

ASTERACEAE

Insights into the evolution of the tribe Arctoteae (Compositae: subfamily Cichorioideae s.s.) using *trnL-F*, *ndhF*, and ITS

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Compositae (Asteraceae) are the largest flowering plant family (23,000 to 30,000 species) and its members are found throughout the world in both temperate and tropical habitats. The subfamilies and tribes of Compositae remained relatively constant for many years; recent molecular studies, however, have identified new subfamilial groups and identified previously unknown relationships. Currently there are 35 tribes and 10 subfamilies (Baldwin & al., 2002; Panero & Funk, 2002). Some of the tribes and subfamilies have not been tested for monophyly and without a clear understanding of the major genera that form each tribe and subfamily, an accurate phylogeny for the family cannot be reconstructed. The tribe Arctoteae (African daisies) is a diverse and interesting group with a primarily southern African distribution (ca. 17 genera, 220 species). They are especially important in that most of the species are found in the Cape Floral Kingdom, the smallest floral kingdom and the subject of intense conservation interest. Arctoteae are part of the monophyletic subfamily Cichorioideae s.s. Other tribes in the subfamily include Eremothamneae, Gundelieae, Lactuceae, Liabeae, Moquineae, and Vernonieae, and these were all evaluated as potential outgroups. Ultimately 29 ingroup taxa and 16 outgroup taxa with a total of 130 sequences (125 newly reported), from three genetic regions, two from chloroplast DNA (*trnL-F* and *ndhF*) and one from the nuclear genome (ITS), were used to evaluate the tribe and its proposed outgroups. Each molecular region is examined separately, the chloroplast markers are examined together, and the data are combined. The data were analyzed with and without outgroups and problem taxa using parsimony and maximum likelihood methods. The analyses showed robust support for two outgroup clades, Liabeae-Vernonieae and Gundelieae-Lactuceae and two main subtribes within Arctoteae: Arctotineae and Gorteriinae. Support for monophyly of Arctoteae is weak. Within Arctoteae, some taxa of interest are easily placed: *Didelta*, *Cuspidia* and *Heterorhachis* are consistently part of subtribe Gorteriinae, *Cymbonotus*, the Australian genus, is nested within subtribe Arctotineae, and *Haplocarpha* is at the base of Arctotineae. *Berkheya*, *Haplocarpha*, and *Hirpicium* are probably paraphyletic. Furthermore, *Platycarpha* most likely does not belong in Arctoteae, and *Heterolepis* and the tribe Eremothamneae are within Arctoteae but not within either of the two main subtribes. After some rearrangements, the two main subtribes, Arctotineae and Gorteriinae, are monophyletic and the latter has three clades. The study shows that the unusual taxa are of critical importance, and they should be included in any molecular analysis. Adequate representation of the ingroup is also important as all previous studies of Arctoteae had involved only a few taxa from the core subtribes, and so did not reveal the problems. Multiple outgroups evaluated in an iterative manner had pronounced effects on the relationships within the ingroup, not only on the position of the root. Finally, unrooted consensus trees and unrooted phylograms were found to be very useful in analyzing the data, allowing for examination of placement of taxa without the bias of a rooted tree.

KEYWORDS: Arctoteae, Asteraceae, Cape Floral Kingdom, Cichorioideae, Compositae, ITS, molecular phylogenetics, *ndhF*, outgroups, South Africa, *trnL*.

INTRODUCTION

The Compositae family has the largest number of species of any family of seed plants (23,000–30,000), and today its members can be found on every continent except Antarctica. The subfamilial classification has had

minor revisions, but until recently it had not seriously changed since the 13 tribes of Bentham (Bentham, 1873a, b). The advent of DNA sequence data from the chloroplast and nuclear genomes has changed Compositae systematics in dramatic ways: first, they have identified basal clades that turned the ideas about

evolution within the family upside down (Jansen & al., 1991; Kim & Jansen, 1995) and second, more recently by revisions that have divided the family into 10 subfamilies and 35 tribes (Baldwin & al., 2002; Panero & Funk, 2002; Panero & al., unpubl.). Many of the traditional tribes had broad distributions covering hemispheres (i.e., Lactuceae, Heliantheae s.l.), or nearly the whole globe (e.g., Senecioneae). Three of the more modestly sized tribes, Arctoteae (southern Africa and Australia), Calenduleae (southern Africa), and Liabeae (Andean South America, Central America, Mexico, and the Caribbean), have rather restricted distributions and therefore lend themselves to detailed systematic and biogeographic investigations. One of these, Arctoteae, is the subject of our investigation. This tribe is particularly interesting not only because it is more or less confined to southern Africa, but also because most of its species are endemic to the Cape Floral Kingdom.

The tribe Arctoteae belongs to the redefined subfamily Cichorioideae s.s. (Panero & Funk, 2002). The subfamily contains from four to seven tribes, depending on where the member taxa are found in the final analysis and on one's personal philosophy with regard to Compositae classification. The four traditional and larger tribes are Arctoteae (African daisies), Lactuceae (or Cichorieae; dandelions), Liabeae (Andean sunflowers), and Vernonieae (ironweeds). The three small tribes (with one or two genera each) are Eremothamneae (*Eremothamnus* and *Hoplophyllum*; authors for all genera are found in Table 1), Gundelieae (*Gundelia*, crown

of thorns), and Moquineae (*Moquinea* and *Pseudostiffia*). None of the relationships among the tribes have been resolved, and some of the taxa are not universally accepted at the tribal level. For purposes of this study, Vernonieae, Liabeae, and Lactuceae were included as potential outgroups for Arctoteae. The tribe Moquineae was assumed to be close to, if not actually in, the Vernonieae clade and was not included. The genera of Eremothamneae were treated as part of Arctoteae and *Gundelia* was originally treated as part of Arctoteae, but it eventually fell outside the tribe.

Data from a variety of sources (Cassini, 1816, 1821; Beauverd, 1915a, b; Robinson & Brettell, 1973a; Norlindh, 1977; Robinson, 1992, 1994; Bremer, 1994; Herman & al., 2000; Leistner, 2000) as well as personal observations show that the tribe Arctoteae does not have an abundance of characters to support its monophyly. Beginning with Cassini, the traditional definition of Arctoteae was based on the presence of a swollen area with a ring of hairs located just below the branch point on the style. While there are hairs in a ring just below the division in the style, this character is also found in some thistles, and the presence of a swollen area is found mostly in the subtribe Arctotinae. Members of the two main subtribes of Arctoteae have sagittate but not tailed anthers (except for *Arctotis* which has calcarate, short caudate anthers), and the heads usually have both ray and disc florets. The stigmatic papillae are spread over the inner surfaces of the style branches, but this character is found in many tribes in the bottom third of the family

Table 1. Highlights of classification and nomenclature of the tribe Arctoteae (Compositae). Numbers in parentheses indicate the number of species in the genus.

