolia Eckl. & Zeyh.	2581	South Africa, Hogsback, Gaika's Kop, on SW facing slope near summit, 20 January 1994, *De Castro 274* (JRAU)	cpDNA: AY838439 ITS: DQ368862
Polemannia montana Schltr. & H. Wolff	1329	South Africa, Natal, Underberg District, 27 January 1975, *Hilliard & Burtt 7751* (E)	ITS: DQ368863
Polemannia simplicior Hilliard & B. L. Burtt	1330	South Africa, Eastern Cape, Barkley East District, 6 February 1983, *Hilliard & Burtt 16487* (E)	ITS: DQ368864
Polemannia simplicior	1331	South Africa, Eastern Cape, Rhodes to Naude's Nek, Dunley, *Hilliard & Burtt 16617* (E)	ITS: DQ368865
Polemanniopsis marlothii (H. Wolff) B. L. Burtt	2459	South Africa, Pakhuis Pass (at Picnic Rocks), near Clanwilliam, 15 January 2003, *Downie 2459* (ILL), *Van Wyk 4105* (JRAU)	cpDNA: AY838440
Pseudocarum eminii (Engl.) H. Wolff	166	Ethiopia, Bale Region, Dello Awraja, in Harenna Forest, Kecha, ca. 45 km on Dello Mena-Goba road, 14 August 1986, *Mesfin 5324* (MO)	cpDNA: AY838441 ITS: DQ368866
Pseudocarum laxiflorum (Baker) B-E. van Wyk	167	Madagascar, Ambatofindrahana, March 1960, *Keraudren 266* (MO)	cpDNA: AY838442 ITS: DQ368867
Steganotaenia araliacea Hochst.	2456	South Africa, Kirstenbosch Botanic Garden (coll. no. 611/77), 14 January 2003, *Downie 2456* (ILL)	cpDNA: AY838443
Stenosemis caffra Sond.	2506	South Africa, Zuurberg National Park, Ferniebrae, 8 January 1986, *B-E & M van Wyk 1210* (JRAU)	cpDNA: AY838444

et al., 2002; Hardway et al., 2004). Pairwise sequence divergence values of between 5% and 15% among the taxa compared usually indicate an appropriate rate of divergence that will often yield readily alignable sequences (Olmstead and Palmer, 1994). Sequences that are substantially more divergent, particularly those with numerous indels, are difficult to align. For those regions of questionable alignment, it is best to exclude them from the analysis (Swofford and Olsen, 1990). The results of the *rps16* intron phylogenetic analyses were used to partition the taxa sequenced for the ITS region into three data sets of supposedly related taxa so that ambiguity of alignment is reduced or avoided within each. These three ITS data sets comprised (1) tribe Heteromorpheae, with one accession of *Annesorhiza* (*Annesorhiza* clade) selected as the outgroup; (2) tribe Pleurospermeae and the *Komarovia* clade and its allies, with *Bupleurum* (tribe Bupleureae) selected as the outgroup; and (3) the *Annesorhiza* clade and its allies, with *Lichtensteinia* (*Lichtensteinia* clade) identified as the outgroup. Alternative selections of outgroups, such as rooting the trees of the Heteromorpheae data set with *Bupleurum* or *Lichtensteinia* or rooting the trees of the *Annesorhiza* clade data set with *Heteromorpha*, did not appreciably change the ingroup tree topologies.

Experimental strategy—Leaf material for DNA extraction was obtained directly from the field, from plants cultivated from seed in the greenhouse, from accessioned plants from botanical gardens, or from herbarium specimens. Voucher information is given in Table 1 for all newly obtained sequences. Total genomic DNA was extracted using the modified hexadecyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987) as detailed in Downie and Katz-Downie (1996, 1999) or using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA).

The region encompassing the *rps16* intron and a portion of its flanking 3′ exon was PCR-amplified using primer pair 5′exon-N (CGTTTGAAACGATGTGGTAG) and rps16 3′exon (CCTGTAGGYTGNGCNCCYTT) or 5′exon-C (TTTGAAACGATGTGGTAGA) and 3′exon-CR (ACCCACGTTGCGAAGAT). All primers are written 5′ to 3′. The first pair is that of Downie and Katz-Downie (1999), but primer 5′exon-N is six bases upstream from the original primer-binding site. The other primer pair was designed from a consensus sequence of previously published *rps16* Apiaceae sequences to minimize self-dimer, hairpin, and pair-dimer formation. Internal primers rps16-C (TAAGAAGCACCGAAGTAATGTC) and rps16-CR (AATGGCGTTTCCTTGTTC) were designed specifically to facilitate some amplifications. PCR amplification methods are the same as described previously (Downie and Katz-Downie, 1996, 1999). For template purification, the QIAquick PCR Purification or the QIAquick Gel Extraction Kits (Qiagen) were used following the manufacturer's instructions. Sequencing reactions were carried out using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). Each of these reactions consisted of 2–5 μl purified PCR product, 5.2 μl of 12.5% glycerol, 2 μl of 5× sequencing buffer, 2 μl of 10 μM sequencing primer, 1.8 μl of sterile water, and 1 μl of Ready Reaction Mix (containing the dye terminators and AmpliTaq DNA polymerase). After an initial denaturation step of 1 min at 95°C, the following 35 thermal cycles were performed: (1) 15 s at 95°C, (2) 5 s at 45°C, and (3) 4 min at 60°C. All sequencing was done using an ABI (Applied Biosystems) 3730XL high-throughput DNA capillary sequencer at the Genetic Engineering Facility of UIUC's Biotechnology Center. The strategies employed to obtain the ITS sequence data are the same as presented elsewhere (Downie and Katz-Downie, 1996; Downie et al., 2000a). With the exception of two accessions of *Chamarea* that had ITS sequence heterogeneity, all *rps16* intron and ITS sequences acquired in this study were deposited in GenBank (Table 1).

Simultaneous consideration of both DNA strands across all sequenced regions permitted unambiguous base determination in all taxa, with two exceptions. The single accession of *Chamarea longipedicellata* demonstrated evidence of ITS sequence additivity at multiple nucleotide sites, as inferred by

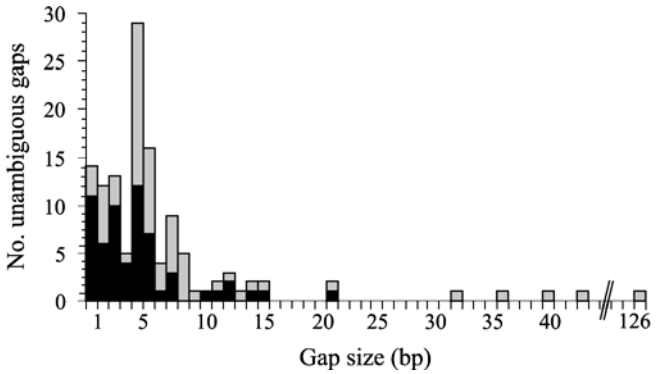

Fig. 1. Frequency of unambiguous gaps in relation to gap size inferred in the alignment of 130 cpDNA *rps16* intron and partial flanking 3′-exon sequences from Apiaceae and Araliaceae. Each bar represents the total number of parsimony informative (solid) and/or uninformative (shaded) gaps, with no overlap between them when they co-occur at the same size interval. Sixty-one gaps, of 1–22 bp in size, were parsimony informative; 64 gaps, of 1–126 bp in size, were uninformative.

overlapping peaks on electropherograms from both forward and reverse sequencing runs. To examine the extent of sequence homogenization among reiterated ITS copies, molecular cloning of this species was conducted. Purified *Taq* polymerase-amplified PCR products were ligated to pCR4-TOPO plasmid vectors and subsequently transformed into chemically competent *Escherichia coli* cells using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, California, USA) following the manufacturer's protocol. Sixty colonies were picked and cultured overnight on selective plates. Five transformed colonies were removed, boiled in 100 μl of sterile water for 15 min, then centrifuged at 4000 rpm for 5 min. To verify the presence of inserts in these transformed colonies, we made a 20-μl PCR cocktail containing 5.0 μl of supernatant, 2.0 μl of 10× *Taq* polymerase reaction buffer, 2 μl of 1.25 mM dNTPs, 1.2 μl of 50 mM $MgCl_2$, 0.5 μl each of 20 μM promoter primers T7 and M13 Reverse, 1 unit of *Taq* polymerase, and approximately 8 μl of sterile water. After an initial denaturation step of 10 min at 95°C, the following PCR cycling program was used: (1) 45 s at 95°C, (2) 60 s at 57°C, and (3) 60 s at 72°C. A 15-min 72°C extension period followed completion of 35 thermal cycles. PCR products were examined on 1% agarose gels for the presence of inserts, and concentrations were estimated by visual comparison with bands containing known amounts of DNA. Three positive transformants were purified using the QIAprep Spin Miniprep Kit (Qiagen) and the manufacturer's instructions, then sequenced using primer T7. One accession of *Chamarea gracillima* (2597) also had ITS sequence heterogeneity at multiple sites, but was not cloned.

Sequence comparisons and phylogenetic analyses—The determination of boundary sequences for the six major structural domains of the cpDNA *rps16* group II intron was based on similar boundary sequences inferred for tobacco, mustard, and other Apiaceae (Michel et al., 1989; Neuhaus et al., 1989; Downie and Katz-Downie, 1999). Both intron and ITS sequences were 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 et al., 1998) and realigned manually as necessary. Gaps were positioned to minimize nucleotide mismatches. In the alignment of *rps16* intron sequences, gaps of equal length in more than one sequence were coded as the same presence or absence character state if they could not be interpreted as different duplication or insertion events. Similarly located but different length indels were coded as multiple binary characters. In several regions, gap coding was particularly problematic because of poly-A's, -G's, or -T's or indirect duplications of adjacent elements in two or more taxa. These gaps were not scored and these ambiguous regions were excluded from subsequent phylogenetic analyses. In the ITS alignments, gaps were not scored as additional binary characters because very few were informative among the ingroup taxa. Uncorrected pairwise nucleotide distances were calculated by PAUP* version 4.0b10 (Swofford, 2002). Sequence characteristics were obtained for each of the six structural domains of the cpDNA *rps16* intron, as well as from the entire intron plus the flanking 3′-exon portion. Similar data were obtained for the three ITS data matrices.

Sequence data from both loci were incomplete for several accessions, and these regions were scored as missing in the analyses. Data for three regions (of 23, 36, and 76 bp) within the *Chamaesium paradoxum rps16* intron could not be obtained despite repeated efforts; moreover, the sequence data we did procure were generally of poor quality, attributable to low DNA concentration. For the two smallest of these regions, data were also unobtainable for *Chamarea gracillima* and *Pseudocarum eminii*. Several additional accessions were missing data (of 5–50 bp) from both ends of the matrix. The *Choritaenia capensis rps16* intron had long stretches of sequence additivity, as inferred by overlapping peaks on the electropherograms. Re-extracting its DNA and repeating the PCR and DNA sequencing did not yield better results; thus this species was excluded from the analysis of intron sequences. In the ITS study, sequence data were unavailable in GenBank for the 5.8S rDNA region for all accessions of tribe Pleurospermeae, the *Komarovia* clade and its allies, and for *Erigenia* and "Oreofraga." This region had little to no variation in the other accessions examined; hence, these missing data did not affect the phylogenetic results. Data for the ITS-2 region could not be obtained for *Glia prolifera* 2511 and *Pseudocarum laxiflorum* 167 despite our repeated but unsuccessful attempts to PCR-amplify this region.

The *rps16* intron and ITS data matrices were analyzed separately using maximum parsimony (MP) as implemented by PAUP*. For the MP analysis of *rps16* intron data (with and without informative gaps scored as binary characters), 1000 heuristic searches were initiated using random addition starting trees with tree-bisection-reconnection (TBR) branch swapping and MulTrees selected, but saving no more than five trees from each search. These trees were subsequently used as starting trees for further TBR branch swapping. The maximum number of saved trees was set to 20 000 and these were permitted to swap to completion. The strict consensus of these 20 000 minimal length trees was then used as a topological constraint in another round of 1000 random addition replicate analyses but, in this case, only those trees that did not fit the constraint tree were saved. No additional trees were found at the length of the initial shortest trees, suggesting that the strict consensus tree adequately summarizes the available evidence, even though the exact number of trees at that length is not known. Bootstrap values (Felsenstein, 1985) were calculated from 500 000 replicate analyses using "fast" stepwise-addition of taxa; only those values compatible with the 50% majority-rule consensus tree were

TABLE 2. Sequence characteristics of the six major structural domains of the cpDNA group II *rps16* intron for 130 accessions of Apiaceae and Araliaceae.

