A phylogeny of the Gochnatieae: Understanding a critically placed tribe in the Compositae

Vicki A. Funk,[1] Gisela Sancho,[2] Nádia Roque,[3] Carol L. Kelloff,[1] Iralys Ventosa-Rodríguez,[4] Mauricio Diazgranados,[1] J. Mauricio Bonifacino[5] & Raymund Chan[1]

1 *U.S. National Herbarium, Department of Botany, Smithsonian Institution-NMNH, MRC 166, Washington, D.C., 20560, U.S.A.*
2 *Museo de La Plata, Paseo del Bosque s/n, 1900 La Plata, BA, Argentina*
3 *Instituto de Biologia, Universidade Federal da Bahia, Campus Universitário de Ondina, 40170-110 Salvador, Bahia, Brazil*
4 *Instituto de Ecología y Sistemática, Carretera Varona Km 3½, Capdevila, Boyeros, AP. 8029, CP 10800, La Habana, Cuba;* current address: *Pasaje El Astillero No. 8, Vaqueros (4401), Salta, Argentina*
5 *Laboratorio de Botánica, Facultad de Agronomía, Universidad de la República, Casilla de Correos 1238, Montevideo, Uruguay*
Author for correspondence: *Vicki A. Funk, funkv@si.edu*
ORCID: V.A.F., http://orcid.org/0000-0002-7975-1450

DOI http://dx.doi.org/10.12705/634.27

Abstract Subfamily Gochnatioideae is the sister group of ~96% of the species in Compositae (Asteraceae). It is of particular interest not only because of its position in the phylogeny, but also because, in recent molecular studies, the node it occupies is not strongly supported making difficult any inferences on the direction of character evolution in the family. The recognition of tribe Gochnatieae was one of the results of a comprehensive molecular analysis of the family that showed the traditional circumscription of the Mutisieae to be non-monophyletic. The four genera of Gochnatieae (*Cnicothamnus*, *Cyclolepis*, *Gochnatia*, *Richterago*) were defined by the presence of apiculate anther appendages and dorsally smooth style branches. *Gochnatia*, which contained about 70 species, was the largest and most complex genus and in the last decade some of its sections have been moved (or returned) to the rank of genus. This study includes a large selection of potential outgroups and over 60% of all species in the tribe, including all the genera and all but one of the sections of *Gochnatia*, to examine evolutionary relationships among the taxa. Both cpDNA and nrDNA were used in a phylogenetic analysis using parsimony, likelihood, and Bayesian approaches. The results suggest a non-monophyletic *Gochnatia* that is here resolved by the recognition of segregate genera. Morphological characters support these new genera and allow the adoption of a new classification for the Gochnatieae. A biogeographic analysis shows a possible southern South American/Andean origin followed by movement in three directions: into the Central Andes, into central and northern Brazil, and into Mexico and the Caribbean. The dating analysis gives an age of the split of the core Gochnatieae from the Wunderlichieae-*Cyclolepis* clade, and hence the age of the tribe, of 36–45 Ma and an age of 23–25 Ma for the first split within the core Gochnatieae (Andean vs. Brazil-Mexico-Caribbean). *Cnicothamnus* remains in Gochnatieae but *Cyclolepis* is designated incertae sedis.

Keywords *Anastraphia*; Asteraceae; biogeography; character evolution; *Gochnatia*; Gochnatieae; molecular phylogenetics; *Moquiniastrum*; Neotropics; *Pentaphorus*; *Richterago*; systematics; taxon age

Supplementary Material Electronic Supplement (Figs. S1–S2, Appendix S1) and alignment are available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

Subfamily Gochnatioideae (currently 103 species: all species numbers are approximate because species are frequently described as new, added to the tribe, or removed from it) was recently determined to be one of the basal lineages of the Compositae (Panero & Funk, 2002, 2007, 2008; Funk & al., 2005) and the sister group of ~96% of the species in the family excluding the Barnadesioideae (92 spp.), Stifftioideae (44), Mutisioideae (619), and Wunderlichioideae (47). Prior to the establishment of Gochnatieae (the only tribe in the subfamily), most of the genera were placed in subtribe Gochnatiinae of tribe Mutisieae (Cabrera, 1977). In 2008 many of the species in subtribe Gochnatiinae were moved into tribe Gochnatieae when the circumscription of Mutisieae was shown to be non-monophyletic (Panero & Funk, 2008). The current recognition of what is now Gochnatieae came about during attempts to resolve the phylogeny of the family, principally the positions of all clades of tribe Mutisieae s.l. (sensu Cabrera, 1977) (Fig. 1).

At the beginning of this study the most recent treatments by Roque & Pirani (2001), Sancho & Freire (2009), and Funk & al. (2009a, b) showed four genera in Gochnatieae: *Cnicothamnus* Griseb., *Cyclolepis* Gilles ex D.Don, *Gochnatia* Kunth, and *Richterago* Kuntze. Based on morphology, *Pentaphorus*

Received: 21 Nov 2013 | returned for first revision: 5 Feb 2014 | last revision received: 4 Jun 2014 | accepted: 4 Jun 2014 | published online ahead of inclusion in print and online issues: 4 Aug 2014 ||

D.Don, described as a genus by Don (1830), was resurrected by Hind (2007) from its placement as a section of *Gochnatia* s.l. (Cabrera, 1971), but the name was not widely used. Recently other segregate genera have been recognized: *Anastraphia* D.Don (Ventosa & Herrera, 2011a, b, c; Robinson & Funk, 2012), and *Moquiniastrum* (Cabrera) G.Sancho (Sancho & al., 2013). These segregate genera are varied in their morphology and habit and result in a total of seven genera in Gochnatieae (Figs. 2–3).

Molecular evidence has shown that other genera once thought to be related to *Gochnatia* s.l. such as *Hyalis* Don ex Hook. & Arn., *Ianthopappus* Roqué & D.J.N.Hind, *Nouelia* Franch, and *Leucomeris* Steetz, are not part of the current tribe Gochnatieae (Panero & Funk, 2008; Funk & al., 2009a). This suggested that the morphological characters previously used to define a wider concept of *Gochnatia* and relatives (e.g., apiculate anther appendages, style branches short, dorsally smooth, and rounded; Table 1; Fig. 4), were not synapomorphies when used in the broad concept of Gochnatieae.

History. — According to the latest study Gochantieae contains four genera (Sancho & Freire, 2009; *Cnicothamnus*, *Cyclolepis*, *Gochnatia*, *Richterago*), however, the large genus *Gochnatia* is key to understanding the tribe. Over the years many different studies that involved *Gochnatia* (Cabrera, 1977;

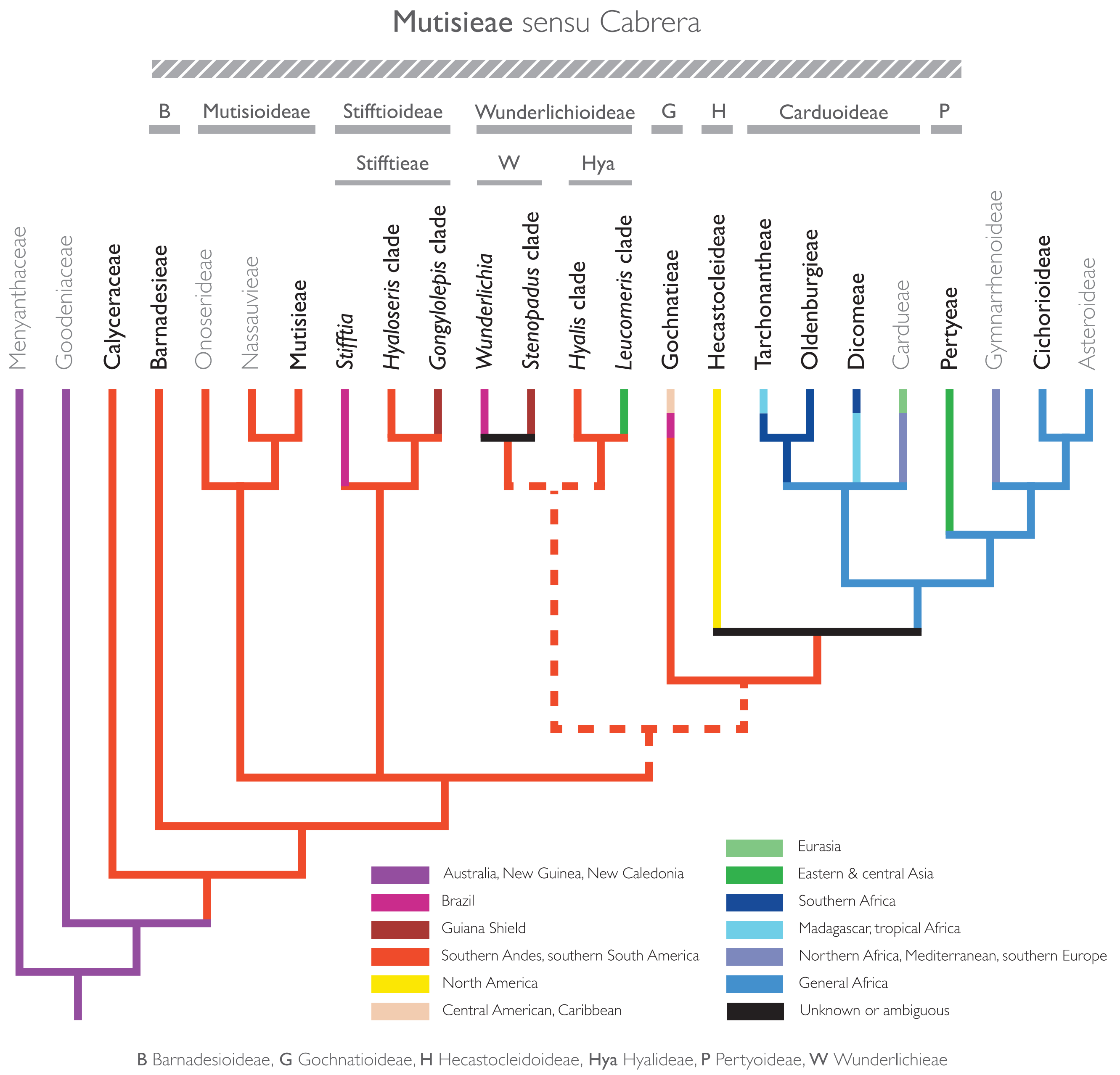


Fig. 1. Simplified tree of the Compositae showing location of Mutisieae s.l. (sensu Cabrera, 1977); modified from Funk & al. (2009a). Bold lettering indicates taxa included in this study; dashed line indicates a branch with only modest support.

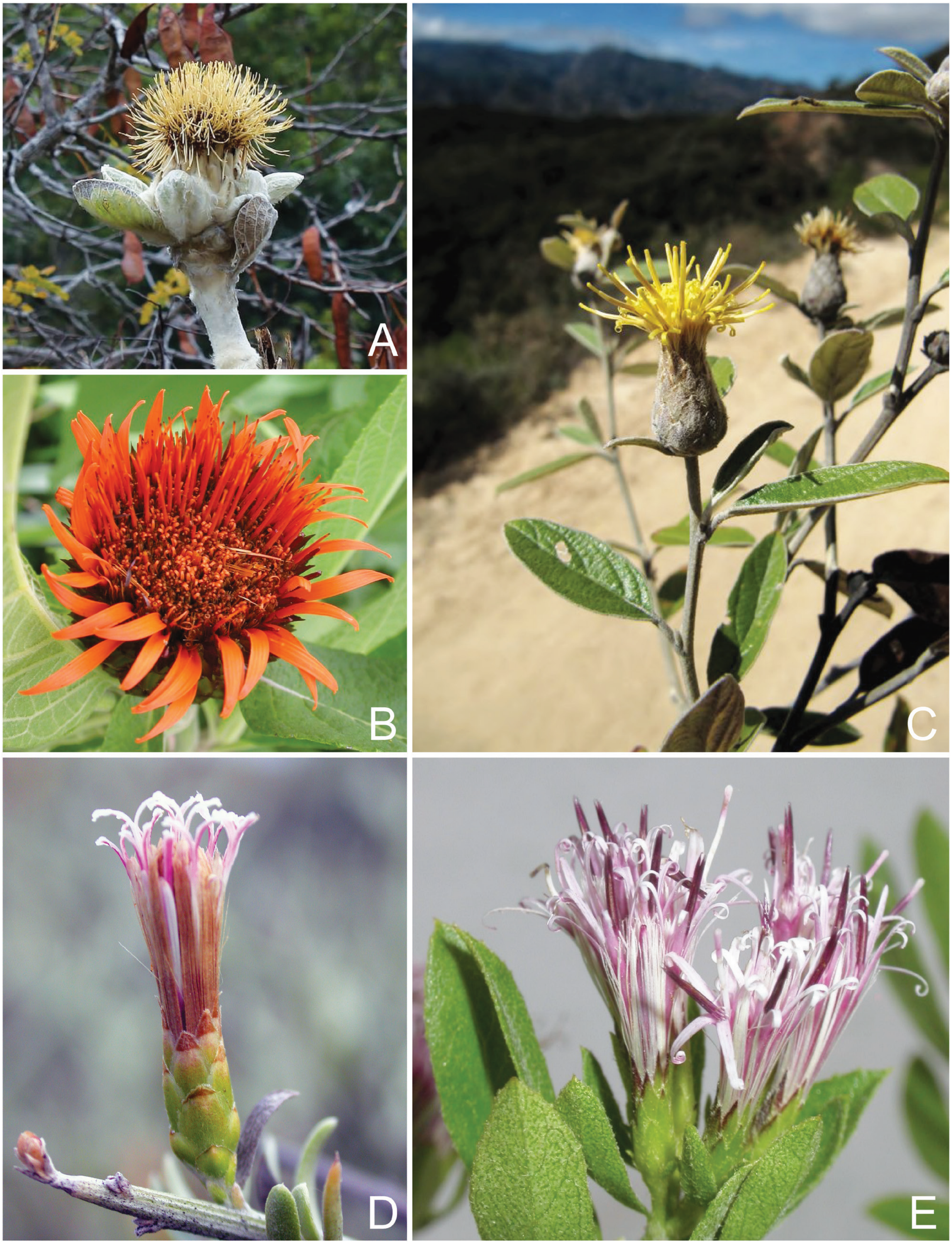


Fig. 2. A, *Wunderlichia mirabilis*, Parque Nacional da Serra do Cipó, Brazil; **B,** *Cnicothamnus lorentzii*, Argentina; **C,** *Gochnatia curviflora*, Perú; **D,** *Cyclolepis genistoides*, Argentina; **E,** *Pentaphorus foliolosus*, Chile. — Image credits: A, M. Mercadante; B, D–E, M. Bonifacino; C, G.A. Parada).

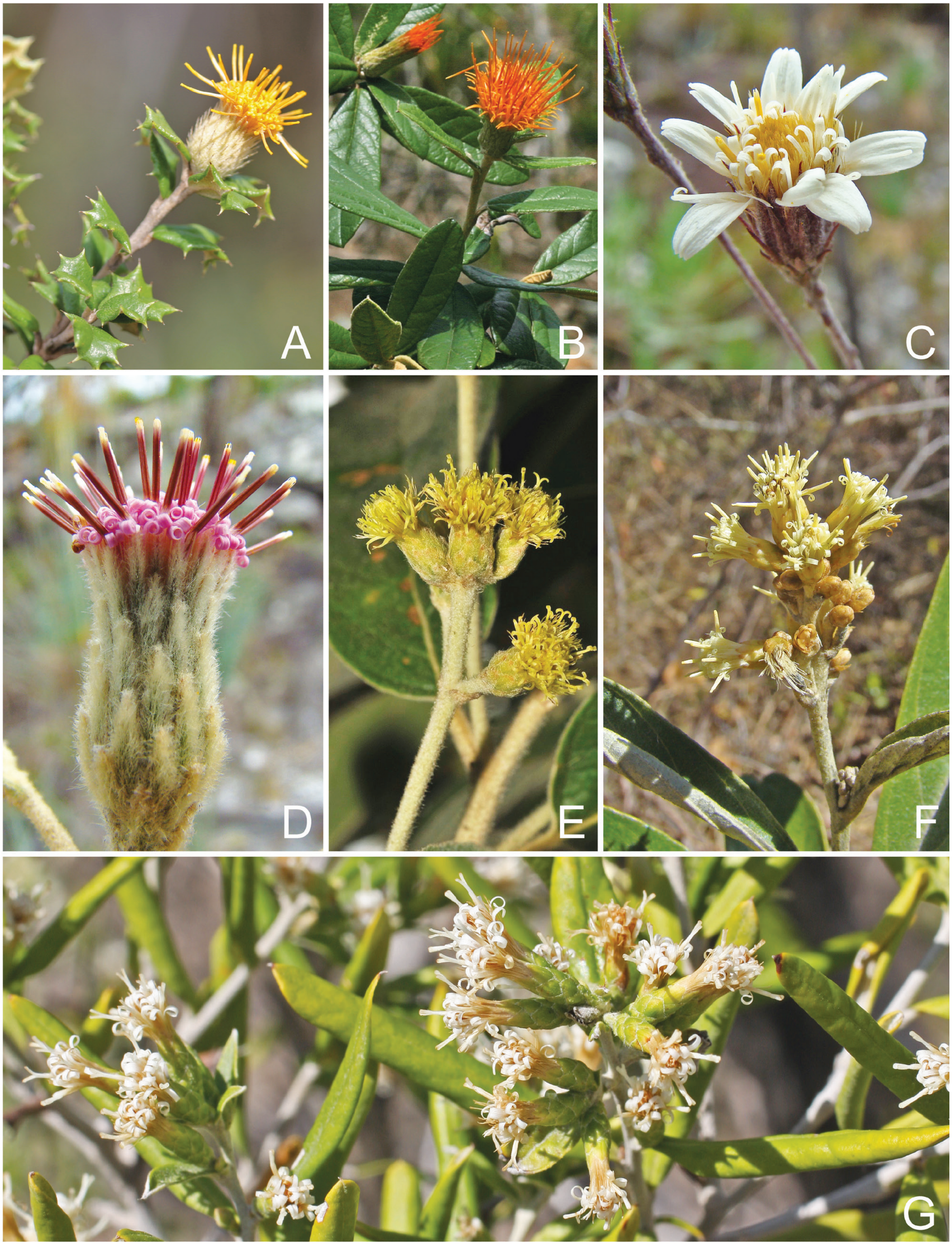


Fig. 3. A, *Anastraphia recurva*, Cuba; **B,** *Anastraphia crassifolia*, Cuba; **C,** *Richterago angustifolia*, Brazil; **D,** *Richterago discoidea*, Brazil; **E,** *Moquiniastrum polymorphum*, Brazil; **F,** *Moquiniastrum densicephala*, Brazil; **G,** *Gochnatia hypoleuca* (Mexican *Gochnatia*), Texas, U.S.A. — Image credits: A–B, I. Ventosa; C–D, F, N. Roque; E, M. Bonifacino; G, T.F. Patterson)

Table 1. Genera proposed as related to *Gochnatia* according to various authors.