Traditional (modified Bentham, 1873b)	Robinson & Brettell, 1973	Heywood & al. (various chapters), 1977	Bremer, 1994
Subtribe Arctotinae Less.			
<i>Arctotheca</i> Wendl. (4)		Arctotinae	Arctotinae
<i>Arctotis</i> L. (~64)		Arctotinae	Arctotinae
<i>Cymbonotus</i> Gaud. (2)		Arctotinae	Arctotinae
<i>Dymondia</i> Compton (1)		Arctotinae	Arctotinae
<i>Haplocarpha</i> Less. (10)		Arctotinae	Arctotinae
Subtribe Gorteriinae Benth. & Hook. f.			
<i>Berkheya</i> Ehrh. (~75)		Gorteriinae	Gorteriinae
<i>Cullumia</i> R. Br. (15)		Gorteriinae	Gorteriinae
<i>Cuspidia</i> Gaertn. (1)		Gorteriinae	Gorteriinae
<i>Didelta</i> L'Herit (2)		Gorteriinae	Gorteriinae
<i>Gazania</i> Gaertn. (17)		Gorteriinae	Gorteriinae
<i>Gorteria</i> L. (3)		Gorteriinae	Gorteriinae
<i>Heterolepis</i> Cass. (3)		Gorteriinae by Norlindh, Mutisieae by Merxmüller	unassigned to subtribe
<i>Heterorhachis</i> Sch. Bip. (1)		Gorteriinae	Gorteriinae
<i>Hirpicium</i> Cass. (12)		Gorteriinae	Gorteriinae
Subtribe Gundeliinae Bentham			
<i>Gundelia</i> L. (1)	Tribe Gundelieae H. Rob. & Brettell	Subtribe Gundeliinae	Subtribe Gorteriinae
<i>Eremothamnus</i> O. Hoffm. (1)	Tribe Eremothamneae H. Rob. & Brettell	Subtribe Eremothamniinae Lenis	unassigned to tribe
<i>Platycarpha</i> Less. (3)	Tribe Cynareae	Tribe Cynareae (Norlindh) (rejected by Dittrich)	unassigned to subtribe
<i>Hoplophyllum</i> DC. (2)		Tribe Vernonieae	unassigned to tribe
<i>Ursinia</i> Gaertn.	Tribe Ursinieae H. Rob. & Brettell	Tribe Anthemideae	Tribe Anthemideae

phylogeny. The pappus is usually made up of scales (absent in *Cymbonotus* and *Cullumia*), but *Heterolepis* has what has been described as “bristle-like” scales. Most members of the tribe are annual or perennial herbs, but some are shrubs or subshrubs; most have yellow flowers, but there are some noticeable exceptions such as *Arctotis* and *Gazania* (Figs. 1B, C, E). All of the species in the Arctoteae, except those in *Cymbonotus* (2–3 species, Australia), are native to southern Africa.

The tribe Arctoteae was first recognized by Cassini (1816) who described a total of 14 genera, three of which were later placed in synonymy. In 1832, Lessing reduced the group to the rank of subtribe within the tribe Cardueae (thistles); he recognized 18 genera, including five new ones. Most modern treatments are based on the work of Bentham (Bentham, 1873a, b) who reinstated Arctoteae at the tribal level with three subtribal groups: Euarctoteae (now Arctotinae), Gorterieae (Gorteriinae), and Gundelieae (Gundelinae). Hoffmann (1890) used Bentham’s subtribes, but he interpreted the genus *Arctotis* in the broad sense, encompassing most of the modern-day genera of the subtribe. Minor movements of problem genera ensued. Bentham (1873b) removed *Heterolepis* from Arctoteae and placed it in Inuleae. Hoffmann placed *Eremothamnus* in Senecioneae (1890). Beauverd (1915a) transferred *Ursinia* from Arctoteae to Anthemideae based on style morphology. Moore (1929) moved *Eremothamnus* into Inuleae, and Merxmüller (1967) put it back into Senecioneae. Stix (1960) suggested that *Platycarpha* should be in Mutisieae. Prior to 1970 *Eremothamnus* had been placed in the tribes Senecioneae, Liabeae, Astereae, Gnaphalieae, and Inuleae, but in 1970 Leins suggested that *Eremothamnus* was in Arctoteae, close to but not inside Gorteriinae. In 1973, Robinson and Brettell (1973a) took *Ursinia* out of Arctoteae and put it into its own tribe based on pollen characters. Also in the same publication, Robinson and Brettell put *Platycarpha* in Cardueae (thistles) and returned *Heterolepis* to Arctoteae. In that same year, Robinson & Brettell (1973b) moved both *Eremothamnus* and *Gundelia* into tribes of their own.

In the book *Biology and Chemistry of the Compositae* (Heywood & al., 1977), each tribe was discussed in one or more chapters, and comments were made concerning the placement of problem taxa. Norlindh (1977) prepared the treatment of Arctoteae; he accepted the three subtribes of Bentham and added the subtribe Eremothamninae while excluding *Platycarpha* and *Ursinia*. Dittrich (1977) in his treatment of the thistles (Cardueae) rejected both *Platycarpha* and *Gundelia* and suggested that they should be placed in Arctoteae. Merxmüller (1977), in his treatment of Inuleae, agreed that *Heterolepis* did not belong in that tribe and suggested a placement in Mutisieae, and Jones (1977) included