Characteristic	Intron domain					
	I	II	III	IV	V	VI
Length variation (bp)	466–517	70–108	48–78	21–150	34–36	21–79
No. aligned positions	712	172	88	206	36	79
No. positions eliminated	138	63	12	64	0	1
No. positions not variable	338	47	37	63	30	64
No. positions autapomorphic	72	15	13	22	2	9
No. positions parsimony informative	164	47	26	57	4	5
No. unambiguous alignment gaps	66	25	8	22	1	3
No. unambiguous alignment gaps parsimony informative	29	16	3	11	0	2
Maximum sequence divergence (%)	12.7	25.5	28.0	25.7	13.2	13.6

Fig. 2. Strict consensus of 20 000 minimal length 1148-step trees derived from equally weighted maximum parsimony analysis of 130 cpDNA *rps16* intron and partial flanking 3′-exon sequences plus 61 binary-scored alignment gaps (CIs = 0.6028 and 0.5366, with and without uninformative characters, respectively; RI = 0.8820). Numbers at nodes are bootstrap estimates for 500 000 replicate analyses using "fast" stepwise-addition of taxa; values <50% are not indicated. The names of the major clades are based on previous studies or are newly recognized in this study.

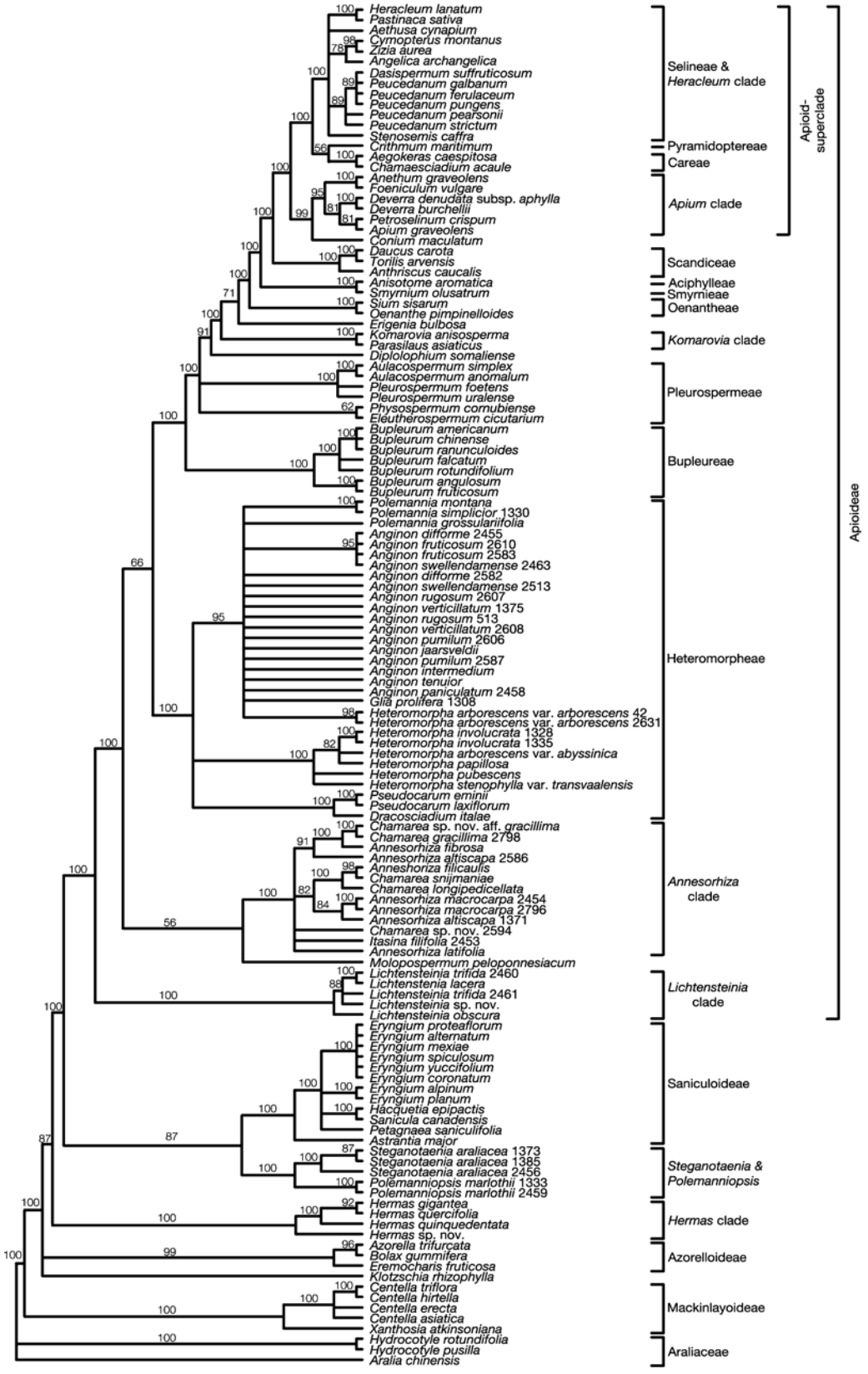

Fig. 3. Fifty-percent majority-rule consensus of 50 320 trees derived from Bayesian analysis of 130 cpDNA *rps16* intron and partial flanking 3′-exon sequences. Numbers at nodes are posterior probability values. The names of the major clades are based on previous studies or are newly recognized in this study.

recorded. For each of the three ITS data sets, heuristic MP searches were replicated 1000 times with random stepwise-addition of taxa, TBR branch swapping, and saving multiple trees. Bootstrap values were calculated from 1000 replicate analyses using TBR branch swapping and simple stepwise-addition of taxa. To examine the extent of conflict between the *rps16* intron and ITS data sets for a comparable set of taxa, the incongruence length difference (ILD) test of Farris et al. (1995) was implemented using the partition homogeneity test of PAUP*. This test was carried out with 1000 replicate analyses, using the heuristic search option with simple addition of taxa and TBR branch swapping. Although serious questions have been raised regarding the value of this test as a criterion for deciding whether data should be combined into a single phylogenetic analysis (e.g., Yoder et al., 2001; Barker and Lutzoni, 2002), it is still a widely used method to assess data heterogeneity and combinability. The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP*.

Bayesian inference of *rps16* intron sequences was conducted using the program MrBayes version 3.0 (Huelsenbeck and Ronquist, 2001). The program was run in parallel on an IBM pSeries 690 system at the National Center for Supercomputing Applications at UIUC. Prior to analysis, the program Modeltest version 3.5 (Posada and Crandall, 1998) was used to select an evolutionary model of nucleotide substitution (among 56 possible models) that best fit these data, as selected by the Akaike Information Criterion estimator (Posada and Buckley, 2004). The settings appropriate for the best-fit TVM+G model were put into a MrBayes block in PAUP* (nst = 6; rates = gamma). The priors on state frequencies and rates and variation across sites (shape of the gamma distribution) were estimated automatically by the program. From a random starting tree, a Bayesian analysis was run for 7 million generations and the trees saved to a file every 100 generations (i.e., 70 000 trees were sampled). Sixteen simultaneous Markov chain Monte Carlo (MCMC) chains were run, and the temperature was adjusted to 0.05 in order to keep an appropriate heat range for the 16 chains. Branch lengths of the trees were saved. Variation in likelihood scores to determine apparent stationarity was examined graphically using the program Tracer version 1.2.1 (A. Rambaut and A. Drummond, University of Oxford, unpublished data). The states of the chain that were sampled before stationarity (i.e., the "burn in" of the chain) were discarded, and the posterior probability values for each bipartition of the phylogeny were determined from the remaining trees. MCMC convergence was also explored graphically using the cumulative option of the program AWTY online (Wilgenbusch et al., 2004), that displays posterior probabilities of splits at selected increments over a MCMC run.

The *rps16* intron data set was also analyzed using the maximum likelihood method as implemented by PAUP* after the program Modeltest was used to select an appropriate model of nucleotide substitution. The results obtained were congruent to those inferred by the Bayesian analysis; hence they will not be discussed further.

Biogeographic analysis—To reconstruct the distribution of the ancestor of subfamily Apioideae, a dispersal–vicariance analysis was carried out with the program DIVA version 1.1 (Ronquist, 1996), using the optimize command and default option settings. We entered the following simplified, fully resolved phylogenetic tree based on the results of the *rps16* intron analysis: (Azorelloideae, (*Hermas* clade, ((*Steganotaenia* & *Polemanniopsis*, Saniculoideae), (*Lichtensteinia* clade, ((*Annesorhiza* clade, *Molopospermum*), (Heteromorpheae, (Bupleureae, (Pleurospermeae, (*Komarovia* clade, (Oenantheae, apioid superclade and allies [the latter representing tribes Scandiceae, Aciphylleae and Smyrnieae])))))))))). With the exceptions of the *Annesorhiza, Lichtensteinia* and *Hermas* clades, which are newly recognized in this study, this tree is also congruent with a summary of relationships within subfamily Apioideae as revealed by phylogenetic analyses of seven molecular data sets (Downie et al., 2001). Because one of our primary goals was to corroborate a southern African origin of subfamily Apioideae, only three unit areas were defined: (a) southern Africa (i.e., Botswana, Lesotho, Namibia, South Africa, and Swaziland), (b) rest of the world, and (c) sub-Saharan Africa (excluding southern Africa). We coded each terminal taxon for its likely ancestral distribution and not for all of the regions in which its members presently occur, because if we had, information important for the optimization of ancestral states would be lost (Ronquist, 1996). In most cases, however, ascertaining the ancestral distribution of a terminal taxon was clear, because it was endemic to one of the defined unit areas. The likely ancestral distribution of the widespread tribe Bupleureae was determined based on a recent phylogenetic study by Neves and Watson (2004), in which a Eurasian (specifically, a western Mediterranean) origin for the group was inferred. Members of tribe Heteromorpheae are distributed primarily in southern Africa, but some genera (i.e., *Heteromorpha, Pseudocarum,* and "Oreofraga") also extend northward to Ethiopia and Yemen (including Socotra) (Winter and Van Wyk, 1996; Van Wyk et al., 1999). Given these distributions and in an effort to determine how each ancestral area affected the reconstruction of the distribution of the ancestor of Apioideae, we ran three different analyses assuming that the ancestor of tribe Heteromorpheae was distributed only in southern Africa, only in sub-Saharan Africa, or in both places.

Evaluating the distribution of the woody habit—Within the independent context of the phylogenies inferred from MP analysis of *rps16* intron sequences, the evolutionary pattern of woodiness was hypothesized within subfamily Apioideae. The character habit, scored as herbaceous or woody for each terminal and designated as unordered, was optimized onto all minimal length trees. The "State Changes & Stasis" chart of MacClade version 4.07 (Maddison and Maddison, 2005) was used to determine the minimal number of times the woody habit had evolved. These reconstructions of character evolution were displayed graphically using the option "Trace Character" in MacClade's tree window.