Genus	Cabrera (1971, 1977) **(1)**	Hansen (1991) **(2)**	Karis & al. (1992) Bremer (1994) **(1)**	Roque & Hind (2001); Roque & Pirani (2001) **(1)**	Freire & al. (2002) **(1, 3)**	Panero & Funk (2002, 2007)	Hind (2007)	Katinas & al. (2008) **(1, 3)**	Funk & al. (this paper) **(1, 3, 4)**
Actinoseris (Endl.) Cabrera	accepted	accepted	accepted	synonym of *Richterago*	accepted	synonym of *Richterago*	synonym of *Richterago*	accepted	synonym of *Richterago*
Chucoa Cabrera	accepted	n/a	accepted	accepted	n/a	not mentioned	n/a	n/a	n/a
Cnicothamnus Griseb.	not mentioned	not mentioned	accepted	accepted	accepted	accepted	n/a	accepted	accepted
Cyclolepis Gilles ex D.Don	accepted	accepted	accepted	accepted	accepted	accepted	n/a	accepted	incertae sedis
Gochnatia Kunth	sect. *Discoseris* sect. *Gochnatia* sect. *Hedraiophyllum* sect. *Leucomeris* sect. *Moquiniastrum* sect. *Pentaphorus*	accepted as defined by Cabrera	accepted as defined by Cabrera	accepted as defined by Cabrera excluding: sect. *Discoseris*	sect. *Anastraphioides* sect. *Discoseris* sect. *Glomerata* sect. *Gochnatia* sect. *Hedraiophyllum* sect. *Leucomeris* sect. *Pentaphorus* sect. *Rotundifolia*	accepted as defined by Cabrera excluding: sect. *Discoseris* sect. *Leucomeris* sect. *Pentaphorus*	accepted as defined by Cabrera excluding: sect. *Discoseris* sect. *Leucomeris* sect. *Pentaphorus*	accepted as defined by Cabrera excluding: sect. *Leucomeris* sect. *Pentaphorus*	*Gochnatia* s.str. *Anastraphia* Mexican *Gochnatia* *Moquiniastrum* *Pentaphorus*
Hyalis D.Don ex Hook & Arn.	n/a	accepted	accepted	accepted	accepted	n/a	n/a	accepted	n/a
Ianthopappus Roque & D.J.N.Hind	in *Actinoseris*	in *Actinoseris*	in *Actinoseris*	accepted	accepted	n/a	n/a	accepted	n/a
Nouelia Franch.	not mentioned	accepted	accepted	accepted	accepted	n/a	n/a	accepted	n/a
Pleiotaxis Steetz	accepted	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
*Richterago*Kuntze [*Actinoseris* (Endl.) Cabrera + *G.* sect. *Discoseris* (Endl.) Cabrera]	synonym	synonym	synonym	accepted	synonym	accepted	accepted	synonym	accepted

Numbers in bold refer to the characters used by the authors to support the proposed relationships among the genera (1, apiculate anther appendages; 2, involucres “cone-like”; 3, style branches short, rounded and dorsally smooth; 4, style branches concave). The diagnostic characters of each genus follow Freire & al. (2002), Katinas & al. (2008) and Roque & Funk (2013).
“accepted” indicates the author(s) used this genus name as related to *Gochnatia* (all authors accept *Gochnatia*); n/a indicates the author(s) did not consider it to be “related” to *Gochnatia* of Cabrera.

Hansen, 1991; Sancho, 2000; Freire & al., 2002; Hind, 2007) (Table 1) tried to address its generic and intrageneric relationships. These morphological treatments, although extensive, did not solve the relationships among the sections of *Gochnatia* and their related genera.

Prior to the use of molecular data, taxa now in *Anastraphia*, *Chucoa* Cabrera, *Cyclolepis*, *Gochnatia*, *Hyalis*, *Ianthopappus*, *Moquiniastrum*, *Pentaphorus*, and *Richterago* (p.p.) from the Americas along with those of the Asian *Leucomeris* and *Nouelia* and the African *Pleiotaxis* Steetz, were at one time or another included within the subtribe Gochnatiinae (most were in *Gochnatia*) that was in the tribe Mutisieae s.l. (Cabrera, 1961, 1971). All these taxa were thought to be related because they possess apiculate anther appendages (Fig. 4), and some authors also used style branches that were short with an apex that was rounded and smooth (Table 1). In 1971 *Gochnatia* s.l. was revised by Cabrera (1971) who divided it into six sections containing 69 species. As defined in Cabrera (1971), *Gochnatia* s.l. species could be found in southern Texas, Mexico, the Caribbean, most of South America, and isolated areas of Asia and Africa. Later, Cabrera (1977) suggested a probable relationship between *Gochnatia* and *Chucoa* [*Chucoa ilicifolia* Cabrera, the type, has recently been transferred into *Onoseris* Willd. (Panero, 2009; Panero & Freire, 2013); for consistency we used *Chucoa* throughout this study.] Hansen (1991) considered only *Actinoseris* (Endl.) Cabrera (sensu Cabrera, 1970; now placed in *Richterago*) and *Cyclolepis* of Cabrera's group of related genera, to be close to *Gochnatia*, excluding *Pleiotaxis* (African) and *Chucoa*. However, he added *Hyalis* and *Nouelia* (Asian) in what he called the "*Gochnatia* group" and characterized it by its cone-like involucre (Table 1; this character was not used by other authors). A morphological cladistic analysis of Mutisieae (Karis & al., 1992) supported the *Gochnatia* complex as an independent lineage and recognized *Cnicothamnus* as the sister group of *Actinoseris* (now included in *Richterago*). These results were used by Bremer (1994), who stated that *Actinoseris*, *Chucoa*, *Cyclolepis*, *Hyalis*, and *Nouelia* were all closely related to *Gochnatia*, and he cited acuminate to apiculate apical anther appendages as the defining characters (Table 1); he also suggested that *Gochnatia* was probably paraphyletic. Roque & Hind (2001), based on the acuminate to apiculate anther appendages, added to Hansen's group *Cnicothamnus* and the new *Ianthopappus* (removed from *Actinoseris*), and they agreed with Bremer (1994) that *Chucoa* should be a member of the group (Table 1).

Roque & Pirani (2001) resurrected and re-circumscribed *Richterago* to include the species formerly placed in *Actinoseris* (sensu Carbrera, 1970) along with the three species found in *Gochnatia* sect. *Discoseris* (Endl.) Cabrera (Table 1). Although *Actinoseris* contained species with radiate heads and those in *Gochnatia* sect. *Discoseris* had discoid heads, they had previously been treated as congeneric by Lessing (1830), so this transfer effectively returned *Richterago* to Lessing's concept. Roque & Pirani (2001) distinguished *Richterago* by a series of characters, the most important being the perennial scapose habit, but they are also mostly rosulate herbs (hemicryptophytes

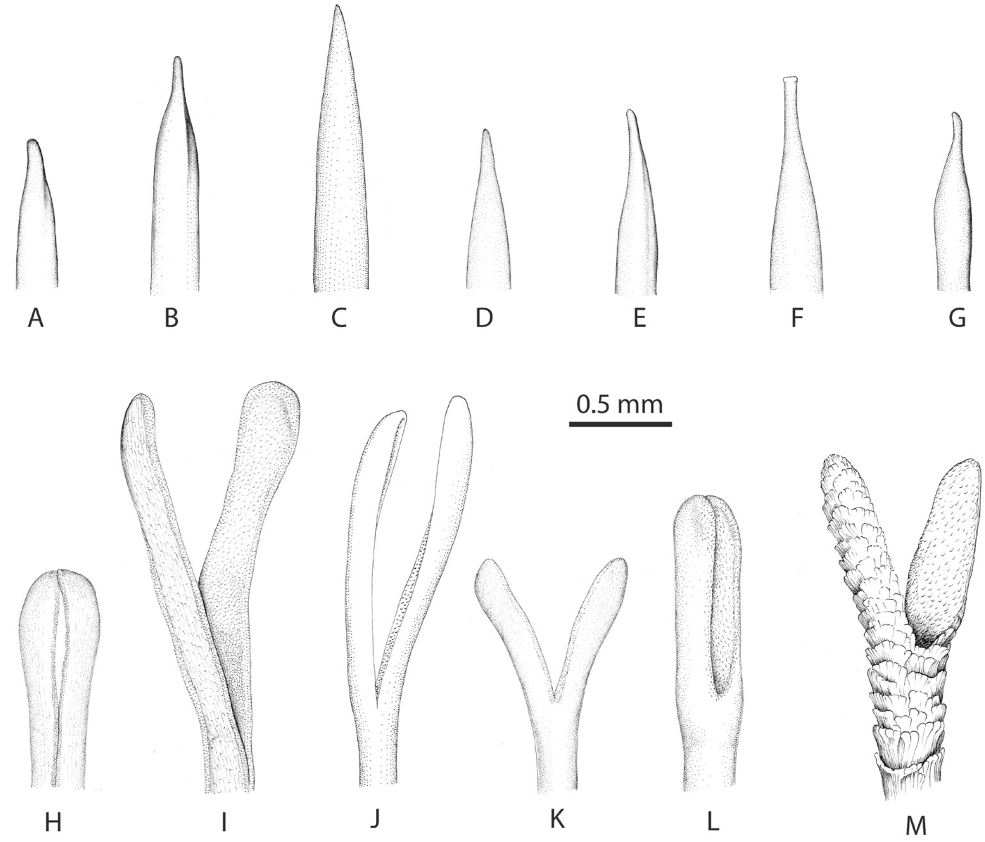


Fig. 4. Illustrations of key characters. Anther apical appendage: **A–B & D–G,** apiculate; **C,** acute anther appendage. Style branches: **H–L,** short, rounded, smooth, concave branches, **M,** obtuse, rounded and patterened style branches. **A,** *Hyalis* (Hyalideae); **B,** *Ianthopappus* (Hyalideae); **C,** *Dinoseris* (Hyaloseris clade, Stifftieae); **D,** *Cyclolepis* (incertae sedis); **E,** *Gochnatia* s.str. (Gochnatieae); **F,** *Cnicothamnus* (Gochnatieae); **G,** *Richterago* (Gochnatieae); **H,** *Ianthopappus* (Hyalideae); **I,** *Cyclolepis* (incertae sedis); **J,** *Cnicothamnus* (Gochnatieae); **K,** *Richterago* (Gochnatieae); **L,** *Gochnatia* s.str. (Gochnatieae); **M,** *Chimantaea* (Wunderlichieae). — Assembled from Figs. 3–5 in Roque & Funk (2013).

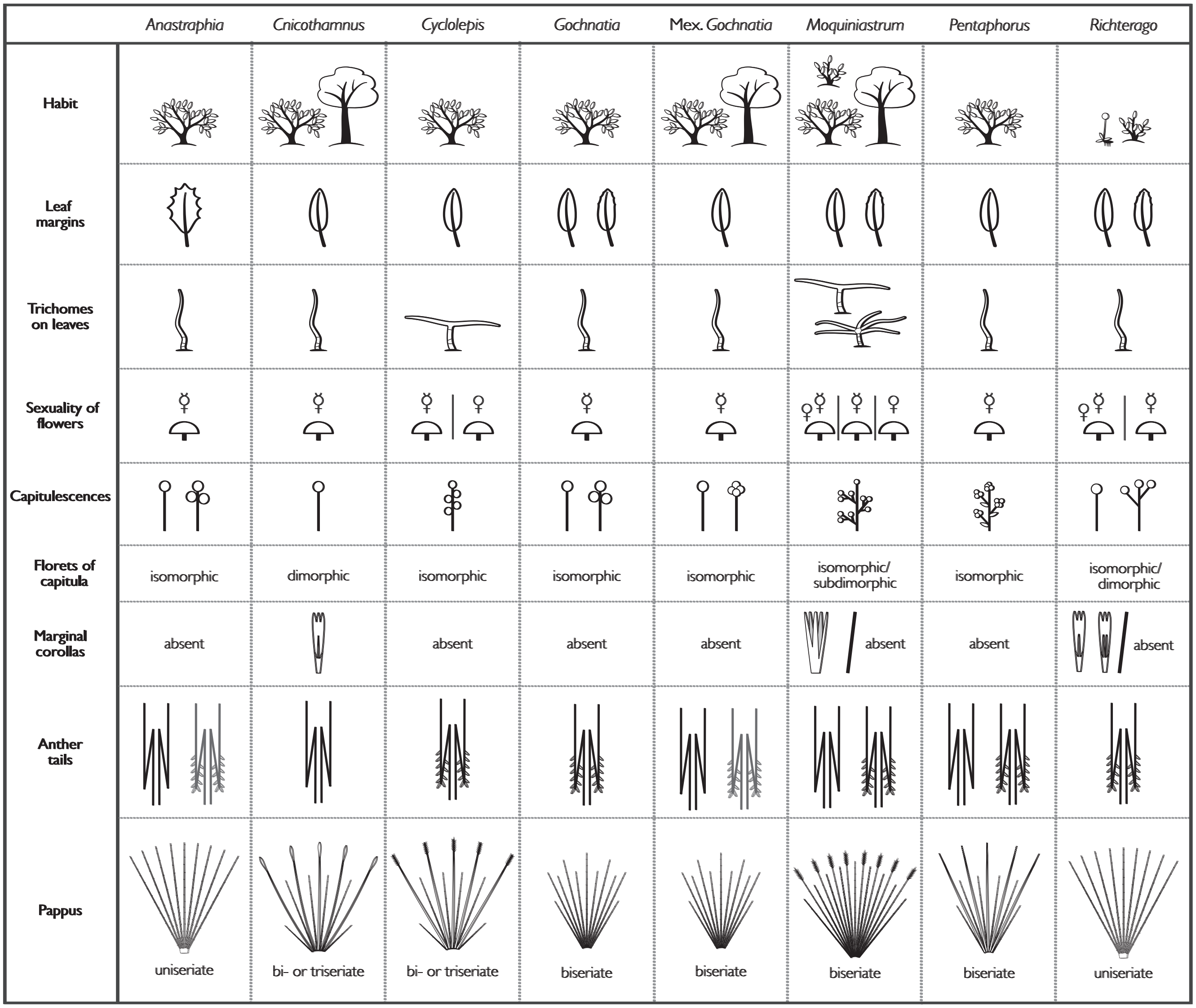
or geophytes) or subshrubs (erect, woody, unbranched stem) and have a uniseriate pappus of 25–42 bristles that are basally connate forming a fleshy ring.

Later, the *Gochnatia* complex was defined by Freire & al. (2002) as all genera with apiculate anther appendages and dorsally smooth style branches (Table 1): *Actinoseris* (*Richterago* p.p.), *Cnicothamnus*, *Cyclolepis*, *Gochnatia* (including sections *Discoseris*, *Leucomeris*, *Pentaphorus* and the Caribbean species of section *Gochnatia*), *Hyalis*, *Ianthopappus*,and *Nouelia*. This morphological study also showed that *Chucoa* differed from the other members of the *Gochnatia* complex by having acute anther appendages and style branches with a papillose dorsal surface. As a result, Freire & al. (2002) excluded *Chucoa* from the *Gochnatia* complex.