Hoplophyllum in Vernoniaeae. Heywood & Humphries (1977) placed *Ursinia* into Anthemideae but considered the placement dubious. More recently, Karis (1992) published a cladistic study based on morphology that suggested that *Hoplophyllum* was sister to *Eremothamnus*, and he had moderate support for the two genera being closely related to Arctoteae. Robinson (1994) used pollen data to support his hypotheses that *Gundelia* and *Eremothamnus* belonged in tribes of their own and not in Arctoteae. Bergqvist & al. (1995) used cpDNA restriction site data from 58 genera of the family to examine the position of *Eremothamnus* and found that it was sister to Arctoteae, but he used only one genus (*Gazania*) from Arctoteae. The most recent classification of Arctoteae was published by Bremer (1994); he recognized only the two main subtribes by sinking Gundelieae into Gorteriinae. He listed *Eremothamnus* and *Hoplophyllum* as belonging to the subfamily Cichorioideae s.l. but “unplaced” as to tribe. Karis & al. (2001) used sequence data from *ndhF* to suggest that *Gundelia* did not belong in Arctoteae but was the sister group of Cichorieae (Lactuceae). Based on these data, *Gundelia* could be included in Lactuceae or left in its own monotypic tribe, Gundelieae. Most recently the Saharan genus *Warionia* has been shown to be closely related to, and possibly the sister taxon of, *Gundelia* (Panero & Funk, 2002), and these two together now constitute Gundelieae and are sister to Lactuceae. *Ursinia* has been placed in Anthemideae based on morphological and molecular data (Heywood & Humphries, 1977; Watson & al., 2000). However, problems remain concerning the placement of several genera: *Eremothamnus*, *Heterolepis*, *Hoplophyllum*, and *Platycarpha*. In addition, *Cuspidia*, *Didelta*, *Haplocarpha*, and *Heterorhachis* are unusual taxa morphologically, and a confirmed placement of these genera would help with understanding the evolution of the morphology of the tribe. *Heterorhachis* and *Cuspidia* are monotypic genera that have unusual features, *Haplocarpha* has a variable chromosome number, and *Didelta* has an unusual condition where its receptacle breaks into parts enclosing the achenes. The outer parts are adnate to one of the outer involucre bracts that become thickened and lignified and new plants may germinate out of these parts (Fig. 1A). Finally, *Cymbonotus*, the only genus found outside of southern Africa, may be designated “endangered” in its native Australia and so its position in the tribe needs to be confirmed. Table 1 presents an historical summary of taxonomy of Arctoteae.

The ultimate goal of our research effort is to produce a detailed morphological study and molecular analysis of Arctoteae and to use the results to investigate the evolution and biogeographical history within the tribe and among tribes of the subfamily Cichorioideae s.s. Obviously, this task will take some time and involve sev-



Fig. 1. Variation in shape and color of heads in the mostly southern African tribe Arctoteae. A, *Didelta*; B, C, *Arctotis*; D, *Berkheya*; E, *Gazania*. All photos by V. Funk.

eral projects. The goals of this paper are to investigate the monophyly of Arctoteae, determine the sister group(s) and/or most appropriate outgroup(s), determine placement of problem and unusual genera, and establish a sound basis for a subtribal classification of the tribe.

MATERIALS AND METHODS

Taxon sampling. — The terms “ingroup” and “outgroup” are, of course, relative to one another and change based on the specific question being addressed. For this study we consider the ingroup to include all taxa that were previously thought to be in the tribe Arctoteae, with the exception of *Ursinia* (now in Anthemideae). These ingroup genera were sampled thoroughly by including several species of each genus so that all “groups” within the genera were sampled. At least two populations were sampled in the monotypic genera. The first set of outgroups for this study included all taxa outside of traditional Arctoteae that are found in the subfamily Cichorioideae s.s. (Appendix). A second set of outgroups from outside the subfamily were added later (Appendix). In general, all outgroup taxa were sampled less thoroughly than the ingroup taxa.

Ingroup. Seventeen ingroup genera were used in this study, including all but one of the genera previously placed in Arctoteae (excluding *Ursinia*: Anthemideae). A total of 76 samples was analyzed and 28 of these ingroup samples (Appendix) were selected to be used in the pruned tree. There are 78 newly reported sequences, 26 ITS, 27 *trnL-F*, and 25 *ndhF*, and two taken from GenBank.

Outgroups. Sixteen species in 15 outgroup genera were sampled for this study (Appendix). There are 44 newly reported sequences, 14 ITS, 15 *trnL-F*, and 15 *ndhF*, one sequence was taken from GenBank and two were sent by J. Panero (pers. comm.).

The tribes of Cichorioideae s.s. (Arctoteae, Eremothamneae, Gundelieae, Lactuceae, Liabeae, Moquineae, and Vernonieae) form a monophyletic group with good support (Panero & Funk, 2002); the relationships among these tribes, however, is poorly understood. All of the tribes were considered as possible outgroups for Arctoteae, but only four were used. The Eremothamneae were considered by Norlindh (1977) to be part of Arctoteae, so it was included in the ingroup. Gundelieae (as *Gundelia*) was also included in Arctoteae by Norlindh (1977) and Bremer (1994), but, based on *ndhF* sequence data, Karis suggested that Lactuceae and *Gundelia* were sister taxa (Karis & al., 2001). Our initial analyses supported this, and so the tribe Gundelieae was moved to the outgroup. The Gundelieae were, until recently, believed to be monotypic, but they have now

been shown to contain *Warionia* as well as *Gundelia* (Panero & Funk, 2002); both genera were used in this study. Lactuceae, morphologically distinct but poorly known molecularly, was represented by three common genera (*Cichorium*, *Hypochaeris*, and *Lactuca*). The taxa from Liabeae, a modest-sized tribe from the Americas, were selected because they represent the two main lineages of the tribe and one member of the paraphyletic base of the tribe (Kim & al., 2003). Vernonieae, a large and diverse tribe with ca. 2500 species, was included as an outgroup. The taxa from Vernonieae used in the study were selected because they were near the base of the preliminary trees produced in an ongoing molecular study of the tribe (Keeley & Chan, pers. comm.). Previously it has been suggested that Vernonieae and Liabeae might be closely related, in fact Cassini had Liabeae as a subunit of Vernonieae (Cassini, 1828, 1830) and other authors have also placed the two together (e.g., Robinson & Funk, 1987; Bergqvist & al., 1995). Moquineae were considered to be part of Vernonieae and were not included in this study. Once the data were combined, additional outgroups could be added from outside of subfamily Cichorioideae including *Barnadesia* and *Dasyphyllum* from subfamily Barnadesioideae, *Gerbera* (two species) from the tribe Mutisieae s.s., and *Carthamus* and *Cirsium* from the tribe Cardueae (thistles).

The DNA sequences were obtained from several sources. Ongoing studies by Keeley and Chan (unpubl.) provided sequences for Vernonieae. Panero (unpubl.) provided the *trnL-F* and *ndhF* for *Warionia*. GenBank provided two ITS sequences (*Hoplophyllum* and *Warionia*) and one *ndhF* (*Eremothamnus*). Although we tried repeatedly to obtain additional sequences from *Eremothamnus*, we were unsuccessful; these two regions, therefore, are missing for this taxon. It does not appear to have created any problems, however; all *ndhF* and combined analyses were conducted with and without the genus, and it made no difference in the results. The remaining 125 sequences are newly reported; the Appendix contains a list of vouchers and the GenBank numbers for all sequences. All plant tissue was collected in the field and stored in silica gel, sampled from herbarium specimens, or freshly collected.