RESULTS

Comparisons and phylogenetic analyses of cpDNA rps16 intron sequences—Among the 130 sequences obtained, the *rps16* intron varied in length from 758 (*Diplolophium somaliense*) to 908 bp (*Annesorhiza altiscapa*) and averaged 862 bp. Juxtaposed was an additional 17 bp of sequence data from the *rps16* 3′exon that were retained in the analysis because they contained phylogenetic information. Alignment of these intron and flanking exon sequences resulted in a matrix of 1332 positions, of which 278 were excluded from subsequent analyses because of alignment ambiguities. These ambiguous regions ranged from 1 to 43 bp in size. Of the remaining 1054 unambiguously aligned positions, 607 were not variable, 135 were variable but uninformative, and 312 were parsimony informative. Percentage G + C content for the intron ranged from 31.3 to 37.3%, averaging 34.3%. The g1 statistic for 10 000 random trees was −0.354. This value is significantly more skewed (i.e., more negative) than random data (g1 = −0.09 for 250 variable positions for more than 25 taxa; $P < 0.01$), indicating that these sequence data contain significant amounts of phylogenetic signal (Hillis and Huelsenbeck, 1992). A total of 125 unambiguous gaps, ranging between 1 and 126 bp, was required for proper alignment of these sequences; 61 gaps of 1–22 bp in size were parsimony informative. The frequency distribution of both informative and uninformative gaps in relation to their sizes is presented in Fig. 1. Relative to the outgroup *Aralia chinensis* L., these gaps represent a minimum of 84 insertion and 39 deletion events (two gaps could not be polarized because they were unique to *Aralia*). Pairwise sequence divergence estimates ranged from identity to 13.3% of nucleotides [the latter between *Hydrocotyle rotundifolia* Wall. and *Torilis arvensis* (Huds.) Link]. Each of the following groups of taxa yielded identical DNA sequences: two species of *Bupleurum* (*B. chinense* and *B. americanum*); three species of *Eryngium* L. (*E. alternatum* J. M. Coult. & Rose, *E. mexiae* Constance, and *E. yuccifolium* Michx.); four accessions of *Anginon* (*A. difforme* 2455, *A. fruticosum* 2583, 2610, and *A. swellendamense* 2463); two accessions of *Heteromorpha arborescens* var. *arborescens* (42, 2631); nine accessions of *Anginon*, *Glia*, and *Polemannia* (*A. difforme* 2582, *A. intermedium, A. paniculatum, A. pumilum* 2606, *A. rugosum* 513, 2607, *A. verticillatum* 2608, *G. prolifera* 1308, and *P. grossularifolia*); two accessions of

TABLE 3. Sequence characteristics of the three nuclear rDNA ITS data sets (A, tribe Heteromorpheae; B, tribes Pleurospermeae and Erigenieae and the *Komarovia* and *Physospermopsis* clades; C, *Annesorhiza* clade plus allies).

Characteristic	ITS data set		
	A	B	C
No. accessions	39	24	20
Length variation (bp)	540–573	430–448	587–625
No. aligned positions	636	467	635
No. positions eliminated	67	39	38
No. positions not variable	425	170	413
No. positions autapomorphic	75	60	82
No. positions parsimony informative	69	198	102
Maximum sequence divergence (%)	19.5	34.9	18.6

Annesorhiza macrocarpa (2454, 2796); and the accessions *Annesorhiza filicaulis* and *Chamarea snijmaniae*.

For each of the six major structural domains of the cpDNA *rps16* group II intron, the characteristics of the aligned sequences are presented in Table 2. Domain I is the largest, ranging between 466 and 517 bp in size, whereas domains V (34–36 bp) and VI (21–38 bp, excluding *Peucedanum pearsonii*) are the smallest. Domains V and VI are also the most conserved evolutionarily, with few informative positions, low sequence divergence, and very few alignment gaps. The small size of the intron in *Diplolophium somaliense* is due to a 126-bp deletion, representing the almost complete removal of domain IV. The large size of the intron in *Annesorhiza altiscapa* is due to a 33-bp direct repeat in domain I, but in only one of two accessions of this species. In *Peucedanum pearsonii*, domain VI is 79 bp in size, resulting from the direct repetition of 44 bp of adjacent sequence, some of which is from domain V.

MP analysis of 1054 unambiguously aligned *rps16* intron and flanking exon nucleotide positions plus 61 binary-scored informative gaps resulted in the preset maximum tree limit of 20 000 trees, each of 1148 steps (CIs = 0.6028 and 0.5366, with and without uninformative characters, respectively; RI = 0.8820). The strict consensus of these trees (with accompanying bootstrap values) is shown in Fig. 2. Repeating the analysis without the 61 scored gaps also resulted in the preset limit of 20 000 trees, each of 1060 steps (CIs = 0.5953 and 0.5212, with and without uninformative characters, respectively; RI = 0.8760). The topology of this strict consensus tree was identical to that when the gaps were included, with the following exceptions: *Crithmum maritimum* L. comprises a clade with *Aegokeras caespitosa* (Sibth. & Sm.) Raf. and *Chamaesciadium acaule*; the two accessions of *Deverra* form a clade with *Apium graveolens* L. and *Petroselinum crispum* (Mill.) A. W. Hill; the branch leading to *Erigenia bulbosa* (Michx.) Nutt. collapses; less resolution is obtained within *Heteromorpha*, *Polemannia*, and *Anginon*, as well as within *Annesorhiza* and *Chamarea*; and *Hermas* is resolved as sister taxon to the major

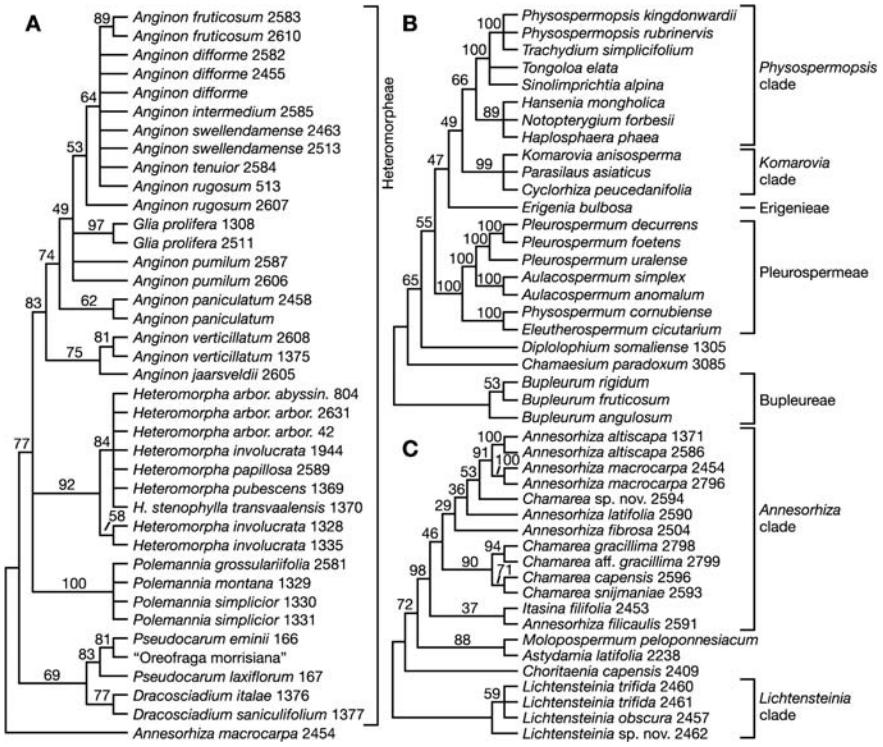

Fig. 4. Strict consensus trees derived from MP analyses of nuclear rDNA ITS sequences of partitioned taxa: Tree A, tribe Heteromorpheae (no. of MP trees = 4; length of MP trees = 203 steps; CI without uninformative characters = 0.7438; RI = 0.9034); tree B, tribes Pleurospermeae and Erigenieae and the *Komarovia* and *Physospermopsis* clades (no. of MP trees = 9; length of MP trees = 661 steps; CI without uninformative characters = 0.5584; RI = 0.7372); tree C, *Annesorhiza* clade plus allies (no. of MP trees = 3; length of MP trees = 329 steps; CI without uninformative characters = 0.6752; RI = 0.7778). Bootstrap values are indicated on branches and were calculated from 1000 replicate analyses. The taxa for which ITS sequences were obtained in this investigation can be identified by their corresponding DNA accession numbers; those without numbers were obtained directly from GenBank. Complete taxon names, including the ranks of infraspecific taxa, are in Table 1.

clade of *Steganotaenia*/*Polemanniopsis*, Saniculoideae and Apioideae. Overall, the inclusion of gap characters in the analysis increased resolution in some portions of the tree (particularly within *Heteromorpha, Anginon, Annesorhiza*, and *Chamarea*), but also decreased it in other portions of the tree (i.e., the phylogenetic position of *Hermas*).

In the Bayesian analysis, the trace plot calculated by the program Tracer showed two apparent stationarity phases, as inferred by a bimodal distribution of the likelihood values: one between generations 90 000 and 1 340 000; the other, with slightly higher likelihood values, beginning from about the 1 968 000th generation. The cumulative graphic produced by the program AWTY online showed that the posterior probabilities of the splits during the first apparent stationarity period are not stabilized, meaning that the MCMC analysis had not converged onto the stationarity distribution during this period. On the contrary, the posteriors do stabilize during the second phase, showing that tree topologies are finally being sampled in proportion to their posterior distribution and that the chains actually reached stationarity by the 1 968 000th generation. Given these results, the first 19 680 trees were discarded as the "burn in" and a 50% majority-rule consensus tree was calculated based upon the remaining 50 320 trees (Fig. 3). The consensus tree from the first stationarity period showed almost the same topology as the one based on the second stationarity period. The only difference between them was found within Saniculoideae. *Eryngium* formed a monophyletic group in the first stationarity period while this alliance collapsed in subsequent generations.

The phylogenies estimated using MP, Bayesian, and maximum likelihood analyses of *rps16* intron sequences are highly consistent with one another. Twenty-two major tribes and clades identified in previous studies (Downie et al., 2001; Plunkett et al., 2004), as well as in this study, are bracketed in Figs. 2 and 3. These include Apioideae, the apioid superclade (comprising tribes Careae, Pyramidoptereae, and Selineae, and the *Apium* and *Heracleum* clades), Scandiceae, Aciphylleae, Smyrnieae, Oenantheae, the *Komarovia* clade, Pleurospermeae (constituting two unresolved lineages in these trees, but monophyletic in previous studies), Bupleureae, Heteromorpheae, *Annesorhiza* clade, *Lichtensteinia* clade, Saniculoideae, *Steganotaenia* + *Polemanniopsis*, *Hermas* clade, Azorelloideae, Mackinlayoideae, and the outgroup Araliaceae. Many of these clades are well supported, with high bootstrap and posterior probability values. The *Annesorhiza, Lichtensteinia,* and *Hermas* clades are newly recognized. The *Annesorhiza* clade comprises *Annesorhiza, Chamarea,* and *Itasina*. Within this clade, pairwise sequence divergence estimates across the three genera range from identity to 1.7%. The *Lichtensteinia* clade is monogeneric. The *Annesorhiza* and *Lichtensteinia* clades are successively basal to tribe Heteromorpheae and represent the earliest known branching lineages within subfamily Apioideae. The *Hermas* clade is also monogeneric and, in the Bayesian tree and the MP strict consensus tree without scored gap characters, arises as a weakly supported sister group to a clade comprised of Saniculoideae, *Steganotaenia*/*Polemanniopsis,* and Apioideae.

The phylogenies inferred from *rps16* intron sequence data help to clarify the phylogenetic placements of several taxa. *Pseudocarum* spp. allies with *Dracosciadium*, which is included along with *Anginon, Glia, Heteromorpha,* and *Polemannia* in tribe Heteromorpheae. *Molopospermum* is sister group to the *Annesorhiza* clade, but this relationship is not very strongly supported (59% bootstrap, 56% posterior probability). The five shrubby and endemic South African species of *Peucedanum* are closely related, with 0.1–1% pairwise sequence divergence among them, and in the Bayesian tree they form a clade with the South African genus *Dasispermum*. *Chamaesciadium acaule*, from the Caucasus, allies strongly with *Aegokeras caespitosa* from Turkey (95% bootstrap, 100% posterior probability) and is a new addition to tribe Careae. *Deverra* occurs in the *Apium* clade of the apioid superclade. The genera *Steganotaenia* and *Polemanniopsis*, treated traditionally in subfamily Apioideae, unite as a well-supported clade that is sister group to subfamily Saniculoideae. This sister group relationship, however, is supported weakly, with 58% and 87% bootstrap and posterior probability values, respectively. Constraining *Steganotaenia, Polemanniopsis,* and all accessions of subfamily Apioideae to monophyly in a subsequent MP search resulted in trees just one step longer than those without the constraint. In these suboptimal trees, *Steganotaenia*/*Polemanniopsis* is sister group to the clade comprised of *Heracleum* through *Lichtensteinia*.