Phylogenetic studies based on molecular data (Jansen & Kim, 1996; Kim & al., 2002; Panero & Funk, 2008) supported *Gochnatia* and some of its proposed relatives as an independent lineage separate from the rest of Mutisieae. Based on these results, Panero & Funk (2002) described tribe Gochnatieae and subfamily Gochnatioideae. However, not everything that had been proposed as closely related to *Gochnatia* was included in the new tribe: *Hyalis*, *Ianthopappus*, *Leucomeris*, *Nouelia*, and *Pleiotaxis* were all placed elsewhere. The value of the characters of "dorsally smooth style branches" and "apiculate anther appendage" needed to be reconsidered (Table 1).

Two main infrageneric classifications have been proposed for *Gochnatia*: Cabrera (1971; six sections) and Freire & al. (2002; eight sections). In Cabrera's treatment, six sections were established on the basis of habit, capitulescence, and capitulum sexuality. Some of Cabrera's sections, such as *Gochnatia* sect. *Leucomeris*, sect. *Pentaphorus* and sect. *Moquiniastrum*, were defined by morphological features and geographical distributions that proved useful in our study. For instance, section *Moquiniastrum* had some diagnostic characters regarding capitulescences and sexuality of capitula that have traditionally been regarded as derived within *Gochnatia* (Sancho, 2000) (Table 2);

Table 2. Genera of the Gochnatieae with diagnostic morphological characters. *Cyclolepis* is incertae sedis.



	Anastraphia	*Cnicothamnus*	*Cyclolepis*	*Gochnatia*	Mex. *Gochnatia*	*Moquiniastrum*	*Pentaphorus*	*Richterago*
Habit								
Leaf margins								
Trichomes on leaves								
Sexuality of flowers								
Capitulescences								
Florets of capitula	isomorphic	dimorphic	isomorphic	isomorphic	isomorphic	isomorphic/ subdimorphic	isomorphic	isomorphic/ dimorphic
Marginal corollas	absent		absent	absent	absent	absent	absent	absent
Anther tails								
Pappus	uniseriate	bi- or triseriate	bi- or triseriate	biseriate	biseriate	biseriate	biseriate	uniseriate

Sancho (2000) suggested that section *Moquiniastrum* might be paraphyletic. Cabrera (1971) thought that *G.* sect. *Gochnatia* and sect. *Hedraiophyllum* were not "natural groups" and in fact, section *Gochnatia* included groups that were morphologically distinct and disjunct with respect to their geographic distribution. Sections *Discoseris* (from Brazil), *Leucomeris* (from Asia), *Pentaphorus* (from Chile and Argentina) and the Caribbean species of sect. *Gochnatia*, all included in *Gochnatia* by Cabrera, are now gaining acceptance as separate genera (*Richterago*: Roque & Pirani, 2001; *Leucomeris*: Panero & Funk, 2002; *Pentaphorus*: Hind, 2007; *Anastraphia*: Ventosa & Herrera, 2011a; *Moquiniastrum*: Sancho & al., 2013).

Freire & al. (2002) based their circumscription of their *Gochnatia* sections on pappus features identified for the first time, although they also included some characters that were used by Cabrera such as capitulescence and habit (Table 2). These authors, like Cabrera, maintained a broad concept of *Gochnatia*. The Freire & al. (2002) treatment reorganized some of the sections and resolved some of the non-natural groups within *Gochnatia* (e.g., sect. *Hedraiophyllum* and sect. *Moquiniastrum*).

Gochnatieae as the sister group to most of the Compositae. — According to the tree obtained by Panero & Funk (2008), Gochnatieae are the sister group of the remainder of the family, excluding the four subfamilies listed above. The tribe is also the last branch of the basal grade of the family that is rooted in South America. It is followed by the monospecific *Hecastocleis* A.Gray, native to the mountains of Nevada and Death Valley (U.S.A.). This genus is, in turn, the sister group of the rest of the family beginning with the Carduoideae (Thistles and African Mutisieae) (Fig. 1), which has a large African-based radiation followed by several introductions into other parts of the world, mainly Eurasia/Asia (Funk & al., 2009a).

The phylogeny of the basal grade of the Compositae (section of the phylogeny between the Barnadesioideae and the Carduoideae; Fig. 1) is not well resolved. Certainly the individual clades are well supported and the three tribes of the Mutisioideae s.str. (Onoserideae, Nassauvieae and Mutisieae, sensu Panero & Funk, 2002) hold together well as a clade, but, based on the work by Panero & Funk (2008) and subsequent analyses (Funk & al., 2009a, b), the relationships among the other clades lacked consistent strong support using parsimony, likelihood, and Bayesian inference methods. In fact, five of the well-supported clades (Mutisioideae s.str., Stifftieae, Hyalideae, Wunderlichieae, and Gochnatieae; Fig. 1) could be interpreted as forming a polytomy (Fig. 1). It is also an area of conflicting morphological characters (Ortiz & al., 2009; Roque & Funk, 2013) where characters thought to be diagnostic are shared by genera in different clades.

One way to better understand the basal grade is to carefully study each clade at the species level so that one can be sure of the contents of the clades and therefore have a clear understanding of the morphology of the members of each clade. The goal of this paper is to achieve such an understanding of the Gochnatieae (Gochnatioideae; Fig. 1). This clade is especially important for two reasons: first, as discussed above, the tribe Gochnatieae is uniquely positioned between the southern South American base of the phylogeny and the rest of the family (Fig. 1), and second, because the Gochnatieae share a number of characters with some members of other clades that confuses our understanding of the evolution at the base of the family (Funk & al., 2009a, b; Roque & Funk, 2013).

In regard to the Gochnatieae, the questions addressed in this study are: (1) What are the phylogenetic relationships within the tribe? (2) Are some of the genera (*Hyalis*, *Ianthopappus*, *Leucomeris*, *Nouelia*, *Pleiotaxis*) previously thought to be closely related to *Gochnatia* (sensu Cabrera) nested in other clades? (3) Do the taxa currently placed in the Gochnatieae form a monophyletic group? (4) Is the tribe the sister group of a clade containing 96% of the species in the family? (5) Are there any apomorphies that define the tribe? (6) Can we use the phylogeny to examine diversification within the tribe? (7) Does a better understanding of the phylogeny and classification of the Gochnatieae help us understand the biogeographic history of the basal grade of the family?

■ MATERIALS AND METHODS

Plant samples. — All leaf samples were collected in the field and stored in silica gel or taken from herbarium specimens. For some samples, removing debris such as epidermal hairs from the plant tissue under a dissecting scope proved essential in avoiding amplification of contaminating fungal ITS (nrDNA). This study involved 122 samples, 46 from outgroup or sister-group taxa and 76 from the ingroup (including *Cyclolepis*). Voucher information (including full species name, country of origin, collector(s) and number, date of collection, location, and herbarium where the voucher is deposited) is given in the Appendix S1 (Electr. Suppl.).

Outgroup selection. — The selection of the outgroups was guided by a recent review of the family phylogeny (Funk & al., 2009a). Four recent collections of Calyceraceae (the sister group of the Compositeae) were included because of the ambiguity near the base of the Compositae phylogeny found in this and previous studies (Panero & Funk, 2008; Funk & al., 2009a; Ortiz & al., 2009). The tribe Gochnatieae was previously a member of the Mutisieae (sensu Cabrera, 1977) (Fig. 1), so a broad selection of the genera that were formerly part of the Mutisieae were included: these taxa are currently members of the Barnadesioideae, Stifftioideae, Mutisioideae, and Wunderlichioideae. Included in these four subfamilies are *Chucoa*, *Hyalis*, *Ianthopappus*, and *Leucomeris*, all of which had, at one time or another, been proposed as being closely related to *Gochnatia*, but were removed by Panero & Funk (2002, 2008).

Sister-group selection. — According to Panero & Funk (2008) the vast majority of the members of the Compositae (~96%) form a clade that is the sister group of the Gochnatieae (Fig. 1). A number of taxa from this large sister group were used, including members of the subfamilies Hecastocleidoideae, Carduoideae, Pertyoideae, and Cichoroideae (Fig. 1). Taxa more highly nested than these (e.g., Asteroideae) could not be aligned with sufficient confidence. Eventually 46 species from 29 genera were included as members of the outgroup/sister group.

Ingroup selection. — Since we began this study a few years ago, several of the co-authors have described or resurrected genera using species that Cabrera included in *Gochnatia* (*Anastraphia*, *Moquiniastrum*, *Pentaphorus*, *Richterago*). In all, 58 species out of 90 (number does not include *Cyclolepis*, *Cnicothamnus*, or genera removed from *Gochnatia* by Panero & Funk, 2008) were included in the study, 64% of all species known to occur in these genera. Also, all genera that had been suggested as closely related to the core *Gochnatia* were included (see details below) and were sampled from the widest possible geographic range.

Anastraphia (formerly contained in *G.* sect. *Gochnatia* p.p): 21 samples representing 17 species out of 33 species total; 51.5%. All the species are found in the Caribbean with the bulk (25) in Cuba. This study contains material from the whole range of the distribution: Cuba (13 samples), Hispaniola (7), and the Bahamas (1). For *Anastraphia*, as well as Mexican *Gochnatia*, leaf material (including material collected in silica) proved very difficult to amplify. Ultimately we included eight samples that were missing *matK* and three samples that were missing both ITS and *matK*. However, *Anastraphia* is such a well-supported group that these data-deficient taxa fell into the clade with the other Caribbean species.

Cnicothamnus: 2 samples/1 species/2 species total; 50%. The genus has two species distributed in Bolivia and northwestern Argentina. The study used two samples of different populations of *C. lorentzii* Griseb., both from Argentina.

Cyclolepis: 2/1/1; 100%. Samples from two populations of the monospecific *Cyclolepis* from Argentina were used in the study. The species is known to occur from Paraguay to northern Patagonia.

Gochnatia (including segregates): It is difficult to provide the number of species found in *Gochnatia* because it has varied greatly over time. If we use everything that has ever been in *Gochnatia* (minus those that have been moved into other subfamilies, but including *Anastraphia*, *Moquiniastrum*, and *Richterago*), we started with potentially ~90 species and sampled 63% of those species. We included representatives from all across the general distribution of the genus. The South American species came from: Peru (2 species sampled out of the 4 species found there), Bolivia (5/5), Chile (1/1), Argentina (3/4), Brazil (29/36), Paraguay (2/3), Uruguay (2/2), and Venezuela (1/1). Some species occur in more than one country, for example both species found in Uruguay were sampled from Brazil. Although *Gochnatia* has been reported from Puerto Rico (Cabrera, 1971) and Guyana (Funk & al., 2007) those records are in error. The Puerto Rican record was a mistaken locality (from the Bahamas) and the collection from Guyana was made in adjacent Brazil (P. Acevedo, pers. comm. & V.A. Funk, pers. comm., respectively). The 33 Caribbean species are now in *Anastraphia* (see above). In Mexico and Texas (U.S.A.) there are seven species, two are known only from the type specimen and one is only known from a few older collections. This study includes samples of the remaining four species (see Electr. Suppl.: Appendix S1). *Gochnatia obtusata* S.F.Blake is sometimes considered a subspecies of *G. hypoleuca* (DC.) A.Gray (Cabrera, 1971) but here we have kept them separate to maximize species diversity and evaluate the relationship between the two.

Regarding the coverage of the sections of *Gochnatia*, we followed the treatments by Cabrera (1971) and Freire & al. (2002). For the six sections of Cabrera (1971) we sampled: sect. *Discoseris* (2 species out of 3), sect. *Gochnatia* (22/34), sect. *Hedraiophyllum* (5/7), sect. *Leucomeris* (as *Leucomeris*; 1/2), sect. *Moquiniastrum* (10/18), and sect. *Pentaphorus* (2/2). For the eight sections of Freire & al. (2002) we sampled: sect. *Discoseris* (2/3), sect. *Gochnatia* (5/7), sect. *Anastraphioides* (17/26), sect. *Glomerata* (2/3), sect. *Leucomeris* (3/5), sect. *Hedraiophyllum* (13/21), and sect. *Pentaphorus* (2/2). In total, we included species from all geographic regions and covering the six sections recognized in Cabrera's treatment (1971) and seven of the eight sections of Freire & al. (2002), missing only sect. *Rotundifolia* (2 spp.). Of course, species have been moved from one section to another and at least nine new species have been described so the various totals do not agree.

Pentaphorus (formerly *G.* sect. *Pentaphorus*): 2/2/2; 100%. Found in Chile and Argentina.

Richterago (consists of *Actinoseris* and *Gochnatia* sect. *Discoseris*): 18/15/17; 88%. It is endemic to central and southern Brazil and the center of diversity is in the Espinhaço Range, in Minas Gerais (Roque & Pirani, 2014).

We also included all the genera that have since been moved out of this tribe (see Outgroup Selection).

Two species, *Gochnatia angustifolia* G.Sancho & al. and *G. rotundifolia* Less., have a distinct morphology and were previously placed alone in a separate section of *Gochnatia* (Freire & al., 2002; Sancho & al., 2005), but we were unsuccessful in extracting any usable DNA.

DNA amplification and sequencing. — For DNA extractions approximately 30 mg of dried leaf tissue was placed in an impact-resistant 2 mL tube with a small amount of 1 mm diam. zirconia/silica beads and about ten 2.3 mm diam. zirconia/silica beads (BioSpec Products, Bartlesville, Oklahoma, U.S.A.). Tissue disruption was with a FastPrep FP120 (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) at a speed setting of 4 m/s and run for 40–60 s. We used the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.) following the manufacturer's directions, but with an extended incubation period (up to 40 min) at 45°C with 30 µl of 20 mg/ml of proteinase K for herbarium material. The Qiagen extractions were further purified using the Ultra Clean 15 DNA purification kit (MO BIO Laboratories, Carlsbad, California, U.S.A.) when the initial PCR amplification was unsuccessful. The Ultra Clean protocol for large DNA fragments was followed to reduce potential shearing.

Amplification and sequencing of ITS used primers ITS5A (Downie & Katz-Downie, 1996), and ITS4 (White & al., 1990). A blocking-primer approach was successfully used to suppress fungal ITS recovery in some samples. For this method 0.1–0.3 µl of 10 µM ITS5 (White & al., 1990), modified with an extension blocking 3′ C3 spacer (Vestheim & Jarman, 2008), was added to the PCR cocktail along with the ITS5A/ITS4 primers. Only then could plant ITS be successfully amplified using a high annealing temperature (up to 58°C). Primers used to amplify

Table 3. Primer sequences used for PCR and cycle sequencing.

Name	Sequence (5′ to 3′)	Reference
ITS5A	GGA AGG AGA AGT CGT AAC AAG G	Downie & Katz-Downie, 1996
ITS5	GGA AGT AAA AGT CGT AAC AAG G	White & al., 1990
ITS4	TCC TCC GCT TAT TGA TAT GC	White & al., 1990
trnL-Fc	CGA AAT CGG TAG ACG CTA CG	Taberlet & al., 1991
trnL-Ff	ATT TGA ACT GGT GAC ACG AG	Taberlet & al., 1991
ndhF1603	CCT YAT GAA TCG GAC AAT ACT ATG C	Jansen, 1992
ndhF+607	ACC AAG TTC AAT GYT AGC GAG ATT AGT C	Jansen, 1992
trnK3914F	GGG GTT GCT AAC TCA ACG G	Johnson & Soltis, 1994
trnK2R	AAC TAG TCG GAT GGA GTA G	Steele & Vilgalys, 1994
matK1240F	ACC TTA CCC AGC TCA TCT G	Bayer & al., 2002
matK1240R	CAG ATG AGC TGG GTA AGG T	Bayer & al., 2002
matK1541F	CGA TCA ACA TCT TCT GGA GC	Bayer & al., 2002
matK1541R	GCT CCA GAA GAT GTT GAT CG	Bayer & al., 2002
matK1240FCu	GAA ATC TTG GTT CAG ACT CTT CGC	Kelloff & G. Johnson*
psbA3′F	GTT ATG CAT GAA CGT AAT GCT C	Sang & al., 1997
trnHF	CGC GCA TGG TGG ATT CAC AAT CC	Tate & Simpson, 2003

* not previously published

and sequence the *trnL-F* region (including *trnL* intron and the *trnL-F* intergenic spacer) were trnL-c and trnL-f (Taberlet & al., 1991). Primers for the 3′ end of the *ndhF* region were ndhF-1603 and ndhF+607 (Jansen, 1992). The *trnH-psbA* intergenic spacer was amplified and sequenced using the primers psbA3′F (Sang & al., 1997) and trnHF (Tate & Simpson, 2003). The entire *matK* gene and part of the flanking *trnK* intron were amplified and sequenced in two fragments using primer pairs trnK3914F/matK1541R and matK1240F/trnK2R (Johnson & Soltis, 1994; Steele & Vilgalys, 1994; Bayer & al., 2002). For samples of Cuban origin an alternative primer matK1240FCu was designed as a substitute for primer matK1240F both in the amplification and sequencing steps. All primer sequences and their sources are shown in Table 3.