DNA amplification, sequencing, and analysis. — DNA extractions were performed using a Qiagen DNeasy Plant Mini Kit following the instructions supplied but with an extended incubation period (up to 40 minutes) for herbarium material. Primer ITS5A (Downie & Katz-Downie, 1996), based on White & al.’s (1990) fungal primer ITS5 and corrected at two positions for angiosperms, was substituted for ITS5 in this study. All primer sequences are given in Table 2. Primers used to amplify and sequence the *trnL-F* and downstream *trnL-F* spacer region of cpDNA were designed by Taberlet & al.

(1991), and those used for the 3' end of the *ndhF* region were designed by Jansen (1992).

For the PCR amplification reactions, each 25 μ l PCR reaction cocktail contained 12.9 μ l of sterile water, 2.5 μ l of 10x PCR reaction buffer A (Promega), 2 μ l of 20 mM dNTPs (Pharmacia) in an equimolar ratio, 2.5 μ l of 25 mM magnesium chloride, 0.5 μ l of 10 mg/ μ l Bovine Serum Albumin (Sigma), 1 μ l of a 10 μ M concentration each of the forward and reverse primer, 0.1 μ l of Taq DNA polymerase enzyme (5 units/ μ l from Promega), and 2.5 μ l of sample DNA. The amount of template DNA was adjusted when necessary to generate sufficient PCR products for DNA sequencing.

The amplification reactions were conducted using thin-walled 0.2 ml PCR reaction tubes in a GeneAmp PCR System 9700 (Perkin Elmer). The PCR program consisted of an initial preheating at 94° C for 2 minutes. Then, the first reaction cycle proceeded as follows: 1 minute at 94° C to denature the template DNA, followed by 1 minute at 48° C (54° C for cpDNA) to allow primer annealing and 45 seconds at 72° C for primer extension. Primer extension time was increased by 4 seconds (7 seconds for cpDNA) for each subsequent reaction cycle. After a total of 40 reaction cycles, an additional 7 minute extension at 72° C was allowed for completion of unfinished DNA strands. All PCR products were quantified by agarose gel electrophoresis with comparison of an aliquot of products with a known quantity of a 100 bp DNA ladder (GeneChoice; visualized with ethidium bromide). The remainder was stored at 4° C until utilized.

PCR products used for sequencing were first purified for sequencing using an enzymatic PCR product pre-sequencing kit (USB). This procedure involved mixing 8 μ l of the PCR product with 1 μ l of each enzyme from the kit and then incubating the mixture first at 37° C for 30 min, to degrade excess primers and dNTPs, and then raising the temperature to 80° C for 15 minutes, to denature the enzymes themselves. This method of purification without loss of PCR products (no filtration, precipitation, or washes are necessary) is especially important for DNA extracted from herbarium vouchers, which is sometimes only weakly amplified and yields barely sufficient PCR product for sequencing.

Table 2. Primer sequences used for PCR and cycle sequencing.

Name	Sequence (5' to 3')
ITS5A	GGA AGG AGA AGT CGT AAC AAG G
ITS4	TCC TCC GCT TAT TGA TAT GC
<i>trnL</i> C	CGA AAT CGG TAG ACG CTA CG
<i>trnL</i> F	ATT TGA ACT GGT GAC ACG AG
<i>ndhF</i> 1603	CCT YAT GAA TCG GAC AAT ACT ATG C
<i>ndhF</i> +607	ACC AAG TTC AAT GYT AGC GAG ATT AGT C

The cycle sequencing reactions were done using 96 well microplates in a PTC-100 thermal cycler (MJ Research). Each one-eighth cycle sequencing reaction cocktail contains 50–150 ng of the purified PCR product, 2 μ l of a 1 mM concentration of the sequencing primer, 0.6 μ l of a 5x reaction buffer (400 mM Tris HCl, 10 mM magnesium chloride at pH 9.0), and 1 μ l of the reagent pre-mix from the BigDye (Version 2/3) dye terminator cycle sequencing pre-mix kit (Applied Biosystems). The cycle sequencing program consisted of an initial preheating at 96° C for 30 seconds. Then, the first reaction cycle proceeded as follows: 10 seconds at 92° C to denature the template DNA, followed by 15 seconds at 55° C to allow primer annealing and 4 minutes at 60° C for primer extension. Unincorporated dye terminators were removed by Sephadex (Sigma) gel filtration using MultiScreen plates (Millipore). The purified cycle sequencing products were then resolved by electrophoresis on a 5% polyacrylamide (MJ Research Kilobasepack) gel using a BaseStation 51 automated DNA sequencer (MJ Research). Sequences from both strands of each PCR product were examined, compared, and corrected using Sequence Navigator software (Applied Biosystems).

All *trnL-F*, *ndhF*, and ITS sequences were aligned visually, with the insertion of gaps where necessary. There were five unambiguous insertions and deletions, mainly in the *trnL-F* data set. Three deletions and one insertion supported all or part of the *Gazania-Gorteria-Hirpicium* clade. The remaining deletion (*ndhF*) was found in all of the ingroup and first set of outgroups (Cichorioideae s.s.) but not in the second set of outgroup taxa. The taxon groups that these indels supported occurred in the phylogenies without coding the indels (see Results), so it did not seem necessary to separately code the indels. Maximum parsimony analysis and parsimony bootstrap analysis (with 1000 replicate runs, each with 10 random taxon additions, TBR branch swapping, and MULPARS in effect) of the aligned *trnL-F*, *ndhF*, and ITS sequences were performed (with and without the outgroups) for each region and for the cpDNA data and the combined data sets via full heuristic searches with PAUP* (Swofford, 2002). No weighting was used. Maximum parsimony analysis using a branch-and-bound search was also performed with a reduced dataset of 17 ingroup and three outgroup taxa selected from previous results. The bootstrap runs employed 1000 replicates with branch-and-bound searches. The likelihood ratio tests were done using the "Tree Scores" function in PAUP* under the likelihood criterion, the Hasegawa-Kishino-Yano model of sequence evolution (Hasegawa & al., 1985), and a gamma distribution of rate variation among sites (with the shape parameter estimated and with four rate categories). The likelihood and the boot-

strap analyses were used on each molecular region separately, the two chloroplast regions combined, and all three regions combined.