The phylogenetic position of *Chamaesium paradoxum* remains unclear. Sequence data were only available for about 80% of its intron and much of it was of poor quality. The results of an initial MP analysis placed *C. paradoxum* on one branch of a four-branched polytomy in a strict consensus tree, along with tribe Heteromorpheae, the *Annesorhiza* clade plus *Molopospermum*, and the large, distal clade comprised of *Heracleum* through *Bupleurum*. In the majority-rule consensus tree, *C. paradoxum* is sister group to the *Heracleum* through *Bupleurum* clade. While these results indicate that *Chamaesium paradoxum* has allies among the basal apioids, its phylogenetic position is far from certain. Moreover, the poor quality of these data precluded their consideration in the final analysis of *rps16* intron sequences.

Nuclear rDNA ITS sequence comparisons and phylogenetic analyses—*Chamarea longipedicellata* and one accession of *C. gracillima* (2597) demonstrated evidence of ITS sequence additivity at multiple nucleotide sites. Molecular cloning of the single accession of *Chamarea longipedicellata* revealed intra-individual ITS polymorphisms. Three sequence types were identified from the ITS-1 region, differing from each other by 7–18 nucleotide substitutions. Gene trees inferred by combining these three sequences with data obtained from direct sequencing of PCR products from other members of the *Annesorhiza* clade revealed the two most similar clones of *C. longipedicellata* comprising a clade with *C. snijmaniae*, a result in accordance with that inferred by phylogenetic analysis of *rps16* intron sequences. The third clone allied with the two accessions of *Annesorhiza altiscapa*, differing from them by four nucleotide substitutions. These intra-individual paralogous ITS sequences plus the sequences obtained from direct sequencing of *C. longipedicellata* and *C. gracillima* (2597) were not included in subsequent analyses because they would mislead phylogenetic inferences.

Details of the alignments of the three ITS data sets of putatively related taxa are presented in Table 3. For each matrix, less than 11% of all positions were excluded because of alignment ambiguities. The first matrix comprised members previously attributable to tribe Heteromorpheae and, as a result of the *rps16* intron analyses, two species of *Pseudocarum*. We also included the genus "Oreofraga" based on sequence similarity. When compared with any other member of this

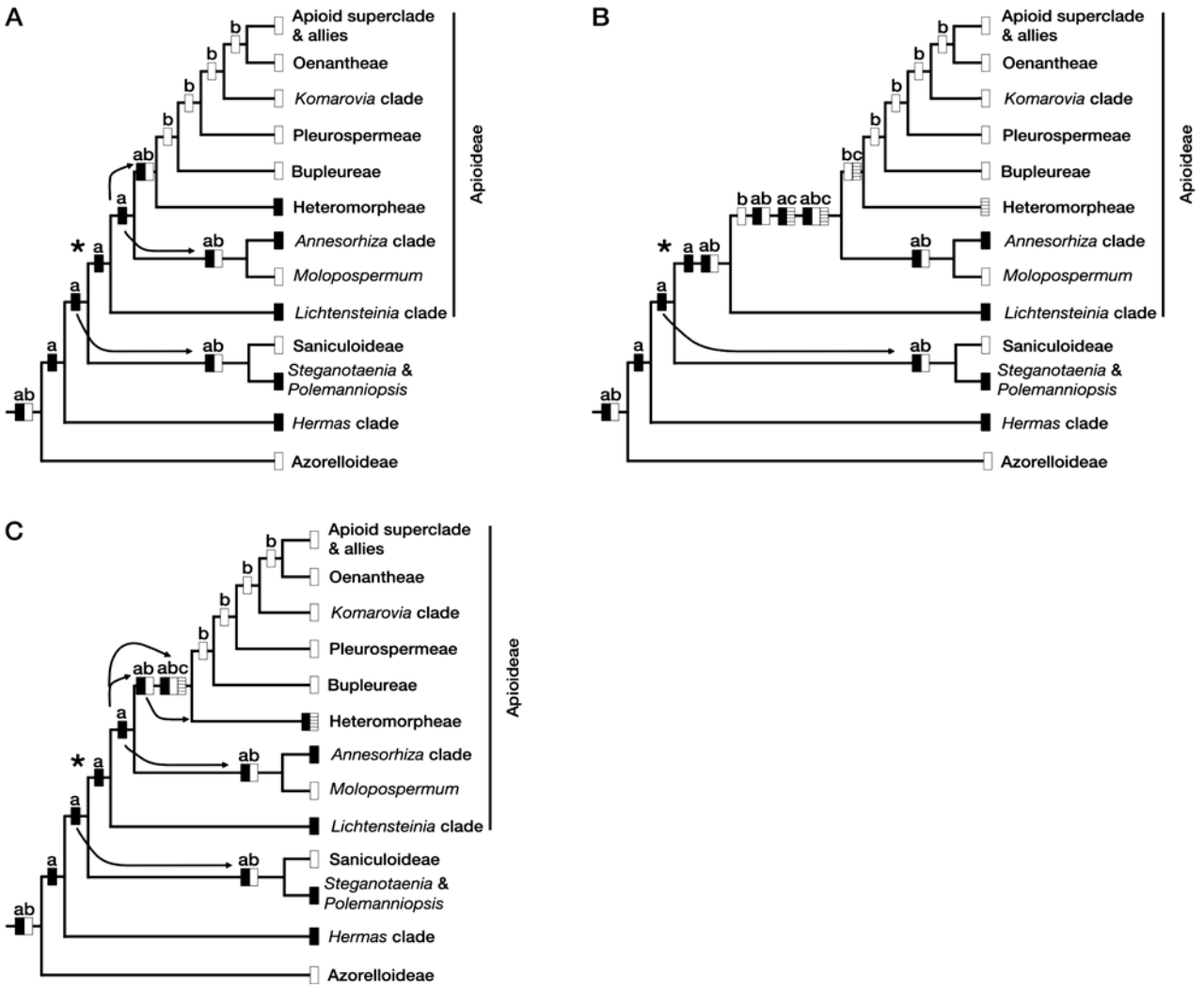

Fig. 5. Optimal reconstructions of the ancestral distributions of Apioideae using dispersal–vicariance analysis assuming that the ancestor of Heteromorpheae was distributed in (A) southern Africa only, (B) sub-Saharan Africa only, or (C) both places. At each node, the optimal distribution prior to vicariance is given; alternative and equally optimal distributions are separated with a space. The historical biogeography of the deepest splits within Apioideae was analyzed in terms of three main areas: southern Africa ("a" and solid bars); rest of the world ("b" and open bars); and sub-Saharan Africa ("c" and striped bars). An asterisk indicates the origin of subfamily Apioideae. Arrows represent dispersal events for one alternative reconstruction in each tree. Dispersal events are not indicated in Fig. 5B because of the complexity of the different scenarios.

group, "Oreofraga" has a sequence divergence value of between 7.4% and 12.9% and is readily aligned. The second matrix included *Erigenia bulbosa* and those accessions of tribe Pleurospermeae and the *Komarovia* clade currently available in GenBank. We also included several Sino-Himalayan and other Asian species of Apioideae having a close affinity to taxa of the *Komarovia* clade (Valiejo-Roman et al., 2002a). *Diplolophium somaliense* was included in this group, because it fell between the aforementioned clades in the *rps16* intron trees, and so was *Chamaesium paradoxum*, because a possible affinity to tribes Bupleureae and Heteromorpheae was established in the *rps16* intron analysis. Pairwise divergence estimates between *C. paradoxum* and the outgroup *Bupleurum* ranged from 5.8 to 7.5%; when *C. paradoxum* was compared to any other member of this matrix, divergence values ranged from 20.8 to 27.2%. Sequence divergence estimates between any ingroup member (other than *C. paradoxum*) and *Bupleurum* were high, with values ranging from 27.2 to 34.9%. A close relationship between *C. paradoxum* and *Annesorhiza* was also established in the intron analysis, but aligning *C. paradoxum* with any member of the *Annesorhiza* clade proved difficult, resulting in several long, ambiguously aligned regions. We acknowledge that the phylogenetic placement of *C. paradoxum* is unclear and that its inclusion in the second matrix is simply based on the similarity of its ITS sequence with those of *Bupleurum*. The third matrix included all representatives of the *Annesorhiza* clade plus *Molopospermum, Astydamia,* and *Choritaenia*. *Molopospermum* is a weakly supported sister taxon to the *Annesorhiza* clade in the *rps16* intron trees, and the *Astydamia* ITS sequence was similar to those of *Annesorhiza, Chamarea,* and *Molopospermum,* with up to 11.4% maximum nucleotide divergence in pairwise comparisons. Across all ITS sequences, *Choritaenia* was most similar to others of this group; here, pairwise comparisons ranged from 14.3–18.6% (the latter between *Choritaenia* and *Molopospermum*).

The strict consensus trees resulting from MP analyses of the three ITS data sets are presented in Fig. 4. The results of the first analysis (Fig. 4A) indicate that *Glia prolifera* arises from within a paraphyletic *Anginon* and that *Heteromorpha* and *Polemannia* are each monophyletic. "Oreofraga" is placed within a paraphyletic *Pseudocarum*, and this clade is sister group (albeit with weak branch support) to *Dracosciadium*. The results of the second analysis (Fig. 4B) maintain tribe Pleurospermeae (*Aulacospermum, Eleutherospermum, Physospermum*, and *Pleurospermum*) as monophyletic (100% bootstrap). The Chinese genera *Physospermopsis, Trachydium, Tongoloa, Sinolimprichtia, Notopterygium,* and *Haplosphaera* unite with north and central Asian *Hansenia* in a clade (66% bootstrap), a group we refer to provisionally as the *Physospermopsis* clade. This clade is a weakly supported sister group to a redefined *Komarovia* clade (i.e., *Komarovia, Parasilaus,* and *Cyclorhiza*). The *Physospermopsis* and *Komarovia* clades comprise a sister group to the North American endemic species *Erigenia bulbosa*. *Diplolophium* and *Chamaesium* arise as successively basal sister groups to all aforementioned clades. The results of phylogenetic analysis of the third data set (Fig. 4C) reveal that the genera *Annesorhiza, Chamarea,* and *Itasina* form a strongly supported clade (98% bootstrap). Pairwise sequence divergence values across these three genera range from identity to 5.2% of nucleotides. Neither *Annesorhiza* nor *Chamarea* are monophyletic. *Annesorhiza filicaulis* allies weakly with *Itasina filifolia*. *Molopospermum* is sister group to *Astydamia* (88% bootstrap). *Lichtensteinia* is monophyletic, and *Choritaenia capensis* is a weakly supported sister group to all other taxa except for the outgroup *Lichtensteinia*.

Comparison of rps16 intron and ITS phylogenies—In the plastid-derived trees, resolution of relationships within tribe Heteromorpheae is poor and internal branch support is weak, as a result of too few informative characters. These trees reveal that the genus *Heteromorpha* is not monophyletic, with the two accessions of *H. arborescens* var. *arborescens* comprising a clade with *Polemannia* in the MP strict consensus tree (Fig. 2) or forming a separate lineage in the Bayesian tree off a large polytomy with *Anginon, Glia,* and *Polemannia* (Fig. 3). All other accessions of *Heteromorpha* comprise a well-supported monophyletic group. *Heteromorpha* is supported as monophyletic, however, in trees just three steps longer than those most parsimonious; thus its monophyly cannot be excluded fully based on these plastid data. Accordingly, the results of the ITS analysis (Fig. 4A) support the monophyly of *Heteromorpha* (92% bootstrap), as well as that of *Anginon* (including *Glia;* with 83% bootstrap) and *Polemannia* (100% bootstrap). The results of a partition homogeneity test for those 31 accessions of tribe Heteromorpheae common to both *rps16* intron and ITS data sets revealed that these two loci do not yield significantly different phylogenetic estimates (ILD probability value = 0.201) and, thus, can be combined for a "total evidence" analysis. MP analysis of combined plastid and nuclear data (using *Annesorhiza macrocarpa* 2454 to root the trees) revealed a strict consensus tree (not shown) with a topology similar to that represented by ITS data alone (Fig. 4A), with the exception that the two accessions of *Heteromorpha arborescens* var. *arborescens* are now a sister group to a clade comprising their congeners (no. of MP trees = 10; length = 265 steps; CI = 0.6953, excluding uninformative characters; RI = 0.8704). The combined analysis supports strongly (96% bootstrap) the monophyly of *Heteromorpha*.