The PCR cocktail comprised 5 µl of 5× PCR buffer (Green GoTaq buffer from Promega (Madison, Wisconsin, U.S.A.) yielding 1× 1.5 mM $MgCl_2$), 2 µl of 20 mM dNTPs (Bioline, Taunton, Massachusetts, U.S.A.) in an equimolar ratio, 1 µl of 25 mM $MgCl_2$ to give a final concentration of 2.5 mM (up to 3.5 µl for cpDNA for a final concentration of 5 mM), 0.5 µl of 10 mg/ml Bovine Serum Albumin (Sigma, St. Louis, Missouri, U.S.A.), 1 µl of a 10 µM concentration of the forward and reverse primers, 0.1 µl of *Taq* DNA polymerase (5 units/µl GoTaq from Promega), 2.5 µl of sample DNA, and sufficient water for a total reaction volume of 25 µl. For some samples, it was necessary to add 5% DMSO (dimethyl sulfoxide) by volume into the reaction cocktail and use a higher annealing temperature (up to 58°C) to improve specificity. The amplification conditions reactions were an initial preheating at 95°C for 2 min, then 40 cycles of 45 s at 94°C to denature the template DNA, followed by 45 s at 54°C (48°C for *ndhF*; up to 58°C for *matK*) to allow primer annealing, and 2 min (3 min for *matK*) at 72°C for primer extension.

PCR products were treated with ExoSAP-IT (USB, Cleveland, Ohio, U.S.A.) with 10 µl of the PCR product with 1 µl of ExoSAP-IT and an incubation of 37°C for 30 min, followed by 80°C for 15 min. This lossless method is especially important for DNAs extracted from herbarium vouchers that sometimes have low yields of PCR products. The cycle sequencing reactions consisted of 50–150 ng of PCR product in 5 µl of water, 2 µl of 1 µM primer, 0.6 µl of a 5× reaction buffer (400 mM Tris-HCl, 10 mM $MgCl_2$ at pH 9.0), and 1 µl of BigDye (Version 3.1) dye terminator pre-mix (Applied Biosystems of Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). The cycle sequencing program consisted of an initial preheating at 96°C for 30 s followed by 10 s at 92°C to denature the template DNA, followed by 5 s at 55°C and 4 min at 60°C for 30 cycles. Reactions were cleaned with Sephadex G-50 (GE Healthcare, Uppsala, Sweden) gel filtration using MultiScreen plates (EMD Millipore of LifeSciences/Biotech, Billerica, Massachusetts, U.S.A.). The purified cycle sequencing products were then resolved by capillary electrophoresis using an Applied Biosystems Hitachi 3730xl DNA Analyzer. Sequences from both strands (six primers for *matK*) of each PCR product were assembled and edited into a consensus sequence using Sequence Navigator software (Applied Biosystems).

Ultimately these efforts resulted in 536 new sequences added to GenBank (ITS 108 sequences; *trnLF* 108; *ndhF* 111; *matK* 91; *psbA* 118).

Phylogenetic reconstruction. — Visual alignments were performed in Mesquite v.2.75 (Maddison & Maddison, 2011). All the sequenced regions were combined in a character matrix, containing 5319 characters in alignment, and five partitions: ITS (662 characters: 1–662), *psbA* (515: 663–1177), *trnL* (872: 1178–2049), *ndhF* (701: 2050–2750), and *matK* (2569:

2751–5319). In addition, a partition that coded the gaps separately (as 307 binary characters) was prepared using DnaSP v.5.10.1 (Rozas, 2009). The matrix including coded gaps contained 5626 characters. After initial explorations using datasets with and without coding gaps, topologies showed no significant differences; therefore, coded gaps were not included in the final phylogenetic reconstructions. Two datasets were used for the analyses: one with 122 samples that included 48 samples (47 taxa) in the outgroup (including the sister group) and 74 samples (59 taxa) in the ingroup; and one with 99 samples that included 46 samples (45 taxa) in the outgroup (including the sister group) and 53 samples (42 taxa) in the ingroup. Phylogenies were constructed under maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). MP analyses were performed in PAUP* v.4.0b10 (Swofford, 2011). Full heuristic searches were configured with MAXTREES set to 100,000 and auto-increase by 100, with the tree-bisection-reconnection (TBR) branch swapping algorithm, 10 random additions, and holding one tree at each step. Non-parametric bootstrap searches were estimated with 10,000 replicates and MAXTREES set to 1. For ML and BI reconstructions, evolutionary models were selected using the Akaike information criterion in jModelTest v.2.0.2 (Posada, 2008; Darriba & al., 2012): GTR+I+ Γ for ITS; TVM+Γ for *psbA* and *trnL*; and TVM+I+Γ for *ndhF* and *matK*. ML reconstructions were performed in Garli v.2.0.1019 (Zwickl, 2006), implemented at the CIPRES Science Gateway v.3.1 (http://www.phylo.org/sub_sections/portal/). The best ML tree was selected from eight independent runs. Bootstrap analyses were performed with 120 replicates, each with eight independent runs. Bayesian reconstructions were performed in MrBayes v.3.1.2 (Ronquist & al., 2011), available also at the CIPRES portal. Settings included two runs, four independent chains, temperatures of 0.02 for the 99-sample dataset and of 0.08 for the 122-sample dataset, 10 million generations sampling every 1000 trees, with a burn-in of 5000 trees. Bootstrap support values and posterior probabilities were summarized and concatenated with the functions Sumtrees and Sumlabels, respectively, implemented in DendroPy (Sukumaran & Holder, 2010). FigTree v.1.4 (Rambaut, 2012) was used to edit the trees.

Divergence dates and clock calibrations. — The divergence time of stem and crown lineages was estimated using the full alignment. Based on the ML reconstruction, the monophyly of the following groups was specified in the priors: Barnadesieae, Carduoideae, Compositae, *Gongylolepis* Schomb.–Stifftieae, Mutisioideae, Wunderlichioideae, Gochnatioideae, and the ingroup. Five calibration points were used for the analysis: 47.46 Ma (SD 0.05 Ma) for Compositae (Barreda & al., 2012); 38 Ma (SD 0.05 Ma) for *Mutisiapollis* sp.-*Dicoma* type (i.e., Carduoideae clade; Zavada & De Villiers, 2000; Scott & al., 2006); 29 Ma (SD 0.05 Ma) for Guayana Highland–centered members (i.e., *Stenopadus*-*Wunderlichia* clade; Barreda & al., 2009); 29 Ma (SD 0.05 Ma) for the Mutisioideae (Barreda & al., 2009); and 24 Ma (SD 0.05 Ma) for the Gochnatioideae (Barreda, 1993; Barreda & Palamarczuk, 2000a, b). Node ages were estimated in BEAST v.1.7.5 (Drummond & al., 2012), with the same partitions and substitution models used for the ML and BI phylogenetic reconstructions. An uncorrelated lognormal-relaxed molecular clock model was used, with clock and tree parameters linked across partitions, and Yule Mode for speciation in the tree prior. The other priors were left as the default values in BEAUti (Drummond & al., 2012), with auto-optimization active. The estimation was run for a total of 10 million generations, sampling every 1000 generations. The convergence and mixing of the MCMC chains were assessed by inspection of the trace plots and effective sample sizes using Tracer v.1.5 (Rambaut & Drummond, 2009). A burn-in of 50% was used to obtain the final dated topology. Mean and 95% highest posterior densities (HPD) of age estimates were visualized using FigTree v.1.4.

■ RESULTS

Although hundreds of trees were produced over the course of this study, only a few are presented here. They represent the different levels of sampling based on the completeness of the data. All outgroups had all of the molecular markers. The most restrictive analyses used 99 samples (all of the samples that had all of the markers) and the least restrictive used 122 samples (all of the taxa that had at least 3 of the 5 markers). Only three taxa were missing two markers (ITS, *matK*), and 19 were missing one (*matK*). One sample, *Moquiniastrum paniculatum* (Less.) G.Sancho (extraction #290), had *matK* but was missing the first few hundred base pairs and as a result was not included in the analysis of 99 samples; Figs. 5–6; Electr. Suppl.: Figs. S1–S2 are based on the ML results of the 99 sample tree. For Figs. 5 and 6 the support values are displayed on the branches in the order of ML/MP/BI; the supplemental figures have only ML values. Figure 5 minimizes the ingroup and shows all the outgroup/sister group taxa (OG/SG). Although all of the OG/SG taxa were used in all the analyses, their relationships never varied and in the interest of space they are only presented in Fig. 5. Figure S1 (Electr. Suppl.) shows the phylogeny using only the chloroplast data and Fig. S2 (Electr. Suppl.) was generated with the nuclear data.

Three interesting things to observe within the OG/SG area of the cladogram (Fig. 5) that differ from Panero & Funk (2008) are: (1) the Hyalideae (*Ianthopappus*, *Hyalis*, *Leucomeris*) are relatively strongly supported as the sister group of the Stifftieae (91/83/1.00) whereas in Panero & Funk (2008) it was less well supported (?/52/91) as the sister group of Wunderlichieae (Fig. 1); (2) the Wunderlichieae are relatively well supported as the sister taxon of the Gochnatieae (72/52/1.00) rather than the Hyalideae (?/52/91); and (3) Gochnatieae plus Wunderlichieae are the sister group of the rest of the family (although without strong support; 72/52/1.00) rather than the better-supported result of the Gochnatieae standing alone as the sister group (?/65/1.00).

Chucoa, *Leucomeris*, *Ianthopappus*, and *Hyalis*, were all at one time or another part of the Gochnatieae but are here excluded. In addition, although *Nouelia* was not included in the analysis (no DNA could be obtained) it is excluded from Gochnatieae because it has been shown to be the sister taxon of

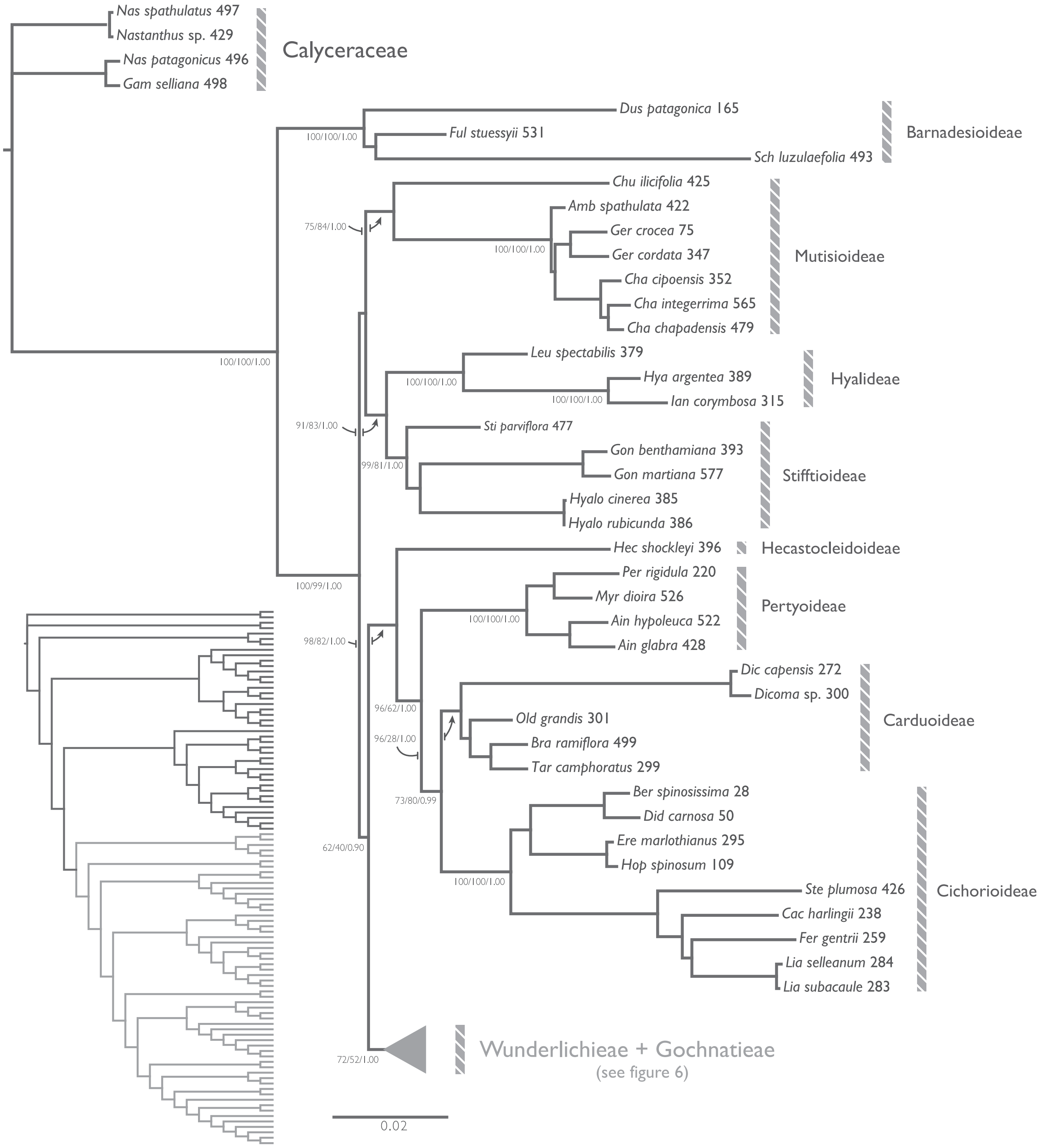
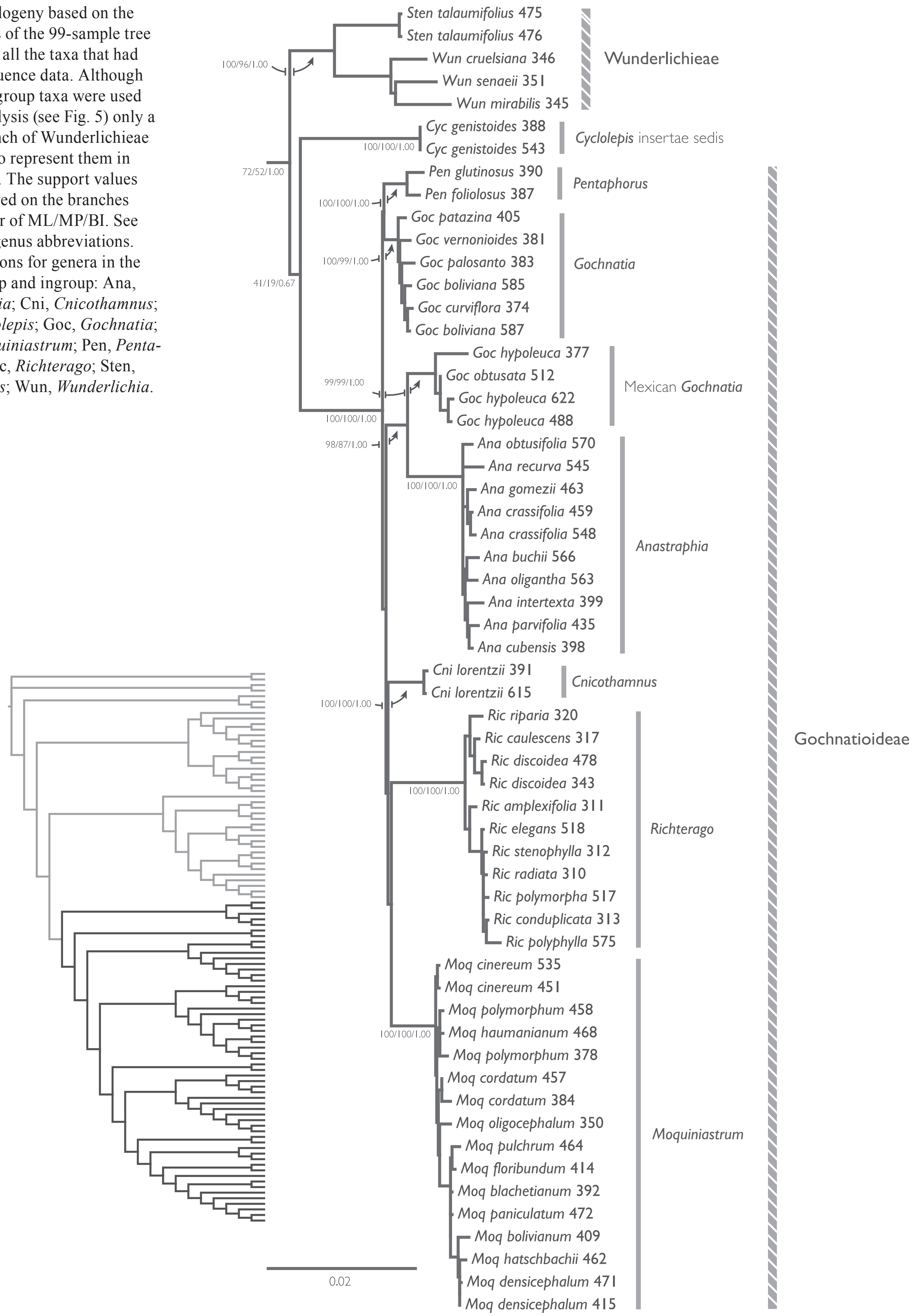


Fig. 5. Phylogeny based on the ML results of the 99-sample tree with all outgroups/sister groups emphasizing placement of taxa that used to be inside of *Gochnatia* (*Leucomeris*, etc.). The support values are displayed on the branches in the order of ML/MP/BI. This figure reduces the ingroup and shows all the outgroup/sister group taxa. The numbers after the taxa are the extraction numbers. Abbreviations for genera in the outgroups: Ain, *Ainsliaea*; Amb, *Amblysperma*; Ber, *Berkeya*; Bra, *Brachylaena*; Cac, *Cacosmia*; Cha, *Chaptalia*; Chu, *Chucoa*; Dic, *Dicoma*; Did, *Didelta*; Dus, *Duseniella*; Ere, *Eremothamnus*; Fer, *Ferreyranthus*; Ful, *Fulcaldea*; Gam, *Gamocarpha*; Ger, *Gerbera*; Gon, *Gongylolepis*; Hec, *Hecastocleis*; Hop, *Hoplophyllum*; Hya, *Hyalis*; Hyalo, *Hyaloseris*; Ian, *Ianthopappus*; Leu, *Leucomeris*; Lia, *Liabum*; Myr, *Myripnois*; Nas, *Nastanthus*; Old, *Oldenburgieae*; Per, *Pertia*; Sch, *Schlechtendalia*; Ste, *Stephanbeckia*; Sti, *Stifftia*; Tar, *Tarchonanthus*.