RESULTS

A number of full heuristic searches were run using various outgroups and adding and subtracting members of the ingroup. These initial runs contained all taxa and all outgroups. These runs produced thousands of equally parsimonious trees, but they consistently showed several things. The core elements of two subtribes formed strongly supported clades, Arctotineae (ARCT) and Gorteriinae (GORT), and within Gorteriinae there are three groups, the *Didelta-Berkheya* clade (Did), *Berkheya-Cullumia* clade (Ber), and the *Gazania-Gorteria-Hirpicium* clade (Gaz). *Berkheya* appears to be paraphyletic and *Hirpicium* and *Haplocarpha* are most likely paraphyletic as well. The 10 outgroup taxa from the first set of outgroups (Appendix 1) consistently divided into two clades, Liabeae and Vernoniaeae (LIA-VER) and Gundelieae and Lactuceae (GUN-LAC). However, the placement of some of the problem taxa, as well as the relationships of the outgroups to the clades within the ingroup and to one another, was unresolved. An examination of many of the equally parsimonious trees showed that most of the alternative trees were the result of closely related taxa changing positions. In order to elucidate relationships among the problem taxa, outgroup taxa, and core subtribes, some taxa were pruned from the dataset. For instance, in the full dataset there were eight samples of *Arctotis*, 18 of *Berkheya*, nine of *Gazania*, and nine of *Hirpicium* and all of these were not pertinent to the goals of this study. In order to decide which taxa to remove from this portion of the study, we ran the data without outgroups. The resulting unrooted phylograms allowed us to select taxa for removal that had similar positions, and so there was no loss of relationships within or among genera. Taxa were selected so as to keep the basic structure of the unrooted phylogram, e.g., all potentially paraphyletic groups were maintained and members of the core subtribes were arranged in the same manner. Because all of the members of each genus indicated to be paraphyletic were contained within a single subtribe, the fact that they are paraphyletic does not affect the questions being asked in this study. After pruning the database there were 28 ingroup samples. The analyses were then re-run using the pruned data set to confirm to the overall branching structure. Figure 2 shows an unrooted phylogram with labels on the major groups plus abbreviations that will be used in most figures; the remaining problem taxa and outgroups are not included. The basic shape of Fig. 2 was taken from

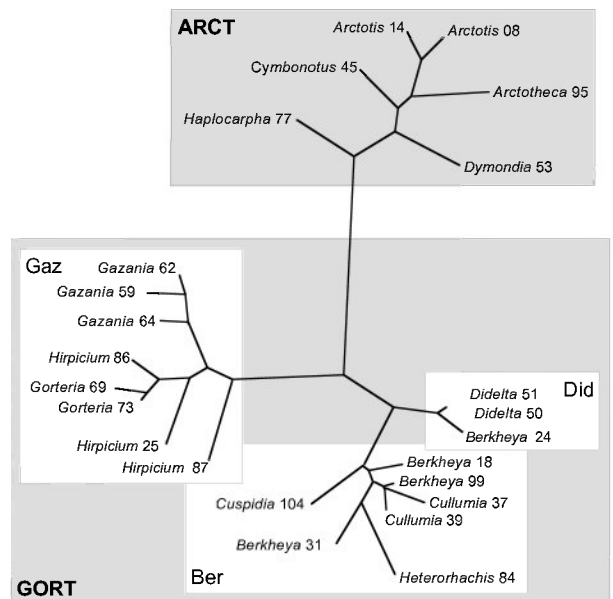


Fig. 2. An unrooted phylogram of Arctoteae showing the location of the two main subtribes, Arctotineae (ARCT), Gorteriinae (GORT), and the three clades of the latter: *Gazania-Gorteria-Hirpicium* (Gaz), *Berkheya-Cullumia* (Ber), and *Didelta* (Did). No outgroups or problem taxa were included. The basic shape was taken from the results of the combined analysis.

results of the combined analysis.

In all analyses conducted for this study, some of the taxa in which we were interested are clearly placed (Fig. 2; no matter what outgroup is used to root this diagram it always attaches somewhere along the long branch between the two subtribes, thus allowing use of the monophyletic subtribes in an unrooted diagram). *Heterorhachis* was consistently part of the *Berkheya-Cullumia* clade (Ber) of the subtribe Gorteriinae, *Haplocarpha* was at the base of the subtribe Arctotineae (ARCT), and *Cymbonotus*, the Australian genus, was always part of Arctotineae. In addition, in the analyses containing outgroups, *Gundelia* was always the sister group of Lactuceae and was, therefore, maintained as part of the outgroup for the rest of the study. The morphologically interesting taxa, *Cuspidia* and *Heterorhachis*, are always found in the *Berkheya-Cullumia* clade and *Didelta* is in the *Didelta-Berkheya* clade; both clades are in the subtribe Gorteriinae. Because they were stable in their placements, they are considered part of one of the main subtribes and their location is not discussed further. However, the positions of the three other taxa of interest, the tribe Eremothamneae (*Eremothamnus* and *Hoplophyllum*), and the genera *Heterolepis* and *Platycarpha*, were not clear nor was their relationship to the two core subtribes or to the outgroups consistent. The position of these three problem taxa was, therefore, a concern for the remainder

of the analysis.

***trnL-F*.** — Data were available for all taxa except *Eremothamnus* (Appendix), resulting in the use of 27 ingroup taxa and 16 outgroup taxa.

In an effort to determine the basic ingroup patterns, the pruned dataset was analyzed without outgroups and excluding the three remaining problem taxa (*Eremothamnus*, *Heterolepis*, and *Platycarpha*). This resulted in an analysis with 35 “parsimony informative” characters (*pi*) which produced 72 trees ($L = 84$, $CI = 0.893$, $RI = 0.936$). The three problem taxa were then added to the same analysis and one of the unrooted phylograms is shown in Fig. 3 (547 trees, $L = 109$, $pi = 38$, $CI = 0.881$, $RI = 0.914$). All three problem taxa were on long branches and very nearly in the same location. When one of the outgroups, the Liabeae-Vernonieae clade (LIA-VER), was added to the analysis, it formed a