In the *rps16* intron trees (Figs. 2 and 3), members of the *Annesorhiza* clade comprise a polytomy, with its largest resolved branches intermixing accessions of *Annesorhiza* and *Chamarea*. *Annesorhiza filicaulis* and *Chamarea snijmaniae* possess identical *rps16* intron sequences and are sister species; this clade is sister group to *C. longipedicellata*. A similar scrambling of taxa occurs in the ITS strict consensus tree (Fig. 4C), but with *Annesorhiza filicaulis* now a weakly supported sister group to *Itasina filifolia*. *Chamarea snijmaniae* unites with three other *Chamarea* species in a strongly supported clade and not with *A. filicaulis*, as it does in the *rps16* intron tree. Results of a partition homogeneity test on a set of 17 accessions common to both data sets (i.e., 12 members of the *Annesorhiza* clade, *Molopospermum*, and four accessions of *Lichtensteinia*) revealed that these matrices yield significantly different phylogenetic estimates (ILD probability value = 0.001). As a result of this significant discordance between data sets, these data were not combined for simultaneous analysis. Otherwise, reduced or erroneous resolution with respect to the true organismal phylogeny would result without further evaluation of the source of conflict.

Biogeographic analyses—The results of the three dispersal–vicariance analyses, assuming that the ancestor of tribe Heteromorpheae was distributed only in southern Africa, only in sub-Saharan Africa, or in both places (Fig. 5A–C, respectively), indicated that the likely ancestral distribution of subfamily Apioideae was in southern Africa. Two alternative biogeographic scenarios were obtained for its distribution, however. One scenario is that the ancestor of Apioideae was strictly distributed in southern Africa. The other scenario, suggesting a "rest of the world" distribution including southern Africa, was recovered as one of two alternatives when the ancestor of Heteromorpheae was inferred to have a strictly sub-Saharan African distribution (Fig. 5B). While we defined the unit area as "rest of the world," the distribution of taxa comprising tribes Bupleureae and Pleurospermeae and the *Komarovia* clade is largely Eurasian. In all analyses, the biogeographical reconstructions of the ancestor of Apioideae to the ancestor of the clade comprising the *Apium* superclade and allies through *Bupleurum* vary depending on the likely distribution of the ancestor of Heteromorpheae. Each reconstruction, however, postulates a migration north to Eurasia that would have required 2–3 dispersal events.

Evolution of the woody habit—Optimization of the character habit onto all 20 000 minimal length *rps16* intron trees showed that a herbaceous habit is ancestral in Apioideae and that woodiness has evolved a minimum of four times within the family. The woody habit has evolved independently in tribe Heteromorpheae, the *Steganotaenia*/*Polemanniopsis* clade, *Bupleurum fruticosum* L. (Bupleureae), and in *Peucedanum* of South Africa. The ancestor of tribe Heteromorpheae is equally parsimoniously reconstructed for both character states. *Diplolophium, Deverra,* and *Stenosemis* are described as both herbaceous and shrubby; when these taxa are scored as woody in the optimizations, the minimum number of times this habit evolved independently increased to seven. Because a completed heuristic search of these data was not possible and not all genera/species with a woody habit were included in this investigation, a precise estimate of the number of times

woodiness has evolved in the subfamily cannot be made. Nevertheless, the character is clearly homoplastic within Apioideae, with numerous derivations of woodiness occurring during the evolution of the group.

DISCUSSION

Subfamilies of Apiaceae and their relationships—Four subfamilies are currently recognized in Apiaceae: Apioideae, Saniculoideae, Azorelloideae (= *Azorella* clade of Downie et al., 2000b, 2001), and Mackinlayoideae (Plunkett et al., 2004). Apioideae and the clade of Saniculoideae plus *Steganotaenia*/*Polemanniopsis* comprise monophyletic sister groups, with subfamilies Azorelloideae (*Azorella* Lam., *Bolax* Comm. ex Juss., and *Eremocharis* Phil.) and Mackinlayoideae (*Centella* L. and *Xanthosia* Rudge) comprising successively more basal branching lineages. The traditionally recognized subfamily Hydrocotyloideae (sensu Drude, 1898) is polyphyletic, with some of its members occurring within Apiaceae subfamilies Azorelloideae and Mackinlayoideae, and others (such as *Hydrocotyle*) now included within Araliaceae (Chandler and Plunkett, 2004). The hydrocotyloid genus *Klotzschia* Cham., forming an isolated lineage in the *rps16* intron trees, has been provisionally included in subfamily Azorelloideae (Downie et al., 2001; Plunkett et al., 2004). However, because of its distinctive fruits (Liu, 2004) and its consistent occurrence as a separate lineage in all studies where it is included, *Klotzschia* may very well comprise a monogeneric subfamily, pending examination of its type species *K. brasiliensis* Cham. & Schltdl. The four included accessions of *Hermas*, a genus endemic to the fynbos region of South Africa and heretofore not included in any molecular systematic study, form a strongly supported clade (100% bootstrap and posterior probability). In the Bayesian tree (Fig. 3) and the MP strict consensus tree without scored gap characters (not shown), the *Hermas* clade arises as a weakly supported sister group to Apioideae plus the clade of Saniculoideae and *Steganotaenia*/*Polemanniopsis*. When gaps are included (Fig. 2), the relationships among many of the basal branching lineages of Apiaceae are unresolved. *Hermas* is traditionally placed in the subfamily Hydrocotyloideae but presents several features, such as multiflowered congested umbels and a haploid chromosome number of seven that have been interpreted as characteristic of subfamily Saniculoideae (B. J. de Villiers et al., University of Johannesburg, unpublished data). Further studies of the polyphyletic subfamily Hydrocotyloideae are in order, especially to confirm the phylogenetic placements of *Klotzschia* and *Hermas*. The results obtained to date, however, are compelling in suggesting that upon further investigation, these genera may be treated as new subfamilies of Apiaceae or constitute members of an expanded subfamily Azorelloideae.

Steganotaenia and Polemanniopsis—Our results continue to support the earlier finding that *Steganotaenia* and the monotypic *Polemanniopsis*, arborescent and shrubby plants that were treated previously in subfamily Apioideae (e.g., Pimenov and Leonov, 1993), unite as a well-supported clade (85% bootstrap, 100% posterior probability) sister group to subfamily Saniculoideae (Downie and Katz-Downie, 1999). Each of the new accessions of *Steganotaenia* and *Polemanniopsis* examined yielded almost identical *rps16* intron sequences to their previously sequenced congeners. Additional synapomorphies supporting the union between *Steganotaenia* and *Polemanniopsis* include the presence of large, distinct marginal fruit wings with enormous intrajugal cavities (Liu et al., 2003). Subfamily Saniculoideae (represented here by *Astrantia* L., *Eryngium, Hacquetia* Neck. ex DC., *Petagnaea* Caruel, and *Sanicula* L., and by *Actinolema* Fenzl, *Alepidea,* and *Arctopus* in other studies [Plunkett and Lowry, 2001; Valiejo-Roman et al., 2002b; C. I. Calviño and S. R. Downie, unpublished data]) forms a strongly supported clade on the basis of molecular evidence (100% bootstrap and posterior probability). The monophyly of Saniculoideae is supported by numerous morphological features (Drude, 1898; Van Wyk, 2001; Liu et al., 2003). The placement of *Steganotaenia* and *Polemanniopsis* into an expanded Saniculoideae, as suggested by Downie and Katz-Downie (1999) and implemented by Liu et al. (2003), cannot easily be reconciled on the basis of morphological data, nor does it receive strong support from the molecular analyses presented herein. While these taxa share the complete absence of commissural and vallecular vittae (oil ducts in the commissure and furrows, respectively), these vittae are also absent in *Lichtensteinia* and many hydrocotyloids. We are not aware of any morphological synapomorphy supporting the union of *Steganotaenia*/*Polemanniopsis* with Saniculoideae that does not also include *Lichtensteinia* and members of subfamilies Azorelloideae and Mackinlayoideae. The only evidence clearly justifying the union of *Steganotaenia* and *Polemanniopsis* with Saniculoideae is that of the *rps16* intron; this relationship, however, is only supported weakly, with 58% bootstrap and 87% posterior probability values. Moreover, a close relationship to subfamily Apioideae cannot be completely ruled out, for *Steganotaenia*/*Polemanniopsis* and Apioideae are monophyletic in trees just one step longer than those maximally parsimonious. Further molecular systematic studies on *Steganotaenia*/*Polemanniopsis* are in progress, and the results should illuminate the proper phylogenetic position of these taxa (C. I. Calviño and S. R. Downie, unpublished data).

Lichtensteinia—The five accessions of *Lichtensteinia* comprise a strongly supported monophyletic group in the *rps16* intron trees (94% bootstrap, 100% posterior probability). We recognize this group as the *Lichtensteinia* clade. The affinity of *Lichtensteinia* has long been problematic because it shares features common to both subfamilies Saniculoideae and Apioideae (Burtt, 1991; Van Wyk, 2001). Emphasizing its large compound umbels and compound leaves, the genus was placed in Apioideae (Drude, 1898; Wolff, 1910; Pimenov and Leonov, 1993). By focusing attention on its fruits instead, the genus was included in Saniculoideae (Koso-Poljansky, 1916; Liu et al., 2003). The fruits of *Lichtensteinia* are characterized by prominent intrajugal vittae (oil ducts in the ribs) and the absence of commissural and vallecular vittae. These same features also occur in subfamily Saniculoideae, the genera *Steganotaenia* and *Polemanniopsis*, and in the hydrocotyloid lineages (Wolff, 1913; Tseng, 1967; Liu et al., 2003). In contrast, almost all other genera in Apioideae have large commissural and vallecular vittae and only some have small intrajugal secretory ducts (Drude, 1898; Liu et al., 2003). The presence of large intrajugal secretary ducts and the lack of both commissural and vallecular vittae among basal members of Apiaceae represent plesiomorphic character states, and the inclusion of *Lichtensteinia, Steganotaenia* and *Polemanniopsis* within an expanded subfamily Saniculoideae, as suggested by Liu et al. (2003), is based on these symplesiomorphies. Further

studies are necessary to corroborate the results obtained here, in which *Lichtensteinia* is sister group to a clade comprising all other taxa of subfamily Apioideae. If this relationship is maintained, a new monotypic tribe of Apioideae will be warranted. Such confirmation of the phylogenetic position of *Lichtensteinia* is also important to enable hypotheses on character state evolution within the subfamily.

Annesorhiza clade—The *Annesorhiza* clade includes *Annesorhiza*, *Chamarea,* and *Itasina* and is well supported in analyses of both chloroplast and nuclear DNA sequences (98–100% bootstrap, 100% posterior probability). These genera are deciduous, perennial herbs endemic to southern Africa. *Annesorhiza* and *Chamarea* have been treated previously as sister taxa (Tilney and Van Wyk, 2001); they are highly similar morphologically, sharing features such as expanded and lignified vascular bundles in the fruit walls, hysteranthous leaves (i.e., leaves that are formed only after flowering and fruiting), heteromorphic fruits in some species, and fleshy pencil-like or tuberous roots that are replaced periodically (Van Wyk and Tilney, 1994; Tilney and Van Wyk, 2001; Liu, 2004). *Annesorhiza* and *Chamarea* differ in their overall size and the shape and relative length of their fruits, with *Annesorhiza* usually having larger leaves, taller scapes, and oblong-shaped fruits slightly longer than the rounded or flask-shaped fruits typical of *Chamarea* (Tilney and Van Wyk, 2001). In practice, however, these characters overlap, and the boundaries between these genera are unsatisfactory. Cladistic analysis of 29 anatomical and morphological characters from leaves, fruits, and roots revealed that the concept of *Annesorhiza* should be broadened to include all known species of *Annesorhiza* and *Chamarea* (Vessio, 2001). This finding is supported by molecular evidence in which these two genera do not comprise monophyletic sister groups. Instead, the three major clades resolved in the ITS strict consensus tree (Fig. 4C) correspond to the three sections of *Annesorhiza* recognized by Vessio (2001), with the only difference being the inclusion of *Chamarea* sp. nov. 2594 (=*Annesorhiza elsiae* Vessio, Tilney & B-E. van Wyk) alongside members of sect. *Annesorhiza* in our study rather than with *A. filicaulis* in sect. *Ternata* in the treatment by Vessio (2001).