Fig. 6. Phylogeny based on the ML results of the 99-sample tree that shows all the taxa that had all the sequence data. Although all the outgroup taxa were used in this analysis (see Fig. 5) only a single branch of Wunderlichieae was used to represent them in this figure. The support values are displayed on the branches in the order of ML/MP/BI. See Fig. 5 for genus abbreviations. Abbreviations for genera in the sister group and ingroup: Ana, *Anastraphia*; Cni, *Cnicothamnus*; Cyc, *Cyclolepis*; Goc, *Gochnatia*; Moq, *Moquiniastrum*; Pen, *Pentaphorus*; Ric, *Richterago*; Sten, *Stenopadus*; Wun, *Wunderlichia*.



Leucomeris (Panero & Funk, 2008). All the OG/SG taxa were used in all analyses but Fig. 6 illustrates only the Wunderlichieae and shows the details of the ingroup.

The 99-sample tree. — In the ML, MP, and BI analyses (Fig. 6) and in the cpDNA tree (Electr. Suppl.: Fig. S1 bootstrap support 63) *Cyclolepis* was the sister group of the rest of the Gochnatieae (14/19/0.67). However, when the indels were coded in the ML and BI analyses (not shown) and in the nrDNA tree (Electr. Suppl.: Fig. S2 bootstrap support 54) *Cyclolepis* was the sister group of the Wunderlicheae. Neither solution has the good support and the relationships among the three taxa do not vary: *Cyclolepis* is always located between Wunderlichieae and Gochnatieae. The difference is where the outgroup branch attaches. Figure 7 contains two unrooted phylogenies (FigTree; Rambaut, 2012) that display the results of the 99-sample analysis as an unrooted diagram. Figure 7A shows the entire 99-sample phylogeny with the Gochnatieae and Wunderlichieae in black and all of the rest of the taxa in grey. Figure 7B is an enlarged Gochnatieae-Wunderlichieae showing the alternative attachment sites for *Cyclolepis* and *Cnicothamnus*.

Figure 6 shows the strongest possible support (100/100/1.0) for the core members of the tribe Gochnatieae (minus *Cyclolepis*). *Cyclolepis* is strongly supported as a clade and is sometimes included in the Gochnatieae but without strong support (see above). The segregate genera (*Anastraphia*, *Moquiniastrum*, *Pentaphorus*, *Richterago*) are strongly supported. After the recognition of the segregate genera, the remaining species still in *Gochnatia* are divided between two clades that are not sister taxa. The type for the genus, *G. vernonioides*, is in the Central Andes, so this clade retains the generic name and is labeled *Gochnatia* (Fig. 6 extraction 381). The second clade that still has species named *Gochnatia* is made up of the Mexican species—although one Mexican species, *G. hypoleuca*, reaches the southern tip of Texas, we refer to this clade as the Mexican *Gochnatia*. The Mexican *Gochnatia* clade is well supported as the sister taxon of *Anastraphia*.

There are eight well-supported clades within the Gochnatieae (Fig. 6):

(1) *Gochnatia* (Fig. 2C): The Central Andean clade (eight species; 100/99/1.0).

(2) *Pentaphorus* (Fig. 2E): At various times, these two species have been recognized as a genus, subgenus, or section. In this analysis the clade has the strongest possible support (100/100/1.0)

(3) Mexican *Gochnatia* species (Fig. 3G): This clade has never been a separate genus but it has strong support (99/99/1.0). Cabrera (1971) placed these species, along with two others, in *G.* sect. *Hedraiophyllum*. The two other species, *G. palosanto* Cabrera and *G. cordata* Less. (both from northern Argentina and southern Bolivia) are now part of *Gochnatia* s.str. and *Moquiniastrum*, respectively. A new species was recently described but is known only from the type collection (Gonzalez-Medrano & al., 2004).

(4) *Anastraphia* (Fig. 3A–B): The 33 Caribbean species (Cuba, Hispaniola, the Bahamas) fall into one strongly supported clade (100/100/1.0) which has already been resurrected as a separate genus (Ventosa & Herrera, 2011a; Robinson & Funk, 2012).

(5) *Moquiniastrum* (Fig. 3E–F): The 21 species in this clade are mainly from central and southern Brazil but extend into extreme northern Brazil, as well as Argentina, Bolivia, Paraguay, Peru, Venezuela and Uruguay (Sancho, 1999, 2000). These taxa previously formed the majority of *Gochnatia* sect. *Moquiniastrum* in Cabrera's (1971) treatment. In this study

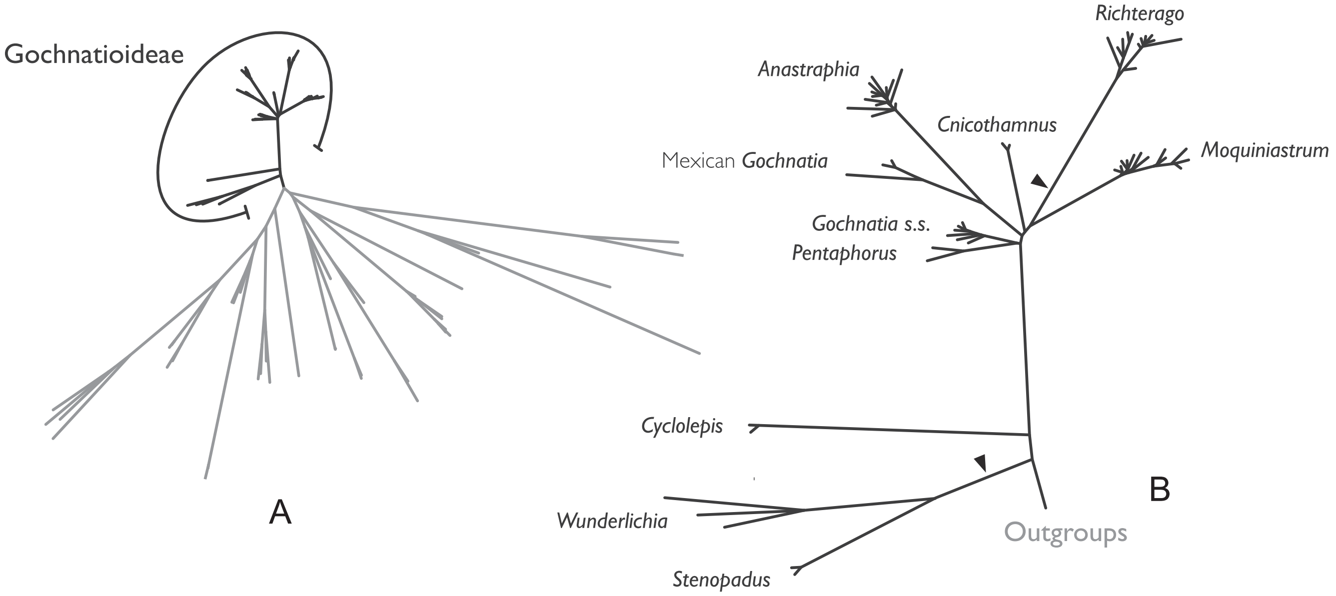


Fig. 7. Two unrooted trees based on the results of the 99-sample analysis (Figs. 5–6): **A,** includes both the ingroup and all outgroups; **B,** includes only the ingroup and shows alternative attachment sites for *Cyclolepis* and *Cniothamnus* (arrow heads). Note in A the difference in branch lengths in the ingroup and outgroups and in B the distance between *Cyclolepis* and the core Gochnatieae.

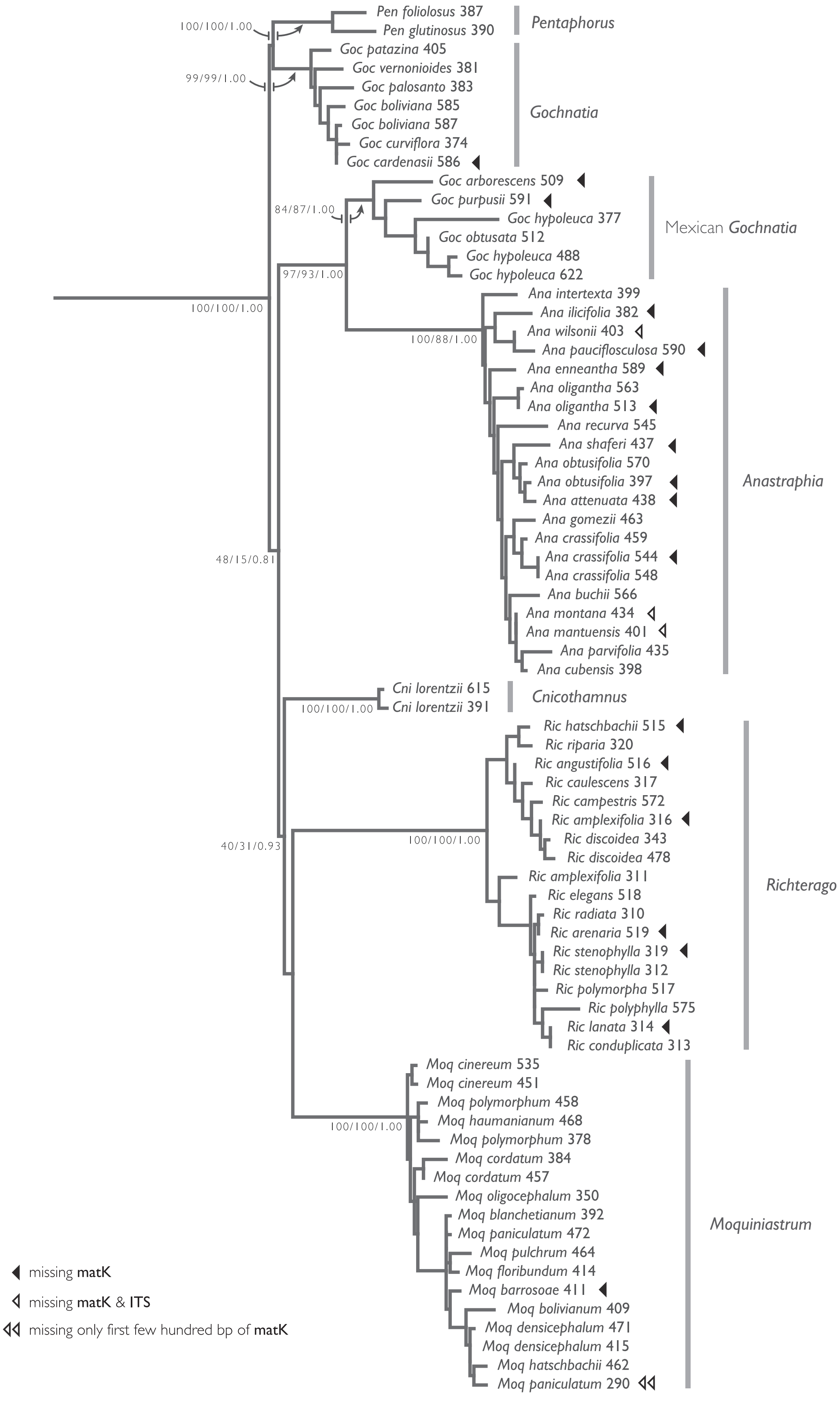


Fig. 8. 122-sample tree, including taxa with all the markers sequenced or missing only one or two markers. For abbreviations see Figs. 5 and 6.

]

the taxa form a monophyletic group with strong support (100/100/1.0). In addition, our results show that *G. cordata*, formerly part of *G.* sect. *Hedraiophyllum*, is nested in *Moquiniastrum*. This clade was recently recognized at the genus level (Sancho & al., 2013).

(6) *Richterago* (Fig. 3C–D): This strongly supported clade (100/100/1.0) of 17 species is endemic to the campos rupestres (high altitudes) of Brazil. The taxa were formerly members of *Actinoseris* and *Gochnatia* sect. *Discoseris*. It was circumscribed by Roque & Pirani (2001) and the taxonomic revision has been completed (Roque & Pirani, 2014).

(7) *Cnicothamnus* (Fig. 2B): Two samples of one species were used; most of the time the clade is found as the sister group of the *Moquiniastrum*+*Richterago* clade (40/31/0.93). However, under certain circumstances, it is poorly supported as the sister group of *Richterago* (e.g., the BEAST species divergence analysis; not shown). *Cnicothamnus* inhabits Bolivia and northwestern Argentina.

(8) *Cyclolepis* (Fig. 2D): Two samples of the monospecific *Cyclolepis* were used and as expected they formed a strongly supported clade but the clade changed positions depending on the method used to analyze the data and the taxa included in the study. None of the resolutions were strongly supported (see discussion and Fig. 7). *Cyclolepis* ranges from Paraguay to northern Patagonia area of Argentina.

The 122-sample tree. — The 23 samples that were excluded because they were missing some of the sequences were added to the 99 samples and the data were reanalyzed (see Materials and Methods and above). The results showed remarkable agreement with the 99-sample phylogeny (Fig. 6). None of the taxa that were missing data fell outside of their predicted group based on morphology and distribution. For a few clades, adding these taxa did lower some of the support values very slightly (ML, MP), but the BI values remained 1.00. This stability in the presence of missing data shows the strength of support for the recognized clades (Fig. 8).

Biogeography. — Figure 9 is a simplified cladogram that has the branches color-coded according to the distribution of the terminal taxa. As described above, the support for the individual clades is strong, although the support for grouping of the clades was not. However, the same tree was obtained from both the ML and BI analyses, providing some support for the discussion on biogeography.