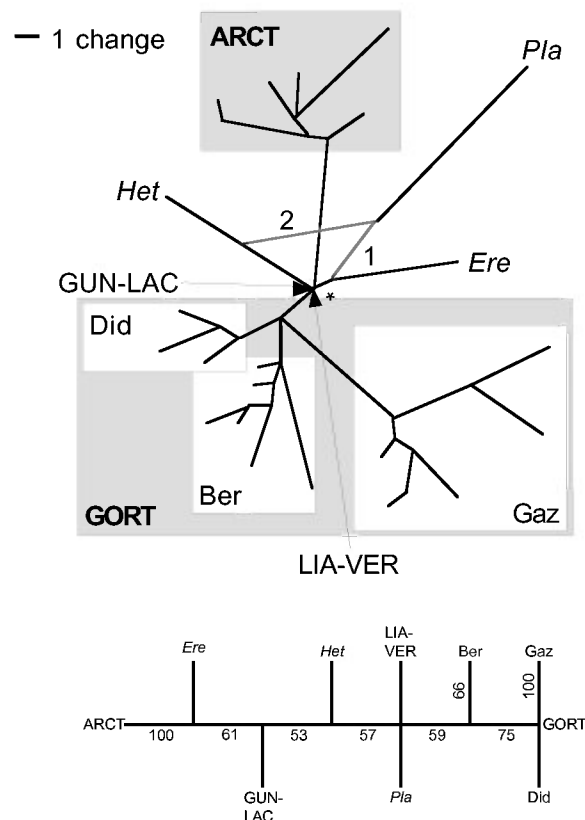


Fig. 3. Unrooted phylogram from *trnL-F* data analyzed with problem taxa but without outgroups. The arrows indicate placement of the two outgroup clades, Gundelieae-Lactuceae (GUN-LAC) and Liabeae-Vernonieae (LIA-VER) when they are added to the analysis singularly. Note that the placement of *Platycarpha* changes from position “1” when the outgroup is LIA-VER to position “2” when the outgroup is GUN-LAC. The * indicates a branch that collapsed in the unrooted strict consensus tree (547 trees, $L = 109$, $pi = 38$, $CI = 0.881$, $RI = 0.914$). The lower diagram is a bootstrap consensus tree reduced to show the main groups.

polytomy with *Eremothamnus*, *Platycarpha* and *Heterolepis*. When the other outgroup clade, the Gundelieae-Lactuceae clade (GUN-LAC), was added it also attached at the same node, however *Platycarpha* changed its position and became the sister taxon of *Heterolepis*. When any single problem taxon or outgroup clade was added to the analysis, it attached at or near the same location; when the taxa were combined, it resulted in hundreds of trees with varying placement of the taxa in question. The maximum likelihood tree for the *trnL-F* data (score of single best tree found = 2612.57292) supported monophyly of the two main subtribes and of the two outgroup clades, but the position of the two outgroup clades and the problem taxa remained unresolved, and Arctoteae were not indicated to be monophyletic. The bootstrap consensus tree, shown in a reduced form at the bottom of Fig. 3, is for an analysis with all problem taxa and both outgroup clades. Note the weak support for the placement of the problem taxa and outgroups.

***ndhF*.** — There were no *ndhF* data for *Heterorhachis* and for one of the *Gorteria* species (*Gorteria* 69) so they were not included in these analyses. There were *ndhF* data available for *Eremothamnus*, so the taxon was added for a total of 26 ingroup samples; all 10 outgroup samples were included (Appendix).

When examined without outgroups or problem taxa, the *ndhF* data set yielded 996 trees ($pi = 42$, $L = 95$, $CI = 0.821$, $RI = 0.906$). It had the same basic structure as the unrooted phylogram from the *trnL-F* sequence data. When all three problem taxa were analyzed together (6029 trees, $L = 122$, $pi = 47$, $CI = 0.803$, $RI = 0.879$) the resulting placement of the taxa put *Platycarpha* closer to *Gorteriinae*, *Eremothamnus* closer to *Arctotinae* and *Heterolepis* between the two (Fig. 4). The two outgroup clades were added separately and their placement is indicated by the arrows on Fig. 4. The Liabeae-Vernonieae clade is placed closer to *Platycarpha* and *Gorteriinae*, and the Gundelieae-Lactuceae clade is closer to *Arctotinae*; *Heterolepis* is between the two. The maximum likelihood tree for *ndhF* (score of single best tree found = 2447.8924) supported the monophyly of the two main subtribes and of the two outgroup clades, but the position of the outgroups and the problem taxa remained unresolved. The Arctoteae could be monophyletic if they included *Eremothamnus* but not *Platycarpha* or *Heterolepis*. The bootstrap consensus tree, shown at the bottom of Fig. 4, is for an analysis with all problem taxa and both outgroup clades; it shows weak support for the placement of *Eremothamnus*, and it has an unresolved cluster at the center of the diagram.

Combined chloroplast dataset. — Neither of the chloroplast datasets had sufficient information to fully resolve the trees. However, the two datasets can be combined to produce a larger number of characters that

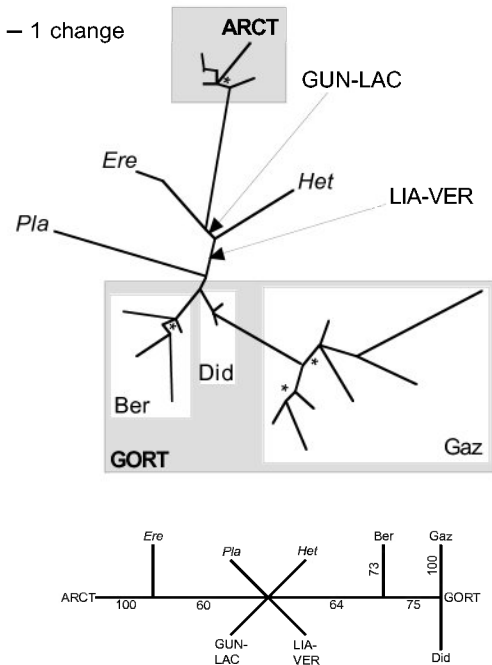


Fig. 4. The upper diagram is an unrooted phylogram from *ndhF* data with problem taxa and without outgroups. The arrows indicate placement of the outgroups when they are added to the analysis singularly. The * indicates branches that collapse in the unrooted strict consensus tree (6029 trees, L = 122, pi = 47, CI = 0.803, RI = 0.879). The lower diagram is a bootstrap consensus tree reduced to show the main groups.

may shed light on the unresolved and conflicting areas of the trees as well as the outgroup placement. As with both *trnL-F* and *ndhF* data, all three problem taxa attached between the two subtribes (Figs. 3, 4). When the outgroup taxa were added separately, the Liabeae-Vernonieae clade connected at the base of the *Platycarpha* and *Heterolepis* clade, and the Gundeliae-Lactuceae grouped with Eremothamneae becoming the sistergroup of Arctotinae. Overall, the Gundeliae-Lactuceae clade was closer to Eremothamneae and Arctotinae and Liabeae-Vernonieae to *Heterolepis* and Gorteriinae. When both outgroup clades were added to the analysis at the same time, two very different basal topologies resulted depending on which outgroup was used to root the tree (Figs. 5, 6; 108 trees, L = 429, pi = 135, CI = .804, RI = .844). When the tree was rooted on Gundeliae-Lactuceae, the Liabeae-Vernonieae clade was nested in the ingroup, sister to Gorteriinae along with *Heterolepis* and *Platycarpha* (Fig. 5). When the Liabeae-Vernonieae clade was used as the root, *Heterolepis* formed a trichotomy at the base of the tree and Gundeliae-Lactuceae was sister to the Arctotinae-Eremothamneae clade (Fig. 6). In both figures, *Platycarpha* was the sister group of Gorteriinae and

Eremothamneae was sister to Arctotinae. In either explanation, the tribe Arctoteae could not be made monophyletic. However, when the data were analyzed with outgroups but without the three problem taxa, the tribe Arctoteae was monophyletic (36 trees, L = 379, pi = 128, CI = 0.815, RI = 0.862). The bootstrap consensus diagram for the chloroplast data with all problem taxa and the two outgroups is shown at the base of Fig. 5.