Annesorhiza filicaulis was recently transferred into *Peucedanum* (as *Peucedanum filicaule* (Eckl. & Zeyh.) Van Wyk and Tilney) because its fruits have a wing configuration quite unlike that of any other species of *Annesorhiza* (Van Wyk and Tilney, 2001). In *Annesorhiza*, the four commissural ribs are somewhat larger than the other ribs, and this asymmetrical development is taken to an extreme in *A. filicaulis* in which they are expanded to form large wings, such as those typical of the genus *Peucedanum* (Tilney and Van Wyk, 2001; Van Wyk and Tilney, 2001). This transfer, however, was done so with some uncertainty, for its retention in *Peucedanum* would depend ultimately upon a revision of *Peucedanum* and related genera in southern Africa and elsewhere (Van Wyk and Tilney, 2001). The cpDNA-derived phylogenies show that *A. filicaulis* is sister group to *Chamarea snijmaniae*, with this clade in turn sister group to *C. longipedicellata*. These same trees also indicate that *A. filicaulis* is quite distantly related to the five South African accessions currently treated in *Peucedanum*, suggesting that the transfer of *A. filicaulis* into *Peucedanum* was premature. Vessio (2001) concurred that *A. filicaulis* should be maintained alongside *Annesorhiza* and *Chamarea* in an expanded *Annesorhiza*.

The relationship between *Annesorhiza* (12 species; Tilney and Van Wyk, 2001) and *Chamarea* (~5 species; Van Wyk, 2000) is complex. Considering their similar morphologies and the significant discordance between the plastid- and nuclear-derived data sets, past hybridization/introgression or polyploidization seems possible, and further study of these taxa is warranted. Other than a chromosome count of $N = 12$ for *A. macrocarpa* (Burtt, 1991), the chromosome numbers are not known for any other member of the *Annesorhiza* clade, and they would be important to know to confirm polyploidy, if it exists. The presence of additive patterns of bands in the ITS electropherograms of two species of *Chamarea* may be associated with allopolyploidy and would be consistent with the retention of multiple rDNA loci from different parental ancestors at the time of speciation (Soltis et al., 1992). Although no interspecific hybridization has been reported for the largely sympatric *Annesorhiza* and *Chamarea*, hypotheses of relationships based on morphology show considerable incongruence in the data, with many species not very well separated (Tilney and Van Wyk, 2001). The high level of incongruence between cpDNA and nuclear rDNA suggests that hybridization and/or introgression may have been events in the early history of these plants, resulting in the similarities observed today among the species. While the extent of hybridization in the southern Africa flora is poorly known, numerous examples of naturally occurring hybrids have been reported (Goldblatt, 1978; Spies et al., 1987; Takatsu et al., 2001; Roodt and Spies, 2003). Given the diverse environments and recurring climatic changes in southern Africa, coupled with the predominant woody and perennial plants that in general can form interfertile hybrids, hybrid speciation was likely important in the flora of southern Africa.

ITS loci are generally assumed to be homogenized by concerted evolution, but factors such as hybridization, polyploidization, pseudogene formation, and incomplete intra- or interarray homogenization may result in different ITS sequences persisting in a genome (Álvarez and Wendel, 2003), such as those detected here for *Chamarea longipedicellata* and, possibly, *C. gracillima*. Divergent sequence types within an individual could interfere with sequencing and may explain why several species of *Annesorhiza* and *Chamarea* (not included in this study) consistently yielded poor data from direct sequencing. The existence of divergent paralogous ITS sequences has also been reported in other Apiaceae. In *Oreomyrrhis* Endl. (tribe Scandiceae), intraindividual paralogous ITS sequences within each of 10 accessions representing nine species coalesced with the sequence obtained via direct sequencing and did not interfere with the phylogeny reconstruction (Chung et al., 2005). These ITS clones differed from direct sequenced ITS by one to four substitutions over the entire ITS region, with these polymorphisms imperceptible from the electropherogram of direct sequencing. Similarly, ITS sequence heterogeneity was detected in multiple accessions of *Sium medium* Fisch. & C. A. Mey. and *Berula erecta* (Huds.) Coville (tribe Oenantheae), but polymorphisms were restricted to very few sites, and, again, the paralogy did not impede phylogenetic inference (Spalik and Downie, 2006). In *Chamarea longipedicellata*, however, each of the three positive transformants sequenced yielded a different sequence type, with up to 18 nucleotide differences detected in ITS-1. Such highly divergent paralogous sequences complicate phylogenetic reconstruction. While two ITS clones formed a clade with *Chamarea snijmaniae*, a relationship supported in part by the

plastid data, the third clone allied unexpectedly with *Annesorhiza altiscapa*, differing by only four substitutions. We expect that further molecular cloning of *C. longipedicellata* will reveal additional ITS polymorphisms. We also suppose that intraindividual ITS polymorphism within accessions of *Annesorhiza* and *Chamarea* may be widespread, given the difficulties we had in generating data from direct sequencing. Future studies of these taxa must be undertaken with low-copy nuclear genes. These markers are with rare exception not subjected to concerted evolution and may elucidate the possible role of hybridization in the evolution of these taxa and parentage of polyploids (Álvarez and Wendel, 2003; Soltis et al., 2003).

Molopospermum, Astydamia, and Choritaenia—*Molopospermum* is a weakly supported sister group to the *Annesorhiza* clade in the *rps16* intron trees and, on this basis, was considered with *Annesorhiza* and *Chamarea* in the ITS analysis. The taxonomic placement of the monotypic *Molopospermum* has long been controversial (reviewed in Downie et al., 2000a). In a revised classification of subfamily Apioideae (Downie et al., 2001), *Molopospermum* was provisionally included in tribe Pleurospermeae based on the serological studies of Shneyer et al. (1992) who found that *Molopospermum* and the "*Physospermum-Pleurospermum* alliance" reported therein were serologically distinct and most distant from all other Apioideae investigated. An earlier ITS study supported the affinity between *Molopospermum* and *Physospermum*, but sampling of putatively allied taxa was minimal, with only *Physospermum* and *Komarovia* considered (Downie et al., 2000a). The *rps16* intron trees support a position for the European *Molopospermum* heretofore unconsidered in any previous study, as sister species to a clade comprising the southern African genera *Annesorhiza, Chamarea*, and *Itasina*. Fruit structural features, such as the presence of lateral wings on one of the two mericarps and an abundance of crystals in the mesocarp, also support this relationship (Liu, 2004). The results of the ITS analysis reveal another surprise, that is, the sister group relationship between *Molopospermum* and the Canary Islands endemic genus *Astydamia*. A comparison of the fruits of these two genera is currently underway. *Choritaenia* is a monotypic genus endemic to southern Africa. It has been treated previously in either subfamilies Hydrocotyloideae (Pimenov and Leonov, 1993) or Apioideae (Bentham, 1867; Van Wyk, 2000), and its inclusion in the same matrix as *Annesorhiza* and allies was based on the similarity of its ITS sequence with these taxa. However, the placement of *Choritaenia* as sister group to the *Annesorhiza* clade plus *Molopospermum* + *Astydamia* may be the result of the relatively small sampling of taxa in this matrix, and its phylogenetic position needs confirmation through analysis of cpDNA data. Unfortunately, the difficulty in obtaining quality sequences from the *Choritaenia rps16* intron despite repeated efforts with direct sequencing of PCR products suggests that molecular cloning will be necessary to obtain these data.

Tribe Heteromorpheae—The previously delimited *Heteromorpha* clade (Downie et al., 1998) was recognized as a tribe by Downie et al. (2000b) to include the endemic African genera *Anginon, Dracosciadium, Glia, Heteromorpha*, and *Polemannia*. The tribe is of evolutionary interest because of its putative sister group relationship to all other examined Apioideae in earlier studies, the predominantly woody habit of its members, and its largely southern African distribution. Downie and Katz-Downie (1999) reported that the genus *Heteromorpha* was not monophyletic, a surprising find given its unusual heteromorphic winged mericarps derived from the expansion of all five sepaline ribs; this type of wing development was described previously as an apomorphy of the genus (Winter et al., 1993; Winter and Van Wyk, 1996). Furthermore, in the study by Downie and Katz-Downie (1999), the relationships among *Anginon, Glia*, *Polemannia*, and *Heteromorpha arborescens* var. *arborescens* could not be resolved because of a lack of informative characters.

With a three-fold increase in sampling from previous studies and additional data from the ITS region, we revise the circumscription of tribe Heteromorpheae to comprise *Anginon*, *Dracosciadium, Glia, Heteromorpha,* "Oreofraga," *Polemannia*, and *Pseudocarum*. We also include the endemic Madagascan genera *Andriana* B-E. van Wyk, *Anisopoda* Baker, *Cannaboides* B-E. van Wyk, *Pseudocannaboides* B-E. van Wyk, and *Tana* B-E. van Wyk. *Anginon*, *Heteromorpha*, and *Polemannia* are each resolved as monophyletic based on analyses of ITS or combined ITS and cpDNA data. We recommend that the concept of *Anginon* be expanded to include *Glia* because *G. prolifera* is the type of the genus and is placed well within *Anginon*, but such a change must await a generic revision of *Glia* currently in preparation (B-E. van Wyk and P. Tilney, unpublished manuscript). *Anginon* and *Glia* share heavily thickened and cutinized outer walls of the fruit epidermal cells and have been previously considered monophyletic sister taxa (Van Wyk et al., 1997). Intrageneric relationships in *Anginon* and *Heteromorpha* are generally unresolved or poorly supported, and it is apparent that more rapidly evolving markers are necessary to resolve relationships within these presumably recently radiated lineages. The eastern African and Madagascan genus *Pseudocarum* is paraphyletic, thus future studies of this genus should include "Oreofraga" and other Socotran endemics. We also include in the tribe the genera *Andriana*, *Cannaboides*, *Pseudocannaboides*, and *Tana* that at one time were considered in *Heteromorpha* (Humbert, 1956) but are now each regarded as distinct and closely related to *Pseudocarum* (Winter and Van Wyk, 1996; Van Wyk et al., 1999). These genera share a similar fruit structure (Liu, 2004), and cladistic analysis of 15 morphological characters indicates a close relationship between them and *Heteromorpha* (Van Wyk, 2001). Furthermore, our ongoing molecular studies of African Apiaceae, while still preliminary, indicate that these Madagascan genera together with *Pseudocarum*, "Oreofraga," *Dracosciadium*, and *Anisopoda bupleuroides* Baker comprise a well-supported clade that is sister group to the clade of *Heteromorpha, Anginon, Glia*, and *Polemannia*.

Tribes Pleurospermeae and Erigenieae and clades Komarovia and Physospermopsis—Tribe Pleurospermeae (*Aulacospermum, Eleutherospermum, Physospermum*, and *Pleurospermum*) is maintained as a strongly supported and well-resolved monophyletic group in the ITS study (Fig. 4B). In the *rps16* intron trees (Figs. 2 and 3), these genera comprise two lineages whose relationship is unresolved, a result attributable to too few supporting characters. The tribe is monophyletic, however, as revealed by phylogenetic analyses of a variety of data sets (Downie et al., 2000b, 2001). Valiejo-Roman et al. (2002a) also include *Hymenidium foetens* (Franchet) Pimenov & Kljuykov, a species usually treated in *Pleurospermum* (Pan and Watson, 2005), alongside members of tribe Pleurospermeae. The *Komarovia* clade (circumscribed

previously to include *Komarovia* and *Parasilaus* and tentatively *Hansenia* and *Physospermopsis*; Katz-Downie et al., 1999; Downie et al., 2000b, c, 2001) is redefined in light of the current study to comprise only *Komarovia, Parasilaus,* and *Cyclorhiza*. This clade received strong bootstrap support. Sister group to the *Komarovia* clade is a moderately supported clade (66% bootstrap) comprising *Physospermopsis, Trachydium, Tongoloa, Sinolimprichtia, Hansenia, Notopterygium*, and *Haplosphaera*. This group encompasses many plants from the Sino-Himalayan region of southwestern China and includes some genera whose taxonomic limits are not at all clear, such as *Physospermopsis, Trachydium*, and *Tongoloa* (Valiejo-Roman et al., 2002a; She et al., 2005). We refer to this group provisionally as the *Physospermopsis* clade.