Divergence dates. — The dating analysis (Fig. 10) shows the same age estimated for Compositae (47.4–47.6 Ma) as does the work based on the only macrofossil for the family (Barreda

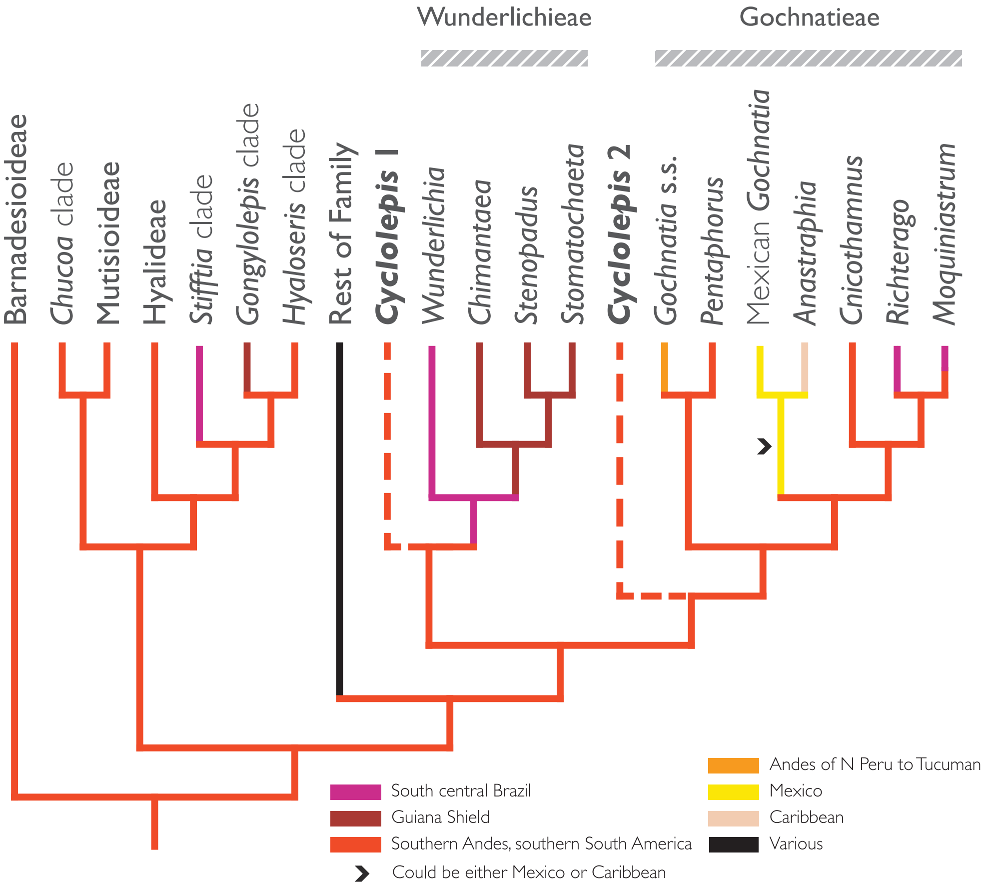
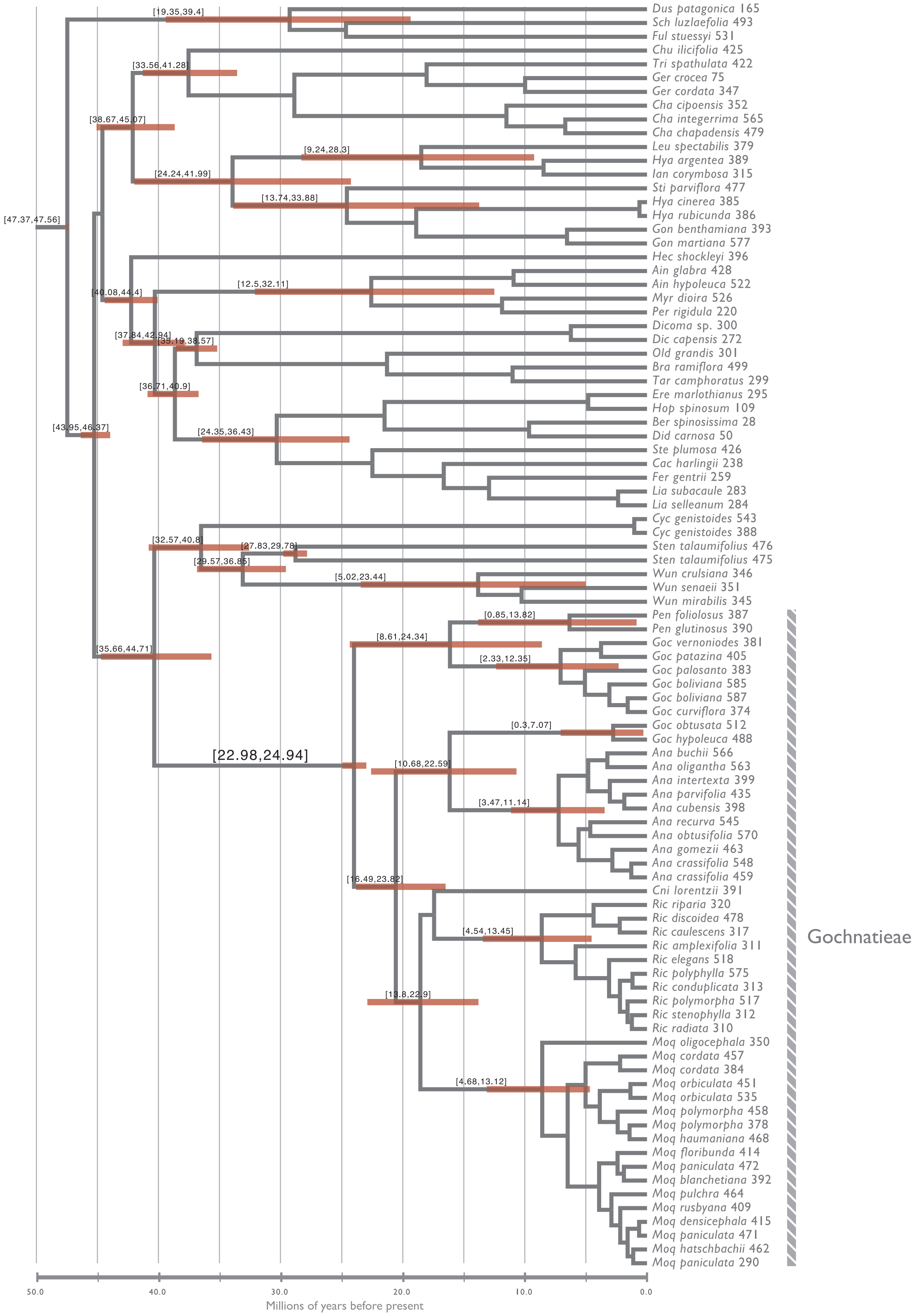


Fig. 9. Color-coded area cladogram. Note options 1 and 2 for the placement of *Cyclolepis*.

Fig. 10. Time-calibrated phylogeny for the Gochnatieae and their relatives. Red bars and text in parentheses on nodes show the range in the age ▶ of diversification with a 95% of highest posterior density (HPD). See text for calibration points used. For abbreviations see Figs. 5 and 6.


[47.37,47.56]
[19.35,39.4]
[33.56,41.28]
[38.67,45.07]
[9.24,28.3]
[24.24,41.99]
[13.74,33.88]
[12.5,32.11]
[40.08,44.4]
[37.84,42.94]
[35.19,38.57]
[36.71,40.9]
[43.95,46.37]
[24.35,36.43]
[32.57,40.8]
[27.83,29.78]
[29.57,36.85]
[5.02,23.44]
[0.85,13.82]
[8.61,24.34]
[35.66,44.71]
[2.33,12.35]
[0.3,7.07]
[22.98,24.94]
[10.68,22.59]
[3.47,11.14]
[16.49,23.82]
[4.54,13.45]
[13.8,22.9]
[4.68,13.12]
Dus patagonica 165
Sch luzlaefolia 493
Ful stuessyi 531
Chu ilicifolia 425
Tri spathulata 422
Ger crocea 75
Ger cordata 347
Cha cipoensis 352
Cha integerrima 565
Cha chapadensis 479
Leu spectabilis 379
Hya argentea 389
Ian corymbosa 315
Sti parviflora 477
Hya cinerea 385
Hya rubicunda 386
Gon benthamiana 393
Gon martiana 577
Hec shockleyi 396
Ain glabra 428
Ain hypoleuca 522
Myr dioira 526
Per rigidula 220
Dicoma sp. 300
Dic capensis 272
Old grandis 301
Bra ramiflora 499
Tar camphoratus 299
Ere marlothianus 295
Hop spinosum 109
Ber spinosissima 28
Did carnosa 50
Ste plumosa 426
Cac harlingii 238
Fer gentrii 259
Lia subacaule 283
Lia selleanum 284
Cyc genistoides 543
Cyc genistoides 388
Sten talaumifolius 476
Sten talaumifolius 475
Wun crulsiana 346
Wun senaeii 351
Wun mirabilis 345
Pen foliolosus 387
Pen glutinosus 390
Goc vernonioides 381
Goc patazina 405
Goc palosanto 383
Goc boliviana 585
Goc boliviana 587
Goc curviflora 374
Goc obtusata 512
Goc hypoleuca 488
Ana buchii 566
Ana oligantha 563
Ana intertexta 399
Ana parvifolia 435
Ana cubensis 398
Ana recurva 545
Ana obtusifolia 570
Ana gomezii 463
Ana crassifolia 548
Ana crassifolia 459
Cni lorentzii 391
Ric riparia 320
Ric discoidea 478
Ric caulescens 317
Ric amplexifolia 311
Ric elegans 518
Ric polyphylla 575
Ric conduplicata 313
Ric polymorpha 517
Ric stenophylla 312
Ric radiata 310
Moq oligocephala 350
Moq cordata 457
Moq cordata 384
Moq orbiculata 451
Moq orbiculata 535
Moq polymorpha 458
Moq polymorpha 378
Moq haumaniana 468
Moq floribunda 414
Moq paniculata 472
Moq blanchetiana 392
Moq pulchra 464
Moq rusbyana 409
Moq densicephala 415
Moq paniculata 471
Moq hatschbachii 462
Moq paniculata 290
Gochnatieae
50.0
40.0
30.0
20.0
10.0
0.0
Millions of years before present

& al., 2012). Figure 10 also suggests an age of 35.7–44.7 Ma for the split between the Wunderlichioideae and Gochnatioideae and this fits with the position of the Gochnatieae-Wunderlichieae as the sister group to the majority of the family (Fig. 9). The core Gochnatieae splits into two clades (the *Pentaphorus*-*Gochnatia* clade and the rest of the core Gochnatieae) 23–25 Ma. The Mexican *Gochnatia* clade separates from the *Anastraphia* clade between 11 and 23 Ma. More or less at the same time (14–23 Ma the *Richterago*-*Cnicothamnus* clade splits from the *Moquiniastrum* clade. As a result most of the genera are dated 14–23 Ma. Most of the first splits within genera are dated (2.3)5–10(13.4) Ma (Fig. 10). While generally interesting, the confidence limits on many branches are very wide and this undermines their value and prevented an examination of diversification rates. However, two events stand out as reliable: The basal split in the core Gochnatieae has an age of 23–25 Ma and the age of the split of the core Gochnatieae from the Wunderlichieae-*Cyclolepis* clade.

■ DISCUSSION

The boundaries of Gochnatieae. — At the beginning of this study (2008), Gochnatieae contained four genera: *Cnicothamnus*, *Cyclolepis*, *Gochnatia*, and *Richterago*. Several genera have been resurrected or described prior to this publication (see Results). However, decisions were still needed, for instance the tribal assignment of *Cyclolepis* was still in doubt. Its position in the phylogeny is ambiguous possibly because of long-branch attraction: it can be the sister group of the Wunderlichieae (Fig. 7B, Fig. 9 Option 1;), or the sister group of the core Gochnatieae (Fig. 7B, Fig. 9 Option 2). Option 2 is supported by the co-occurence of certain morphological characters in *Cyclolepis* and *Gochnatia*, such as deeply 5-lobed corollas, apiculate anther appendages, and dorsally smooth style branches (Fig. 4), while the styles of most Wunderlichieae are usually very different, i.e., rugulose, with rounded and swollen papillae beyond the bifurcation point of the branches (Fig. 4) (Katinas & al., 2008; Roque & Funk, 2013). However, after additional study it seemed that the floral and style characters supporting Option 2 were plesiomorphic (Fig. 11). Other characters such as the apiculate anther appendages and concave style branches seem to be apomorphic, but occur more than once (Figs. 4, 11). Option 1 is supported because *Cyclolepis* shares with *Wunderlichia* Riedel ex Benth. & Hook.f. some pappus features (e.g., half of the bristles flat and longer, and the other half thin and short; Freire & al., 2002), but this character is poorly explored across the family. However, *Cyclolepis* has some peculiar features that separate it from most members of the core Gochnatieae: gynodioecy, almost leafless stems, spiny branches, deciduous leaves with spiny tips, and female corollas with relatively short lobes. So it may be that this odd little genus is just very different from everything else. The only other taxon in the Gochnatieae with gynodioecy is *Moquiniastrum*, but this appears to be the result of parallel evolution. Indeed, the corollas in female plants of *Cyclolepis* are different in morphology from those of *Moquiniastrum* (i.e., shortly lobed in *Cyclolepis* and deeply lobed in *Moquiniastrum*; Freire & al., 2002). In a separate study traditional and new morphological characters are being evaluated in order to define them as plesiomorphic or apomorphic.

In the molecular results *Cyclolepis* usually grouped with the core Gochnatieae but under certain circumstances (coding of indels or time-calibrated analysis) it grouped with Wunderlichieae. Figure 7 clearly shows the uncertain placement of *Cyclolepis* and the importance of the attachment of the outgroup. Also illustrated in Fig. 7B is a second far less frequent occurrence: the placement of *Cnicothamnus* as the sister group of *Richterago* rather than the sister group of *Richterago* +*Moquiniastrum*. This alternate placement happens only in the time-calibrated analysis (Fig. 10). The placement of both *Cyclolepis* and *Cnicothamnus* occur at poorly supported nodes.

In the light of our findings there are four options for *Cyclolepis*: (1) Leave it in Gochnatieae; (2) put it in its own tribe in Wunderlichioideae; (3) recognize it as a separate tribe and subfamily; or (4) label it incertae sedis. The low support for options 1 and 2 might indicate that this genus would best be treated as an independent lineage as has happened with other distinctive monospecific genera in the family that are apparently the sister group of large radiations, such as *Hecastocleis* and *Gymnarrhena* Desf. However, until our detailed morphological analysis is complete, we have selected "option (4)" and are listing it as incertae sedis: not placed in any tribe or subfamily.

The Mexican species of *Gochnatia* s.l. form a clade that is the sister group of the Caribbean *Anastraphia*. One option would be to include these species in *Anastraphia*, but they lack the characters used to define this genus (leaf margins spinose-dentate, heads solitary to 2–4 and located at apex of branches; anther tails smooth or shortly laciniate; achenes cylindrical or subturbinate with angular corners and prominent veins in some species, tomentose with biseriate non-glandular hairs and biseriate glandular hairs with vesicles; pappus uniseriate with all bristles of equal length and width, in some species biseriate but external bristles exist only in the corner of the achene). Another more plausible option is to place them in a newly described genus. However, the morphological analysis of Mexican *Gochnatia* must be completed before a decision can be made.

The results of this study support the recognition of *Pentaphorus* (Don, 1830; Hind, 2007), *Richterago* (Roque & Pirani, 2001), and *Moquiniastrum* (Sancho & al., 2013). Finally, while there is some ambiguity in the position of *Cnicothamnus*, which like *Cyclolepis* may be the result of long-branch attraction, there is no doubt that it is a separate genus, and it is nested high enough in the Gochnatieae that it clearly belongs in this tribe.

Re-circumscription of *Gochnatia*. — The most comprehensive treatments of *Gochnatia* (Jervis, 1954; Cabrera, 1971; Freire & al., 2002) included about 70 species distributed in America, Africa, and Asia. Recent studies based on morphology as well as molecular evidence had decreased *Gochnatia* to about 40 species. Unpublished preliminary results of this molecular study have been available to the authors for several years as we worked to produce the comprehensive phylogenetic analysis that was needed to confirm the monophyly of the excluded groups, understand their relationships to one another, and investigate the real limits of *Gochnatia*. Our results confirm as monophyletic most of the groups once included in *Gochnatia*: *Anastraphia*, *Moquiniastrum*, and *Pentaphorus*. But, our analysis goes further by re-assigning some taxa and excluding one additional group of species, the Mexican *Gochnatia*. Thus, we are left with a drastically narrower concept of *Gochnatia* that includes only the Central Andean group of seven species. *Gochnatia* shows a cyclic history of inclusion and exclusion of genera or subgenera based on the criteria of the authors. It is interesting to note that in this treatment we have returned to some of the concepts of Lessing (1830, 1832) albeit with many additional taxa and different names. Except for the presence of *Cnicothamnus*, most of the species from the Americas that Cabrera (1971) placed in *Gochnatia* are part of a monophyletic group, it is just that all of the current workers prefer to recognize the morphological and biogeography differences while Cabrera preferred to summarize them into one genus. Of course he also included taxa that have since been moved to other areas of the family phylogeny.