ITS. — Nuclear sequence data were gathered for all ingroup taxa except *Eremothamnus* (28 taxa) and all 10 outgroup taxa. The initial analysis, without outgroups or problem taxa, produced four trees (4 trees, L = 546, pi = 179, CI = 0.663, RI = 0.809), and in all four the three problem taxa attach, when analyzed separately, between the two subtribes as in the chloroplast sequences. Figure 7 illustrates one of the three unrooted phylograms produced when all three problem taxa are included without the outgroup clades (3 trees, L = 729, pi = 196, CI = 0.595, RI = 0.741). Note that while *Platycarpha* remains between the two subtribes, *Heterolepis* and

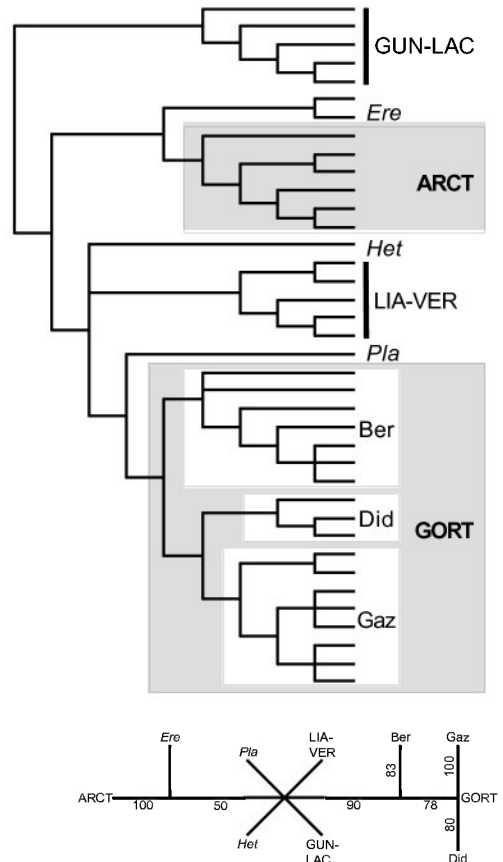


Fig. 5. The upper diagram is a strict consensus tree from the chloroplast data (*trnL-F* and *ndhF*) with both outgroup clades and all three problem taxa rooted on the Gundeliae-Lactuceae clade (108 trees, L = 429, pi = 135, CI = 0.804, RI = 0.844). The lower diagram is a bootstrap consensus tree reduced to show the main groups.

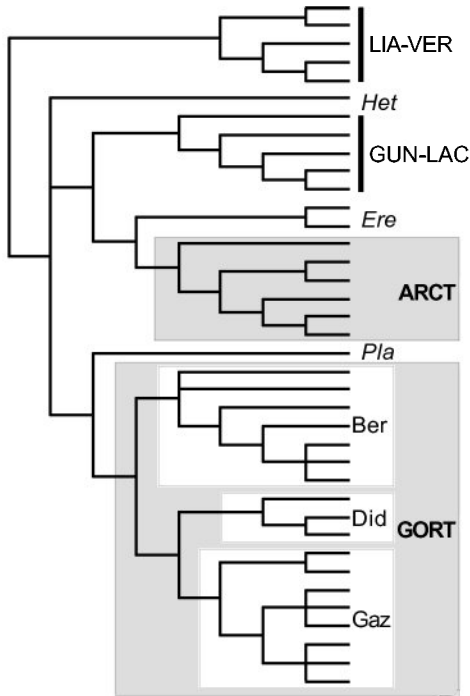


Fig. 6. A strict consensus tree from the chloroplast data (*trnL-F* and *ndhF*) with both outgroup clades and all three problem taxa rooted on the Liabeae-Vernonieae clade. [108 trees, L = 429, pi = 135, CI = 0.804, RI = 0.844]

Eremothamneae are sister to one another and are nested within Gorteriinae. The position of the outgroups (if added separately) is indicated with arrows (Fig. 7). The rooted trees that were run with both outgroups included at the same time showed the outcome of rooting with one outgroup or the other (Figs. 8, 9; 2 trees, L = 1443, pi = 314, CI = 0.463, RI = 0.628). In Fig. 8, when the tree was rooted with the Liabeae-Vernonieae clade, *Platycarpha* was basal followed by monophyletic Arctotinae, while the Gundeliae-Lactuceae clade was between Arctotinae and Gorteriinae. When the same unrooted phylogram was rooted on the Gundeliae-Lactuceae clade (Fig. 9), *Platycarpha* became sister to Liabeae-Vernonieae and the two together were then sister to Arctotinae. The Gorteriinae clade (including the Eremothamneae and *Heterolepis*) was the same with either rooting of the tree. Note that in the analysis of the nuclear DNA data, *Platycarpha* was most closely related to either the Liabeae-Vernonieae clade or Arctotinae, while in the chloroplast DNA it was closer to Gorteriinae. The results of the bootstrap consensus analysis involving all problem taxa and both outgroup clades is shown at the base of Fig. 7; note the position of the *Gazania-Gorteria-Hirpicium* clade in the unresolved center of the diagram. The maximum likelihood tree for the ITS data (score of single best tree found =

8009.8773) supported the monophyly of the two main subtribes and of the two outgroup clades, but the position of the outgroups remained problematic. *Platycarpha* is sister to Liabeae-Vernonieae, and a weak clade of *Heterolepis* and *Hoplophyllum* is sister to Gorteriinae. Arctoteae are not monophyletic, with Gundeliae-Lactuceae closer to Arctotinae and Liabeae-Vernonieae closer to Gorteriinae, depending on the rooting.