The North American monotypic genus *Erigenia* is sister group to the *Komarovia* and *Physospermopsis* clades in the ITS trees, and in the *rps16* intron trees, it is a sister group to the *Heracleum* through *Oenanthe* clade, adjacent to the *Komarovia* clade. In all molecular phylogenies to date where *Erigenia* is included (e.g., Downie and Katz-Downie, 1999; Katz-Downie et al., 1999; Downie et al., 2000b, 2002), the genus constitutes an isolated lineage. Such phylogenies include those resulting from analyses of all North American umbellifers (S. R. Downie, unpublished data). We recognize *Erigenia bulbosa* as constituting the monotypic tribe Erigenieae, as erected by Rydberg (1932).

Stenosemis, Dasispermum, Deverra, and Peucedanum—The southern African endemic genus *Stenosemis* was recognized by Sonder (1862) and soon thereafter was reduced to *Annesorhiza* by Bentham (1867). Although Drude (1868) commented that the generic characters of *Stenosemis* were suggestive of his Peucedaneae subtribe Angelicinae and not *Annesorhiza*, he maintained *Stenosemis* as a subgenus of the latter. Burtt (1979, 1991) recognized *Stenosemis* and *Annesorhiza* as distinct genera, and their separation is corroborated in the *rps16* intron trees (Figs. 2 and 3) where *Stenosemis caffra* is placed quite a distance away from the *Annesorhiza* clade. The two South African members of *Deverra* examined fell within the *Apium* clade. This placement is consistent with previous studies (Downie et al., 2000a, c) that show *Deverra triradiata*, from Saudi Arabia, also in the same clade. The genus *Peucedanum* sensu lato, as commonly circumscribed, contains some 100–120 species of largely African and Eurasian distribution (Pimenov and Leonov, 1993); the close relationship between the African and Eurasian species, however, has been challenged (e.g., Ostroumova and Pimenov, 1997a, b). In the Bayesian tree (Fig. 3), the five woody species of South African *Peucedanum* and *Dasispermum suffruticosum* constitute a well-supported monophyletic group. Preliminary molecular results support the idea that the woody southern African species of *Peucedanum* (together with several other African genera, such as *Dasispermum, Sonderina,* and *Stenosemis*) are monophyletic and that this clade is not directly related to the Eurasian *Peucedanum* species (P. Winter et al., University of Johannesburg and South African National Biodiversity Institute, unpublished manuscript). It is supposed that this group of woody southern African peucedanums may represent a new genus subendemic to the Cape Floristic Region.

Southern African origin of Apioideae—All biogeographic reconstructions support a southern African origin of subfamily Apioideae with subsequent migration northward into Eurasia. Whether the ancestors of Apioideae had a more widespread distribution in Africa, however, cannot be ruled out completely. Such a scenario was reconstructed as one of two alternatives (the other being strictly southern) when a sub-Saharan African distribution was assumed for the ancestors of tribe Heteromorpheae. We consider this reconstruction to be the least likely. Many members of tribe Heteromorpheae are distributed exclusively in southern Africa, with only very few species occurring in Ethiopia and Yemen (including Socotra). Moreover, a strictly southern African origin of the subfamily is also supported by a dispersal–vicariance analysis of the results of the Heteromorpheae ITS analysis (Fig. 4A), in which it was similarly revealed that the ancestors of the tribe were likely distributed in southern Africa (data not shown). We suggest the biogeographic hypothesis presented in Fig. 5A is the most likely reconstruction. In this scenario, two dispersal events from the same ancestor within Apioideae are implied, one taking place in the lineage of the *Annesorhiza* clade + *Molopospermum* (plus *Astydamia* in the ITS trees; Fig. 4C) and the other in its sister group (i.e., Heteromorpheae through the apioid superclade and its allies). This means that the subsequent diversification of these clades took place through two dispersal routes: (1) from southern Africa through northwestern Africa to the Canary Islands (where *Astydamia* is endemic) and from there to Europe (*Molopospermum* is endemic to the Pyrenees, Massif Central, and the southern Alps) and (2) from southern Africa through northeastern Africa to Eurasia, whereupon most of the successive lineages of Apioideae diversified. The northward migration of the subfamily from southern Africa to Eurasia via the Middle East and the Caucasus was hypothesized previously by Plunkett et al. (1996a) based on present-day patterns of distribution. The African taxa that occur within the most recently radiated clades (i.e., *Dasispermum, Deverra, Peucedanum*, and *Stenosemis*) likely represent later dispersal events to Africa. It appears that the African continent was the center of origin of the first ancestors of Apioideae that subsequently migrated northward to Eurasia (possibly via two different dispersal routes), but it also received successive lineages that dispersed from other continents back to Africa.

On the origin of the woody habit—Traditionally, the largely herbaceous family Apiaceae has been envisioned as a specialized group derived from the mostly woody family Araliaceae. As a consequence, many workers interpreted the features predominating in Araliaceae as ancestral and those in Apiaceae as derived. Therefore, umbellifers with a woody habit were postulated to be "primitive" within Apiaceae. On the basis of previous molecular systematic studies showing the woody members of subfamily Apioideae endemic to southern Africa as sister group to all other apioid taxa, researchers have suggested that this character-state was potentially plesiomorphic and that the subfamily probably had a woody ancestry (Downie and Katz-Downie, 1996, 1999; Plunkett et al., 1996a; Chandler and Plunkett, 2004). The placement of herbaceous *Lichtensteinia* and *Annesorhiza* among the earliest diverging lineages within Apioideae, however, presents a different hypothesis on the ancestral habit of the subfamily. Optimization of the character habit onto the plastid-derived trees rejects the prevailing hypothesis that the woody habit is plesiomorphic and instead supports an alternative hypothesis that the ancestors of subfamily Apioideae were herbaceous. The latter

is supported even if the ancestors of subfamilies Azorelloideae and Mackinlayoideae, and even those of the *Hermas* clade, were coded as woody. The ancestors of the predominantly woody tribe Heteromorpheae are ambiguously reconstructed, further suggesting that woodiness might be a derived state within the tribe, contrary to what has been assumed generally.

Woodiness (shrubs or small trees) occurs in about 20 genera from all three traditionally recognized subfamilies of Apiaceae, and within Apioideae the woody habit has arisen multiple times from herbaceous ancestors in a diversity of lineages (Rodriguez, 1971; Oskolski, 2001). In our study, such woody taxa include *Heteromorpha* and its allies (Heteromorpheae), South African *Peucedanum*, *Bupleurum fruticosum* (Bupleureae), and *Steganotaenia/Polemanniopsis* (the last two genera form a clade that may be sister group to subfamily Saniculoideae). Other genera, such as *Diplolophium, Deverra,* and *Stenosemis,* have been described as either herbaceous or woody. The endemic plants of southern Africa are not a random assemblage with regard to growth form and other biological attributes (Cowling and Hilton-Taylor, 1997). Shrubs are dominant in the region; this dominance is largely explained by the nutrient-poor soils that favor shrub growth (Goldblatt and Manning, 2002). It is then not surprising that this habit has arisen independently, and multiple times in this environment, within Apiaceae. Investigations of other plants are providing greater insight into the evolution of the woody habit from herbaceous ancestors. Using genomic and molecular tools in *Arabidopsis* Heynh. and *Populus* L., Kirst et al. (2003) reported that secondary growth may appear as a result of modifying the expression of genes already present in herbaceous progenitors. Although this does not discard the possibility of woody growth arising de novo by convergent evolution, the former explanation is more likely in cases where the frequency and rapidity of such events is high, as might be the case within a single lineage.

LITERATURE CITED

ALLISON, I., AND B-E. VAN WYK. 1997. A revision of the genus *Anginon* (Apiaceae). *Nordic Journal of Botany* 17: 561–577.

ÁLVAREZ, I., AND J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

BARKER, F. K., AND F. M. LUTZONI. 2002. The utility of the incongruence length difference test. *Systematic Biology* 51: 625–637.

BENTHAM, G. 1867. Umbelliferae. *In* G. Bentham and J. D. Hooker [eds.], Genera plantarum, vol. 1, 859–931. Lovell Reeve, London, UK.

BURTT, B. L. 1979. Umbelliferae. Notes on some plants of southern Africa chiefly from Natal: VIII. *Notes from the Royal Botanic Garden Edinburgh* 37: 319–325.

BURTT, B. L. 1991. Umbelliferae of southern Africa: an introduction and annotated checklist. *Edinburgh Journal of Botany* 48: 133–282.

CHANDLER, G. T., AND G. M. PLUNKETT. 2004. Evolution in Apiales: nuclear and chloroplast markers together in (almost) perfect harmony. *Botanical Journal of the Linnean Society* 144: 123–147.

CHUNG, K.-F., C.-I. PENG, S. R. DOWNIE, K. SPALIK, AND B. A. SCHAAL. 2005. Molecular systematics of the trans-Pacific alpine genus *Oreomyrrhis* (Apiaceae): phylogenetic affinities and biogeographic implications. *American Journal of Botany* 92: 2054–2071.

COWLING, R. M., AND C. HILTON-TAYLOR. 1997. Phytogeography, flora and endemism. *In* R. M. Cowling, D. M. Richardson, and S. M. Pierce [eds.], Vegetation of southern Africa, 43–61, Cambridge University Press, Cambridge, UK.

DOWNIE, S. R., R. L. HARTMAN, F.-J. SUN, AND D. S. KATZ-DOWNIE. 2002. Polyphyly of the spring-parsleys (*Cymopterus*): molecular and morphological evidence suggests complex relationships among the perennial endemic genera of western North American Apiaceae. *Canadian Journal of Botany* 80: 1295–1324.

DOWNIE, S. R., AND D. S. KATZ-DOWNIE. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 83: 234–251.

DOWNIE, S. R., AND D. S. KATZ-DOWNIE. 1999. Phylogenetic analysis of chloroplast *rps16* intron sequences reveals relationships within the woody southern African Apiaceae subfamily Apioideae. *Canadian Journal of Botany* 77: 1120–1135.

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND K. J. CHO. 1996. Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast *rpoC1* intron. *Molecular Phylogenetics and Evolution* 6: 1–18.

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND K. SPALIK. 2000a. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 87: 76–95.

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND M. F. WATSON. 2000b. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: towards a suprageneric classification of subfamily Apioideae. *American Journal of Botany* 87: 273–292.

DOWNIE, S. R., G. M. PLUNKETT, M. F. WATSON, K. SPALIK, D. S. KATZ-DOWNIE, C. M. VALIEJO-ROMAN, E. I. TERENTIEVA, A. V. TROITSKY, B.-Y. LEE, J. LAHHAM, AND A. EL-OQLAH. 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. *Edinburgh Journal of Botany* 58: 301–330.

DOWNIE, S. R., S. RAMANATH, D. S. KATZ-DOWNIE, AND E. LLANAS. 1998. Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. *American Journal of Botany* 85: 563–591.

DOWNIE, S. R., M. F. WATSON, K. SPALIK, AND D. S. KATZ-DOWNIE. 2000c. Molecular systematics of Old World Apioideae (Apiaceae): relationships among some members of tribe Peucedaneae sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade. *Canadian Journal of Botany* 78: 506–528.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.

DRUDE, C. G. O. 1898. Umbelliferae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, vol. 3, 63–250. Wilhelm Engelmann, Leipzig, Germany.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570–572.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

GOLDBLATT, P. 1978. An analysis of the flora of southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* 65: 369–436.