Two species, *G. rotundifolia* and *G. angustifolia*, could not be placed in a genus. They lack any known characters that would allow us to select a genus, in fact they are distinct enough to be placed in a section of their own: Freire & al. (2002) placed *G. rotundifolia* in a section by itself (sect. *Rotundifolia*) and

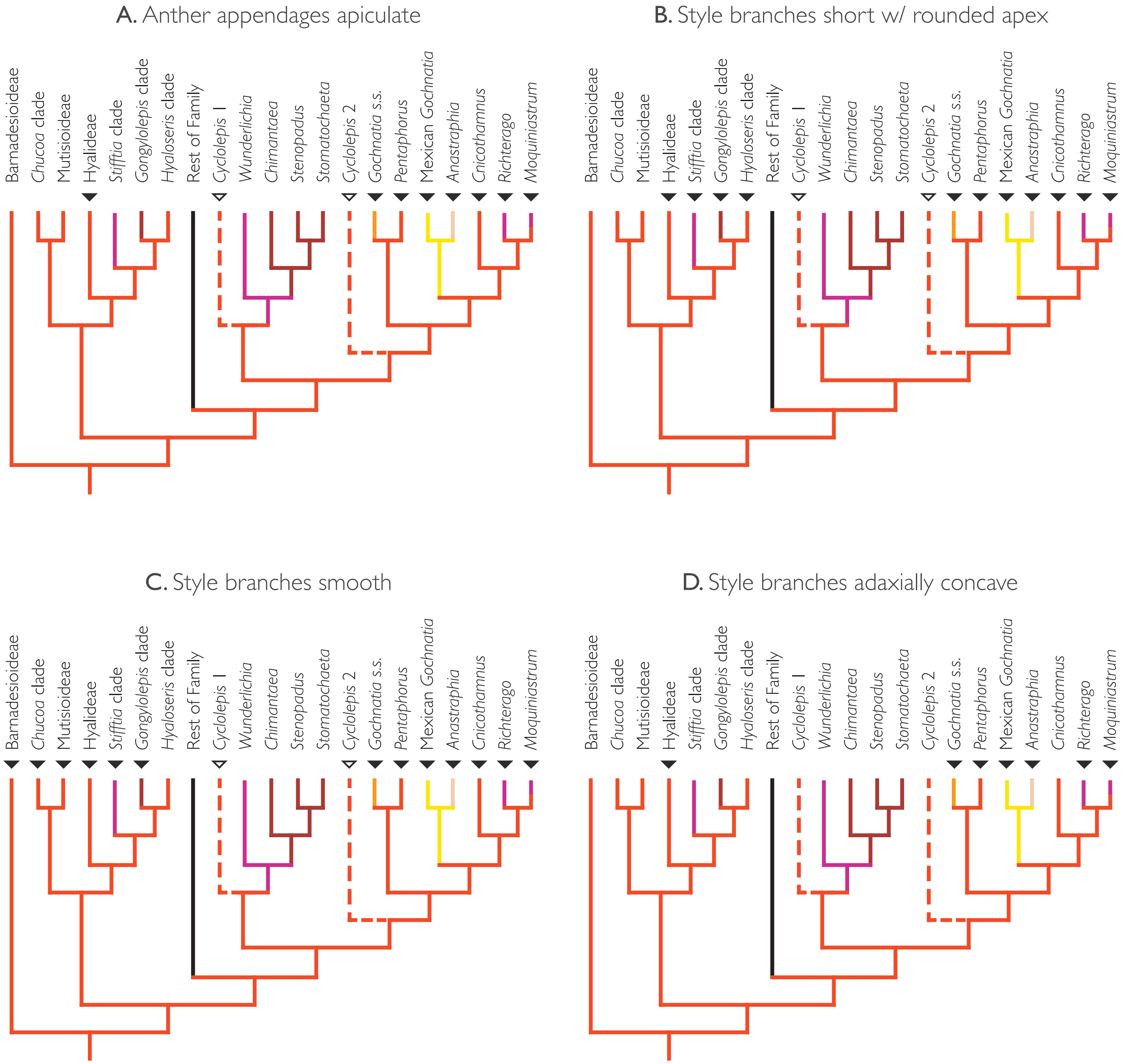


Fig. 11. Characters used to define the Gochnatieae mapped on a simplified biogeography tree (Fig. 9). Open triangles indicate the taxon is found in more than one location.

Sancho & al. (2005) placed *G. angustifolia* in the same section. They have only been collected a few times and we were unable to extract usable DNA from existing material. They are treated as insertae sedis.

Morphological definition of the Gochnatieae clades. — Developing a classification of the Mutisieae s.l. (including what is now Gochnatieae) has been a challenge throughout the history of taxonomic work in the Compositae. Botanists have proposed various classifications based mainly on corolla and head types (Bentham, 1873; Hoffmann, 1890–1894; Cabrera, 1951, 1977; Carlquist, 1957, 1958). Cassini (1816, 1819) and Lessing (1832) both emphasized style characteristics over the corolla morphology, and Jeffrey (1967), who was the first to propose an informal arrangement for Mutisieae, used style morphology alone to characterize nine of his twelve series. The most recent treatment of the Mutisieae s.l. (Katinas & al., 2008) presented an overview of Mutisioideae (a wide concept of the traditional Mutisieae) considering all available information, mainly morphological, about the genera. The authors concluded that the best characters to use were style features followed by pollen morphology, and corolla shape. Their results partly agreed with Cassini's and Jeffrey's ideas about style characteristics as some of the best to use to define groups of Compositae as well as Roque & Silvestre-Capelato's (2001) comments on the pollen. New morphological studies (Roque & Funk, 2013) have discussed the set of characters that define the basal clades once included in the Mutisieae and a detailed analysis of the morphology is underway.

The above-mentioned characters (style and anther features) have also been traditionally used to relate *Gochnatia* to other genera in the basal grade of Compositae. However, in light of the family phylogeny obtained by molecular studies, and now with the present studies in Gochnatieae, the diagnostic morphological characters have to be re-evaluated. Our studies show that, after the removal of certain taxa (e.g., Hyalideae), the subfamily Gochnatioideae is monophyletic and composed of seven well-supported clades. All groups within Gochnatioideae have the characters used traditionally to define the "*Gochnatia* complex": apiculate apical anther appendages and short, rounded and dorsally glabrous style branches. Figure 11 illustrates the distribution of the characters with the addition of one new one: style branches that are concave (Figs. 4, 11D; based on information from Cabrera, 1971; Freire & al., 2002; Katinas & al., 2008; and Roque & Funk, 2013). The apiculate anther appendages are restricted to Gochnatieae and Hyalideae as are the smooth style branches that are concave (Fig. 11A, C). The rounded apex and short branches of the style are common across the basal grade, but when used in conjunction with the "smooth" character join the apiculate anther character to provide diagnostic characters for the Gochnatieae and Hyalideae. As regards style branch length, styles of the Gochnatieae and Hyalideae tend to be shorter (most are 0.2–1.2 mm long) while those of the Stifftieae tend to be longer (most are 1.0–3.0 mm). It is easy to see why, based on morphology, the Gochnatieae and Hyalideae were thought to be closely related. The addition of a new character, i.e., style branches "concave" (Figs. 4, 11D), supports the same two clades, however the "concave" branches appear to be missing in *Cyclolepis* and *Cnicothamnus*. Both the apiculate anther appendages and the smooth rounded style branches support *Cyclolepis* as the sister group of the Gochnatieae. Considering the pappus features, as suggested by Lessing (1830), the elements and series numbers of the pappus can corroborate *Cyclolepis* as sister group of Gochnatieae and not Wunderlichioideae (Roque & Funk, 2013). However, Gochnatieae seems to be the only tribe under discussion with a pappus composed of 1–3 series and 25–80 (rarely 90) bristles or scales, instead of a pappus with (3)4–5 series and 100–200 bristles or scales as is found in Hyalideae, Wunderlichieae, and Stifftieae. If true this is an excellent addition because it would separate Hyalideae and Gochnatieae. However, not all the species have been checked for these pappus characters. Other additional characters, such as number of ribs on the achenes or achene pubescence, must also await a more detailed analysis as there is too much missing information to determine their utility.

The value of pollen features in Gochnatieae was recently discussed (Tellería & al., 2013), so here we provide only a brief summary of the results of that study. Tellería & al. (2013) identified two types of pollen within the tribe: type I, pollen grains generally prolate to subprolate, elliptic in equatorial view, and subcircular or triangular in polar view, exine echinate; and type II, pollen grains prolate to spheroidal, elliptic or spheroidal in equatorial view, circular in polar view, exine microechinate. Most clades in Gochnatieae have type I pollen. Type II is present only in *Moquiniastrum*, with the exception of one or two other species. According to these results, the type II pollen character could be regarded as apomorphic within Gochnatieae. *Cyclolepis* shares type II pollen with *Moquiniastrum*, which suggests parallel evolution in the two lineages. One interesting point is that type II pollen supports the inclusion of *M. cordata* (Less.) G.Sancho in *Moquiniastrum*, a new placement for that species based on the phylogeny presented here.

Biogeographic analysis and divergence dates. — The key to understanding the phylogeny of the Gochnatieae lies in the native ranges of the taxa, because each clade occupies a different biogeographic area, although with some overlap. As a result, the biogeography of the Gochnatieae is rather straightforward. Figure 9 has the branches color-coded according to the distribution of the terminal taxa. It seems that the tribe had a southern South American origin (including the southern Andes and southern Brazil) with independent movements into the Central Andes, Mexico and the Caribbean (Cuba, Hispaniola, the Bahamas), and into central Brazil and on into the northernmost areas of Brazil (Roraima) and lowland Venezuela (Anzoátegui). There are no reported collections from Central America, Guyana, Suriname, French Guiana, or Colombia.

The ages for each clade suggest that the core Gochnatieae separated from its relatives in the late Eocene, between 36 and 45 Ma. Later, between 23 and 25 Ma, the Central Andean Clade split from the rest of the tribe, separating the primarily Brazilian and Caribbean-Mexican taxa from the Andean taxa. In the early Miocene, between 14 and 23 Ma, the *Moquiniastrum* group split from *Richterago*. More or less simultaneously (between 11 and 23 Ma) the Mexican *Gochnatia* split from *Anastraphia*. The early Miocene is characterized by a climate warming from initial cooler and drier conditions (Petuch, 2003). Various major diversification events within the above-mentioned genera and clades followed in the late Miocene (between 2.3 and 13.4 Ma). During this time, the Panama isthmus completed its formation, isolating the Atlantic Ocean from the warmer currents of the Pacific. As a result, the global climate cooled, culminating in the Pleistocene glaciations (Petuch, 2003). Cooler and drier conditions might favor the geographic expansion of the group and its diversification because in general the Compositae prefer open habitats and dryer conditions (Funk & al., 2009a).

■ CONCLUSIONS

Can we answer the questions posed in the introduction?

(1) What are the phylogenetic relationships within the tribe? There are eight well-supported clades and while the relationships among the clades are not strong, the same pattern is found in most of the analyses. *Cyclolepis* is always placed between Wunderlichieae and Gochnatieae

(2) Are some of the genera, previously thought to be closely related to *Gochnatia* (sensu Cabrera), actually nested in other clades? This analysis supports recognizing several genera made up of species formerly in *Gochnatia* and the removal from the tribe of other genera (including the four genera of the Hyalideae: *Leucomeris*+*Nouelia*, *Ianthopappus*+*Hyalis*). *Chucoa* is related to the Mutisieae s.str. and *Cyclolepis* is on a long branch that attaches in between the Gochnatieae and Wunderlichieae. The morphology seems to favor its inclusion in the Gochnatieae but some molecular data place it closer to Wunderlichieae. Until we have more information it is designated incertae sedis.

(3) Do the genera of Gochnatieae form a monophyletic group? There are seven clades within the former *Gochnatia* (sensu Cabrera) that are now (or soon will be) separate genera. In addition, two of the original independent genera remain in the Gochnatieae: *Cnicothamnus* and *Richterago* (composed of the former *Actinoseris* plus *Gochnatia* sect. *Discoseris*). There are now (or soon will be) nine monophyletic genera.

(4) Is the tribe the sister group of the clade containing ~96% of the species in the family? In this study Gochnatieae along with its sister group Wunderlicheae and the unplaced *Cyclolepis* form the sister group of the rest of the family, although the support is not strong. *Hecastocleis* remains with the “rest of the family” (see Fig. 9).

(5) Are there any apomorphies that define the tribe? Two characters (apiculate anther appendages and concave style branches) are believed to have evolved in parallel in the Hyalideae and Gochnatieae. The rounded, smooth style apex appears to be plesiomorphic or gained and lost 2–3 times. We can use a combination of the style and anther apices along with the number of series of pappus bristles and the number of bristles to define the tribe but we need to study the bristle characters in more detail.

(6) Can we use the phylogeny to examine diversification within the tribe? We obtained an age of 36–45 Ma for the split between the core Gochnatieae and its sister group. The basal split within the core Gochnatieae was 23–25 Ma. Dates within the tribe are less reliable because the confidence limits of many of the splits are too great to be very useful.

(7) Does a better understanding of the phylogeny and classification of the Gochnatieae help us understand the biogeographic history of the basal grade of the family and its movement from South America to Africa? The phylogeny supports the previous suggestion (Funk & al., 2005; Panero & Funk, 2008) that the extant taxa are derived from a basal radiation in southern South America but it adds a stronger influence from Brazil. It appears that the Gochnatieae phylogeny is following the path of other Compositae tribes such as Liabeae (Funk & al., 2012) and Arctotideae (Funk & Chan, 2008) in that it has very well-supported groups of taxa with poorly supported connections among them.

In addition to the broad-scale morphological analysis mentioned above, the next steps include continuing to work at the species level in some of the less well-known clades (from the former Mutisieae s.l.) and utilizing NextGen Sequencing methods to identify additional genes that might be useful among the taxa of the basal lineages (Mandel & al., 2014).

It is fitting that we conclude by mentioning Angel Lulio Cabrera (1908–1999; Bonifacino & al., 2009), the first person to bring order to the Mutisieae, then thought to be a highly derived tribe within the Compositae (Cabrera, 1970, 1977). Before his revisionary work, very little had been done on these largely southern South America taxa: only an unpublished thesis from the 1950s (Jervis, 1954). Many of Cabrera’s sections turned out to be mostly monophyletic and nearly all of the species he recognized are accepted today: his work provides a foundation for all studies in the Mutisieae s.l. However, we have new data that allow us to evaluate the utility of various morphological characters because we have a better understanding of the direction of evolution. As a result we are able to identify taxa that were included in *Gochnatia* (sensu Cabrera) based on parallel or convergent evolution, those that have lost characters, and those that share true synapomorphies. Finally, we have chosen to recognize the morphological differences among the groups and to emphasize the well-supported monophyletic groups. Some criticize phylogenetic systematists for “splitting” but in reality we are neither “splitters” nor “lumpers”, we simply seek to insure that our classifications reflect what we know about evolution (Funk, 1985).

■ ACKNOWLEDGEMENTS

The authors thank Gabriel Johnson (US) for his help with designing the alternative Cuban *matK* primer and the fungal blocker primer idea and reference. Also, thanks to Thomas F. Patterson (TEX) who made several trips to obtain vouchers, leaf material preserved on silica, and photos of *Gochnatia hypoleuca* in southern Texas; Tom Wendt (TEX) for his help with the voucher table; and Ken Wurdack (US) for his help with editing the Materials and Methods. We thank the following individuals for supplying leaf samples: Walter Judd (FLAS), Pedro Acevedo-R. (US), Charles Zartman (INPA), Susana Freire (SI), and Jun Wen (US). We thank G.A. Parada (MO) and TROPICOS (http://

www.tropicos.org/Name/2706326) for permission to use the photograph of *Gochnatia curvifolia* and M. Mercadante (Brazil) for permission to use his photograph of *Wunderlichia*. Funding for this research was supplied by several agencies and institutions: the office of the Associate Director for Science, Department of Botany, and the Laboratory of Analytical Biology, National Museum of Natural History, Smithsonian Institution (to V.A.F.); ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica) and CONICET (Comisión Nacional de Investigaciones Científicas y Técnicas) to G.S.; and CNPq/PQ, REFLORA and PRONEM (to N.R.). Two reviewers and the Editors (Editor-in-Chief, Managing Editor, Production Editor) provided helpful comments and we appreciate their assistance.

■ LITERATURE CITED

Barreda, V. 1993. Late Oligocene–Miocene pollen of the families Compositae, Malvaceae and Polygonaceae from the Chenque Formation, Golfo San Jorge basin, southeastern Argentina. *Palynology* 17: 169–186. http://dx.doi.org/10.1080/01916122.1993.9989426

Barreda, V.D. & Palamarczuk, S. 2000a. Palinomorfos continentales y marinos de la Formación Monte León en su área tipo, provincia de Santa Cruz, Argentina. *Ameghiniana* 37: 3–12.

Barreda, V.D. & Palamarczuk, S. 2000b. Palinoestratigrafía de depósitos del Oligoceno tardío-Mioceno, en el área sur del Golfo San Jorge, provincia de Santa Cruz, Argentina. *Ameghiniana* 37: 103–117.

Barreda, V., Palazzesi, L. & Marenssi, S. 2009. Palynological record of the Paleogene Río Leona Formation (southernmost South America): Stratigraphical and paleoenvironmental implications. *Rev. Palaeobot. Palynol.* 154: 22–33. http://dx.doi.org/10.1016/j.revpalbo.2008.11.005

Barreda, V., Palazzesi, L., Katinas, L., Crisci, J.V., Tellería, M.C., Bremer, K., Passala, M.G., Bechis, F. & Corsolini, R. 2012. An extinct Eocene taxon of the daisy family (Asteraceae): Evolutionary, ecological and biogeographical implications. *Ann. Bot. (Oxford)* 109: 127–134. http://dx.doi.org/10.1093/aob/mcr240

Bayer, R.J., Greber, D.G. & Bagnall, N.H. 2002. Phylogeny of Australian Gnaphalieae (Asteraceae) based on chloroplast and nuclear sequences, the *trnL* intron, *trnL*/*trnF* intergenic spacer, *matK* and ETS. *Syst. Bot.* 27: 801–814.

Bentham, G. 1873. Compositae. Pp. 163–533 in: Bentham, G. & Hooker, J.D. (eds.), *Genera plantarum ad exemplaria imprimis in herbariis kewensibus servata definite*, vol. 2. London: Reeve.