GOLDBLATT, P., AND J. C. MANNING. 2002. Plant diversity of the Cape region of southern Africa. *Annals of the Missouri Botanical Garden* 89: 281–302.

HARDWAY, T. M., K. SPALIK, M. F. WATSON, D. S. KATZ-DOWNIE, AND S. R. DOWNIE. 2004. Circumscription of Apiaceae tribe Oenantheae. *South African Journal of Botany* 70: 393–406.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189–195.

HOLMGREN, P. K., N. H. HOLMGREN, AND L. C. BARNETT. 1990. Index herbariorum. New York Botanical Garden, New York, New York, USA.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes. Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

HUMBERT, H. 1956. Contributions à l'étude de la flore de Madagascar et des Comores. Fascicule 5. *Notulae Systematicae (Paris)* 15: 113–134.

JEANMOUGIN, F., J. D. THOMPSON, M. GOUY, D. G. HIGGINS, AND T. J. GIBSON. 1998. Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences* 23: 403–405.

KATZ-DOWNIE, D. S., C. M. VALIEJO-ROMAN, E. I. TERENTIEVA, A. V. TROITSKY, M. G. PIMENOV, B. LEE, AND S. R. DOWNIE. 1999. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences. *Plant Systematics and Evolution* 216: 167–195.

KIRST, M., A. F. JOHNSON, C. BAUCOM, E. ULRICH, K. HUBBARD, R. STAGGS, C. PAULE, E. RETZEL, R. WHETTEN, AND R. SEDEROFF. 2003. Apparent homology of expressed genes from wood-forming tissues of loblolly pine (*Pinus taeda* L.) with *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 100: 7383–7388.

KOSO-POLJANSKY, B. M. 1916. Sciadophytorum systematis lineamenta: mantissa prior. *Bulletin de la Société Impériale des Naturalistes (Moscou)* 30: 277–290.

LIU, M. R. 2004. A taxonomic evaluation of fruit structure in the family Apiaceae. Ph.D. dissertation, Rand Africaans University, Auckland Park, South Africa.

LIU, M. R., B-E. VAN WYK, AND P. M. TILNEY. 2003. The taxonomic value of fruit structure in the subfamily Saniculoideae and related African genera (Apiaceae). *Taxon* 52: 261–270.

MADDISON, D. R., AND W. P. MADDISON. 2005. MacClade 4: analysis of phylogeny and character evolution, version 4.07. Sinauer, Sunderland, Massachusetts, USA.

MICHEL, F., K. UMESONO, AND H. OZEKI. 1989. Comparative and functional anatomy of group II catalytic introns: a review. *Gene* 82: 5–30.

NEUHAUS, H., A. SCHOLZ, AND G. LINK. 1989. Structure and expression of a split chloroplast gene from mustard (*Sinapsis alba*): ribosomal protein gene *rps16* reveals unusual transcriptional features and complex RNA maturation. *Current Genetics* 15: 63–70.

NEVES, S. S., AND M. F. WATSON. 2004. Phylogenetic relationships in *Bupleurum* (Apiaceae) based on nuclear ribosomal DNA ITS sequence data. *Annals of Botany* 93: 379–398.

OLMSTEAD, R. G., AND J. D. PALMER. 1994. Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* 81: 1205–1224.

OSKOLSKI, A. A. 2001. Phylogenetic relationships within Apiales: evidence from wood anatomy. *Edinburgh Journal of Botany* 58: 201–206.

OSTROUMOVA, T. A., AND M. G. PIMENOV. 1997a. Carpological diversity of African *Peucedanum* s.l. (Umbelliferae) I. The species of southern Africa. *Feddes Repertorium* 108: 299–318.

OSTROUMOVA, T. A., AND M. G. PIMENOV. 1997b. Carpological diversity in African *Peucedanum* s.l. (Umbelliferae) II. The species of tropical Africa. *Feddes Repertorium* 108: 533–547.

PAN, Z., AND M. F. WATSON. 2005. *Pleurospermum*. *In* Flora of China Editorial Committee [eds.], Flora of China, vol. 14, 40. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

PETERSEN, G., O. SEBERG, AND S. LARSEN. 2002. The phylogenetic and taxonomic position of *Lilaeopsis* (Apiaceae), with notes on the applicability of ITS sequence data for phylogenetic reconstruction. *Australian Systematic Botany* 15: 181–191.

PIMENOV, M. G., AND M. V. LEONOV. 1993. The genera of the Umbelliferae: a nomenclator. Royal Botanic Gardens, Kew, UK.

PLUNKETT, G. M., G. T. CHANDLER, P. P. LOWRY II, S. M. PINNEY, AND T. S. SPRENKLE. 2004. Recent advances in understanding Apiales and a revised classification. *South African Journal of Botany* 70: 371–381.

PLUNKETT, G. M., AND P. P. LOWRY II. 2001. Relationships among "ancient araliads" and their significance for systematics of Apiales. *Molecular Phylogenetics and Evolution* 19: 259–276.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1996a. Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. *Systematic Botany* 21: 477–495.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1996b. Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbcL* sequences. *American Journal of Botany* 83: 499–515.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

RODRÍGUEZ, R. L. 1971. The relationships of the Umbellales. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 63–91. Academic Press, London, U.K.

RONQUIST, F. 1996. DIVA, version 1.1 for MacOS and Win32. Computer program and documentation distributed by the author, website http://www.ebc.uu.se/systzoo/research/diva/diva.html [Accessed 21 June 2005].

ROODT, R., AND J. J. SPIES. 2003. Chromosome studies in the grass subfamily Chloridoideae II. An analysis of polyploidy. *Taxon* 52: 736–746.

RYDBERG, P. A. 1932. Ammiaceae. Flora of the prairies and plains of central North America, 586. New York Botanical Garden, New York, New York, USA.

SHE, M., F. PU, Z. PAN, M. F. WATSON, J. F. M. CANNON, I. HOLMES-SMITH, E. V. KLJUYKOV, L. R. PHILLIPPE, AND M. G. PIMENOV. 2005. Apiaceae. *In* Flora of China Editorial Committee [eds.], Flora of China, vol. 14, 1–205. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

SHNEYER, V. S., G. P. BORSCHTSCHENKO, M. G. PIMENOV, AND M. V. LEONOV. 1992. The tribe Smyrnieae (Umbelliferae) in the light of serotaxonomical analysis. *Plant Systematics and Evolution* 182: 135–148.

SOLTIS, D. E., P. S. SOLTIS, AND J. A. TATE. 2003. Advances in the study of polyploidy since *Plant Speciation*. *New Phytologist* 161: 173–191.

SOLTIS, P. S., J. J. DOYLE, AND D. E. SOLTIS. 1992. Molecular data and polyploid evolution in plants. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, 177–201. Chapman and Hall, New York, New York, USA.

SONDER, W. 1862. Umbelliferae. *In* W. H. Harvey and W. Sonder [eds.] Flora capensis, vol. 2, 524–567. Hodges, Smith and Co., Dublin, Ireland.

SPALIK, K., AND S. R. DOWNIE. 2006. The evolutionary history of *Sium* sensu lato (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. *American Journal of Botany* 93: 747–761.

SPIES, J. J., C. H. STIRTON, AND H. DU PLESSIS. 1987. The genus *Rubus* (Rosaceae) in southern Africa. IV. Natural hybridization. *Bothalia* 17: 105–120.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0 b10. Sinauer, Sunderland, Massachusetts, USA.

SWOFFORD, D. L., AND G. J. OLSEN. 1990. Phylogeny reconstruction. *In* D. M. Hillis and C. Moritz [eds.], Molecular systematics, 411–501. Sinauer Associates, Sunderland, Massachusetts, USA.

TAKATSU, Y., M. MIYAMOTO, E. INOUE, T. YAMADA, T. MANABE, M. KASUMI, M. HAYASHI, F. SAKUMA, W. MARUBASHI, AND M. NIWA. 2001. Interspecific hybridization among wild *Gladiolus* species of southern Africa based on randomly amplified polymorphic DNA markers. *Scientia Horticulturae* 91: 339–348.

TILNEY, P. M., AND B-E. VAN WYK. 2001. A revision of the genus *Annesorhiza* (Apiaceae). *Nordic Journal of Botany* 21: 615–649.

TSENG, C. C. 1967. Anatomical studies of flower and fruit in the Hydrocotyloideae (Umbelliferae). *University of California Publications in Botany* 42: 1–58.

VALIEJO-ROMAN, C. M., V. S. SHNEYER, T. H. SAMIGULLIN, E. I. TERENTIEVA, AND M. G. PIMENOV. 2006. An attempt to clarify taxonomic relationships in "Verwandtschaftskreis der Gattung *Ligusticum*" (Umbelliferae-Apioideae) by molecular analysis. *Plant Systematics and Evolution* 257: 25–43.

VALIEJO-ROMAN, C. M., E. I. TERENTIEVA, T. H. SAMIGULLIN, AND M. G. PIMENOV. 2002a. nrDNA ITS sequences and affinities (Umbelliferae) of Sino-Himalayan Apioideae. *Taxon* 51: 685–701.

VALIEJO-ROMAN, C. M., E. I. TERENTIEVA, T. H. SAMIGULLIN, AND M. G. PIMENOV. 2002b. Relationships among genera in Saniculoideae and selected Apioideae (Umbelliferae) inferred from nrITS sequences. *Taxon* 51: 91–101.

VAN WYK, B-E. 2000. Apiaceae (Umbelliferae). *In* O. A. Leistner [ed.],

Seed plants of southern Africa, 62–71. National Botanical Institute, Pretoria, South Africa.

VAN WYK, B-E. 2001. A preliminary analysis of evolution of African and Madagascan Apiaceae. *Edinburgh Journal of Botany* 58: 291–299.

VAN WYK, B-E., I. ALLISON, AND P. M. TILNEY. 1997. Morphological variation and phylogenetic relationships in the genus *Anginon*. *Nordic Journal of Botany* 17: 511–526.

VAN WYK, B-E., AND P. M. TILNEY. 1994. The taxonomic value of fruit wall structure in the genus *Annesorhiza*. *South African Journal of Botany* 60: 240–244.

VAN WYK, B-E., AND P. M. TILNEY. 2001. The generic position of *Annesorhiza filicaulis* Eckl. & Zeyh. (Apiaceae). *Edinburgh Journal of Botany* 58: 383–387.

VAN WYK, B-E., AND P. M. TILNEY. 2004. Diversity of Apiaceae in Africa. *South African Journal of Botany* 70: 433–445.

VAN WYK, B-E., P. M. TILNEY, AND P. J. D. WINTER. 1999. Four new genera of woody Apiaceae of Madagascar. *Taxon* 48: 737–745.

VESSIO, N. 2001. The generic affinities of deciduous species of the genera *Annesorhiza* Cham. & Schlechtd., *Chamarea* Eckl. & Zeyh. and *Peucedanum* L. (Apiaceae). M.S. thesis, Rand Africaans University, Auckland Park, South Africa.

WILGENBUSCH, J. C., D. L. WARREN, AND D. L. SWOFFORD. 2004. AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Website http://ceb.csit.fsu.edu/awty [Accessed 17 June 2005].

WINTER, P. J. D., AND B-E. VAN WYK. 1996. A revision of the genus *Heteromorpha* (Apiaceae). *Kew Bulletin* 51: 225–265.

WINTER, P. J. D., B-E. VAN WYK, AND P. M. TILNEY. 1993. The morphology and development of the fruit of *Heteromorpha* (Apiaceae). *South African Journal of Africa* 59: 336–341.

WOLFF, H. 1910. Umbelliferae-Apioideae-*Bupleurum*, *Trinia* et reliquae Ammineae heteroclitae. *In* A. Engler [ed.], Das Pflanzenreich, vol. IV 228, 1–214. Wilhelm Engelmann, Leipzig, Germany.

WOLFF, H. 1913. Umbelliferae-Saniculoideae. *In* A. Engler [ed.], Das Pflanzenreich, vol. IV 228, 1–305. Wilhelm Engelmann, Leipzig, Germany.

YODER, A. D., J. A. IRWIN, AND B. A. PAYSEUR. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* 50: 408–424.