Bonifacino, J.M., Robinson, H., Funk, V.A., Lack, H.W., Wagenitz, G., Feuillet, C. & Hind, D.J.N. 2009. A history of research in Compositae: Early beginnings to the Reading Meeting (1975). Pp. 3–38 in: Funk, V.A., Susanna, A., Stuessy, T.F., & Bayer, R.J. (eds)., *Systematics, evolution, and biogeography of Compositae*. Vienna: IAPT.

Bremer, K. 1994. *Asteraceae: Cladistics and classification*. Portland: Timber Press.

Cabrera, A.L. 1951. Notas sobre compuestas de America Austral. *Darwiniana* 9: 363–386.

Cabrera, A.L. 1961. Compuestas argentinas. Clave para la determinación de los géneros. *Revista Mus. Argent. Ci. Nat., "Bernardino Rivadavia" Inst. Nac. Invest. Ci. Nat., Bot.* 2: 291–362.

Cabrera, A.L. 1970. *Actinoseris*, un nuevo género de Compuestas. *Bol. Soc. Argent. Bot.* 13: 45–52.

Cabrera, A.L. 1971. Revisión del género *Gochnatia* (Compositae). *Revista Mus. La Plata, Secc. Bot.* 12: 1–160.

Cabrera, A.L. 1977. Mutisieae: Systematic review. Pp. 1039–1066 in: Heywood, V.H., Harborne, J.B. & Turner, B.L. (eds.), *The biology and chemistry of the Compositae*, vol. 2. London: Academic Press.

Carlquist, S. 1957. Systematic anatomy of *Hesperomannia*. *Pacific Sci.* 11: 207–215.

Carlquist, S. 1958. Anatomy of the Guayana Mutisieae. Part II. *Mem. New York Bot. Gard.* 10: 157–184.

Cassini, H. 1816. Quatriéme memoire sur le famille des synantherées, contenant l'analyse de l'ovaire et de ses accesoires. *J. Phys. Chim. Hist. Nat. Arts* 85: 5–21.

Cassini, H. 1819. Suit du sixiéme mémoire sur la familie des Synanthérées, contenat les caractères des tribus. *J. Phys. Chim. Hist. Nat. Arts* 88: 189–204.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Meth.* 9(8): 772. http://dx.doi.org/10.1038/nmeth.2109

Don, D. 1830. Descriptions of the new genera and species of the Class Compositae belonging to the floras of Peru, Mexico, and Chile. *Trans. Linn. Soc. London* 16: 169–303. http://dx.doi.org/10.1111/j.1095-8339.1829.tb00136.x

Downie, S.R. & Katz-Downie, D.S. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 83: 234–251. http://dx.doi.org/10.2307/2445943

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molec. Biol. Evol.* 29: 1969–1973. http://dx.doi.org/10.1093/molbev/mss075

Freire, S.E., Katinas, L. & Sancho G. 2002. *Gochnatia* (Asteraceae: Mutisieae) and the *Gochnatia* complex: Taxonomic implications from morphology. *Ann. Missouri Bot. Gard.* 89: 525–550. http://dx.doi.org/10.2307/3298594

Funk, V.A. 1985. Cladistics and generic concepts in the Compositae. *Taxon* 34: 72–80. http://dx.doi.org/10.2307/1221566

Funk, V.A. & Chan, R. 2008. Phylogeny of the Spiny African Daisies (Compositae, tribe Arctotideae, subtribe Gorteriinae) based on *trnL-F*, *ndhF*, and ITS sequence data. *Molec. Phylogen. Evol.* 48: 47–60. http://dx.doi.org/10.1016/j.ympev.2008.03.035

Funk, V.A., Bayer, R.J., Keeley, S., Chan, R., Watson, L., Gemeinholzer, B., Schilling, E., Panero, J.L., Baldwin, B.G., Garcia-Jacas, N., Susanna, A. & Jansen, R.K. 2005. Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae. *Biol. Skr.* 55: 343–374.

Funk, V.A., Berry, P., Hollowell, T. & Kelloff, C. 2007. A checklist of the plants of the Guiana Shield. *Smithsonian Contr. Bot.* 55: 1–584.

Funk, V.A., Anderberg, A.A., Baldwin, B.G., Bayer, R.J., Bonifacino, J.M., Breitwieser, I., Brouillet, L., Carbajal, R., Chan, R., Coutinho, A.X.P., Crawford, D.J., Crisci, J.V., Dillon, M.O., Freire, S.E., Galbany-Casals, M., Garcia-Jacas, N., Gemeinholzer, B., Gruenstaeudl, M., Hansen, H.V., Himmelreich, S., Kadereit, J.W., Källersjö, M., Karaman-Castro, V., Karis, P.O., Katinas, L., Keeley, S. C., Kilian, N., Kimball, R.T., Lowrey, T.K., Lundberg, J., McKenzie, R.J., Mort, M.E. Nordenstam, B., Oberprieler, C., Ortiz, S., Pelser, P.B., Randle, C.P. Robinson, H., Roque, N., Sancho, G., Semple, J.C., Serrano, M., Stuessy, T.F., Susanna, A., Tadesse, M., Unwin, M., Urbatsch, L., Urtubey, E., Vallès, J., Vogt, R., Wagstaff, S., Ward, J.M. & Watson, L.E. 2009a. Compositae metatrees: The next generation. Pp. 747–777 in: Funk, V.A., Susanna, A., Stuessy, T. & Bayer, R. (eds)., *Systematics, evolution, and biogeography of Compositae*. Vienna: IAPT.

Funk, V.A., Susanna, A., Stuessy, T. & Robinson, H. 2009b. Classification of Compositae. Pp. 171–189 in: Funk, V.A., Susanna, A., Stuessy, T. & Bayer, R. (eds.), *Systematics, evolution, and biogeography of Compositae*. Vienna: IAPT.

Funk, V.A., Kelloff, C. & Chan, R. 2012. Phylogeny and biogeography of the tribe Liabeae (Compositae subfamily Cichoroideae). *Taxon* 61: 437–455.

González-Medrano, F., Villaseñor, J.L. & Medina, R. 2004. A new species of *Gochnatia* (Asteraceae, Mutisieae) from the desert scrubland of the state of Hidalgo, Mexico. *Novon* 14: 434–436.

Hansen, H.V. 1991. Phylogenetic studies in Compositae tribe Mutisieae. *Opera Bot*. 109: 1–50.

Hind, D.J.N. 2007. Tribe Mutisieae. Pp. 90–123 in: Kadereit, J.W. & Jeffrey, C. (eds.), *Families and genera of vascular plants*, vol. 8. Berlin, Heidelberg: Springer.

Hoffmann, O. 1890–1894. Compositae. Pp. 87–391 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol. 4(5). Leipzig: Engelmann.

Jansen, R.K. 1992. Current research. *Pl. Molec. Evol. Newslett*. 2: 13–14.

Jansen, R.K. & Kim, K.J. 1996. Implications of chloroplast DNA data for the classification and phylogeny of the Asteraceae. Pp. 317–339 in: Hind, D.J.N. & Beentje, H. (eds.), *Proceedings of the International Compositae Conference, Kew, 1994*, vol. 1, *Compositae: Systematics*. Kew: Royal Botanic Gardens.

Jeffrey, C. 1967. Notes on Compositae, II. The Mutisieae in east tropical Africa. *Kew Bull*. 21: 177–223. http://dx.doi.org/10.2307/4108506

Jervis, R.N. 1954. A summary of the genus *Gochnatia* including a revision of the West Indian species which comprise the section *Anastraphioides*. Michigan: unpub. thesis.

Johnson, L.A. & Soltis, D.E. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s.str. *Syst. Bot*. 19: 143–156. http://dx.doi.org/10.2307/2419718

Karis, P.O., Källersjö, M. & Bremer, K. 1992. Phylogenetic analysis of the Cichorioideae (Asteraceae), with emphasis on the Mutisieae. *Ann. Missouri Bot. Gard*. 79: 416–427. http://dx.doi.org/10.2307/2399778

Katinas, L., Pruski, J.F., Sancho, G. & Tellería, M.C. 2008. The subfamily Mutisioideae (Asteraceae). *Bot. Rev. (Lancaster)* 74: 469–716. http://dx.doi.org/10.1007/s12229-008-9016-6

Kim, H.G., Loockerman, D.J. & Jansen, R.K. 2002. Systematic implications of *ndhF* sequence variation in the Mutisieae (Asteraceae). *Syst. Bot*. 27: 598–609.

Lessing, C.F. 1830. Synanthereis herbarii regii berolinensis, Dissertatio Tertia, *Linnaea* 5: 237–263.

Lessing, C.F. 1832. *Synopsis generum compositarum*. Berlin: Duncker and Humblot.

Maddison, W.P. & Maddison, D.R. 2011. Mesquite: A modular system for evolutionary analysis, version 2.75 http://mesquiteproject.org

Mandel, J.R., Dikow, R.B., Funk, V.A., Masalia, R.R., Staton, S.E., Kozik, A., Michelmore, R.W., Rieseberg, L.H. & Burke, J.M. 2014. A target enrichment method for gathering phylogenetic information from hundreds of loci: An example from the Compositae. *Applic. Pl. Sci*. 2(2): 1300085. http://dx.doi.org/10.3732/apps.1300085

Ortiz, S., Bonifacino, J.M., Crisci, J.V., Funk, V.A., Hansen, H., Hind, D.J.N., Katinas, L., Roque, N., Sancho, G., Susanna, A. & Telleria, M.C. 2009. The basal grade of Compositae: Mutisieae (sensu Cabrerra). Pp. 193–213. in: Funk, V.A., Susanna, A., Stuessy, T.F. & Bayer, R.J. (eds.), *Systematics, evolution, and biogeography of Compositae*. Vienna: IAPT.

Panero, J.L. 2009. *Chucoa ilicifolia*, a spiny *Onoseris* (Asteraceae, Mutisioideae, Onoserideae). *Phytologia* 91: 537–541.

Panero, J.L. & Freire, S.E. 2013. *Paquirea*, a new Andean genus for *Chucoa lanceolata* (Asteraceae, Mutisioideae, Onoserideae). *Phytoneuron* 2013–11: 1–5.

Panero, J.L. & Funk, V.A. 2002. Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). *Proc. Biol. Soc. Washington* 115: 909–922.

Panero, J.L. & Funk, V.A. 2007. New infrafamilial taxa in Asteraceae. *Phytologia* 89: 356–360.

Panero, J.L. & Funk, V.A. 2008. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. *Molec. Phylogen. Evol*. 47: 757–782. http://dx.doi.org/10.1016/j.ympev.2008.02.011

Petuch, E.J. 2003. *Cenozoic seas: The view from eastern North America*. Boca Raton: CRC Press. http://dx.doi.org/10.1201/9780203495858

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molec. Biol. Evol*. 25: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Rambaut, A. 2012. FigTree, version 1.4. Program distributed by the author. United Kingdom: Molecular Evolution, Phylogenetics and Epidemiology. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A. & Drummond, A.J. 2009. Tracer, version 1.5. Program distributed by the author. United Kingdom: Molecular Evolution, Phylogenetics and Epidemiology. http://tree.bio.ed.ac.uk/software/tracer

Robinson, H. & Funk, V.A. 2012. Asteraceae. Pp. 89–142 in: Acevedo-Rodríguez, P. & Strong, M.T. (eds.), Catalogue of seed plants of the West Indies. *Smithsonian Contr. Bot*. 98: i–xxv, 1–192.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2011. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol*. 61: 539–542. http://dx.doi.org/10.1093/sysbio/sys029

Roque, N. & Funk, V.A. 2013. Morphological characters add support for some members of the basal grade of Asteraceae. *Bot. J. Linn. Soc*. 171: 568–586. http://dx.doi.org/10.1111/boj.12000

Roque, N. & Hind, D.J.N. 2001. *Ianthopappus*, a new genus of the tribe Mutisieae (Compositae). *Novon* 11: 97–101. http://dx.doi.org/10.2307/3393216

Roque, N. & Pirani, J.R. 2001. Reinstatement of the name *Richterago* Kuntze and recircumscription of the genus to include species formerly treated as *Actinoseris* (Endl.) Cabrera (Compositae, Mutisieae). *Taxon* 50: 1155–1160. http://dx.doi.org/10.2307/1224734

Roque, N. & Pirani, J.R. 2014. Taxonomic revision of *Richterago* Kuntze (Asteraceae, Gochnatieae). *Syst. Bot*. 39: 997–1026

Roque, N. & Silvestre-Capelato, M.S.F. 2001. Generic delimitation of *Gochnatia*, *Richterago* and *Ianthopappus* (Compositae-Mutisieae) based on pollen morphology. *Grana* 40: 197–204. http://dx.doi.org/10.1080/001731301317223132

Rozas, J. 2009. DNA sequence polymorphism analysis using DnaSP. Pp. 337–350 in: Posada, D. (ed.), *Bioinformatics for DNA squence analysis*. Methods in Molecular Biology Series 537. New Jersey: Humana Press. http://dx.doi.org/10.1007/978-1-59745-251-9_17

Sancho, G. 1999. Novedades taxonómicas en *Gochnatia* (Asteraceae, Mutisieae). *Novon* 9: 557–561. http://dx.doi.org/10.2307/3392165

Sancho, G. 2000. Revisión y filogenia de la sección *Moquiniastrum* Cabrera del género *Gochnatia* Kunth (Asteraceae, Mutisieae). *Fontqueria* 54: 61–122.

Sancho, G. & Freire, S.E. 2009. The tribes Gochnatieae and Hyalideae. Pp. 249–260 in: Funk, V.A., Stuessy, T.F., Susanna, A. & Bayer, R.J. (eds.), *Systematics, evolution, and biogeography of Compositae*. Vienna: IAPT.

Sancho, G., Freire, S.E., Katinas, L. & Tellería, M.C. 2005. A new species and a new combination in Andean Mutisieae (Asteraceae). *Taxon* 54: 85–90. http://dx.doi.org/10.2307/25065304

Sancho, G., Funk, V.A. & Roque, N. 2013. *Moquiniastrum* (Gochnatieae, Asteraceae): Disentangling the paraphyletic *Gochnatia*. *Phytotaxa* 147(1): 26–34. http://dx.doi.org/10.11646/phytotaxa.147.1.3

Sang, T., Crawford, D.J. & Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). *Amer. J. Bot*. 84: 1120–1136. http://dx.doi.org/10.2307/2446155

Scott, L., Cadman, A. & McMillan, I. 2006. Early history of Cainozoic Asteraceae along the Southern African West coast. *Rev. Paleobot. Palynol*. 142: 47–52. http://dx.doi.org/10.1016/j.revpalbo.2006.07.010

Steele, K.P. & Vilgalys, R. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. *Syst. Bot*. 19: 126–142. http://dx.doi.org/10.2307/2419717

Sukumaran, J. & Holder, M.T. 2010. DendroPy: A Python library for phylogenetic computing. *Bioinformatics* 26: 1569–1571. http://dx.doi.org/10.1093/bioinformatics/btq228

Swofford, D.L. 2011. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0a114, 12 Mar 2011. Sunderland, Massachusetts: Sinauer.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109. http://dx.doi.org/10.1007/BF00037152

Tate, J.A. & Simpson, B.B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Syst. Bot.* 28: 723–737.

Tellería, M.C., Sancho, G., Funk, V.A., Ventosa, I. & Roque, N. 2013. Pollen morphology and its taxonomic significance in the tribe Gochnatieae (Compositae, Gochnatioideae). *Pl. Syst. Evol.* 299: 935–948. http://dx.doi.org/10.1007/s00606-013-0774-1

Ventosa-Rodriguez, I. & Herrera-Oliver, P.P. 2011a. Do the Antillean species of *Gochnatia* Kunth (Asteraceae) truly belong in that genus? A phylogenetic analysis based on morphological characters. *Compositae Newslett.* 49: 8–22.

Ventosa-Rodriguez, I. & Herrera-Oliver, P.P. 2011b. Restoration of the name *Anastraphia* to define the species in the section *Anastraphioides* of *Gochnatia* (Gochnatioideae, Asteraceae). *Compositae Newslett.* 49: 23–37.

Ventosa-Rodriguez, I. & Herrera-Oliver, P.P. 2011c. Five new species of *Anastraphia* D.Don (Gochnatioideae, Asteraceae) from Cuba. *Compositae Newslett.* 49: 38–55.

Vestheim, H. & Jarman, S.N. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples: A case study on prey DNA in Antarctic krill stomachs. *Frontiers Zool.* 5: 12. http://dx.doi.org/10.1186/1742-9994-5-12

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M., Gelfand, D., Sninsky, J. & White, T. (eds.), *PCR protocols: A guide to methods and applications*. San Diego: Academic Press.

Zavada, M.S. & De Villiers, S.E. 2000. Pollen for the Asteraceae from the Paleocene–Eocene of South Africa. *Grana* 39: 39–45. http://dx.doi.org/10.1080/00173130150503795

Zwickl, D.J. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. Dissertation, University of Texas, Austin, U.S.A.