Combined data analysis of all sequences. —

All 28 ingroup taxa and 10 outgroup taxa were used for the combined data analysis (*trnL-F*, *ndhF*, ITS). Figure 2 is an unrooted phylogram for the combined dataset (without problem taxa and outgroups), and the two main subtribes and the three clades within Gorteriinae are distinct. When the problem taxa were added (2 trees, L = 976, pi = 281, CI = 0.644, RI = 0.767), they were spread out along the branch that separates the two subtribes. The position of each outgroup clade was examined individually and the Liabeae-Vernonieae clade was

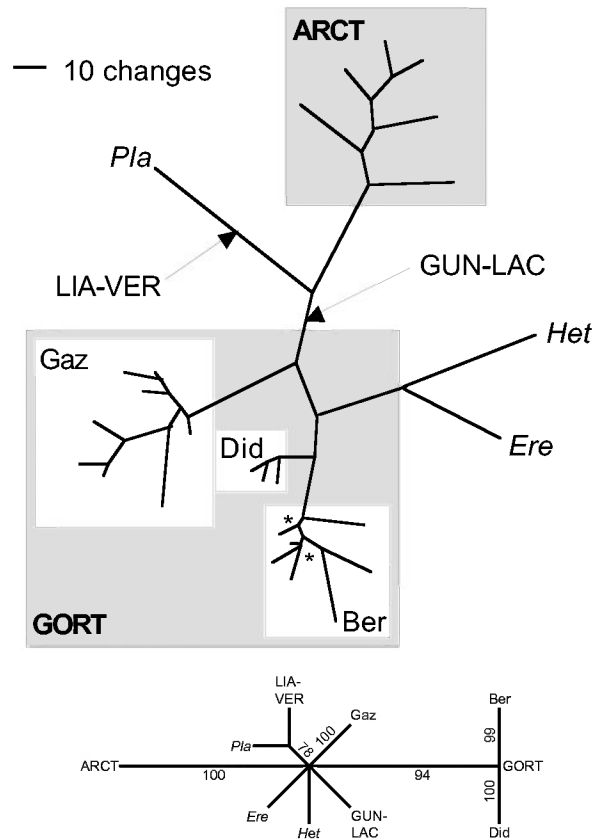


Fig. 7. The upper diagram is an unrooted phylogram from ITS data with problem taxa but without outgroups. The arrows indicate placement of the outgroups when they are added to the analysis singularly. The * indicates branches that collapse in the strict consensus tree (3 trees, L = 729, pi = 196, CI = 0.595, RI = 0.741). The lower diagram is a bootstrap consensus tree reduced to show the main groups.

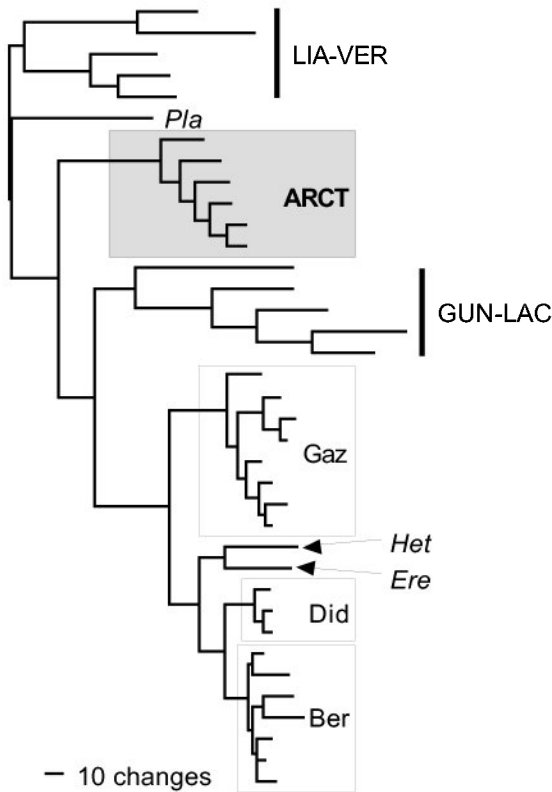


Fig. 8. A phylogram from the ITS data with problem taxa and outgroups, rooted with Liabeae-Vernonieae (LIA-VER). The other outgroup (GUN-LAC) is sister to the subtribe Gorteriinae.

always the sister group of *Platycarpha*; however the Gundelieae-Lactuceae clade attached in five different places. When the two outgroups were analyzed together, a four-branched polytomy resulted with the two outgroup clades, the subtribe Arctotinae, and on a short branch the remainder of the ingroup (Fig. 10; 8 trees, $L = 1850$, $\pi = 441$, $CI = 0.530$, $RI = 0.665$). The bootstrap consensus analysis of the same dataset produced the diagram shown at the bottom of Fig. 10. This analysis was, as usual, less resolved than the parsimony analysis so it lacks the branch between the Ere-Het-GORT clade and the rest of the taxa. Since the position of the Liabeae-Vernonieae clade was unambiguous in the parsimony analysis, one could argue that it would make a “better” outgroup and root the tree using that clade. In such an analysis, Arctoteae (including *Heterolepis* and *Eremothamneae* but excluding *Platycarpha*) are monophyletic. Another alternative, and the one used here, was to gather sequence data from additional taxa that were outside the subfamily Cichorioideae. Prior attempts to use these taxa in this study had failed because with *trnL-F* and *ndhF* data there were too few characters to resolve the

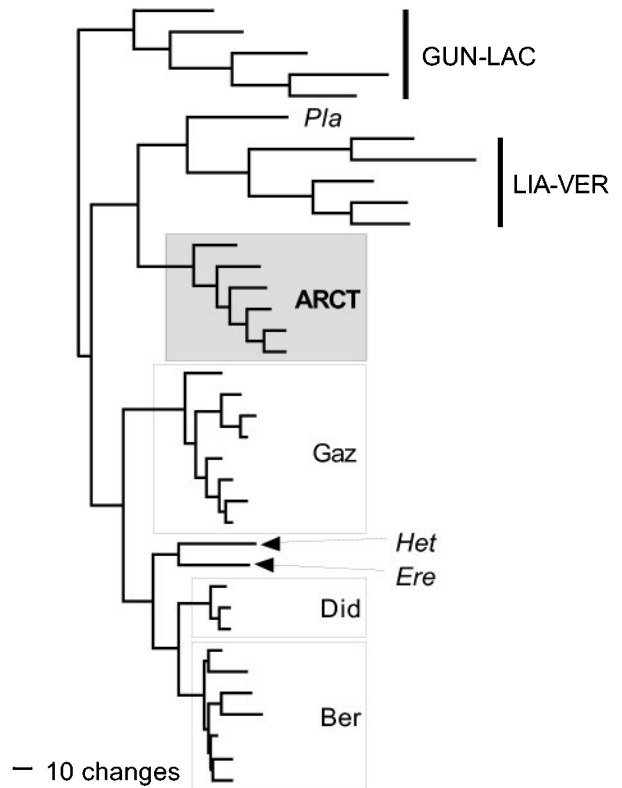


Fig. 9. A phylogram from ITS data with problem taxa and outgroups, rooted with Gundelieae-Lactuceae. The other outgroup (LIA-VER), along with *Platycarpha*, is sister to the subtribe Arctotinae.

tree, and with the ITS some of the new outgroups were so different that the results were ambiguous. However, with the combined dataset there was a sufficiently large number of “parsimony informative” characters, shorter branches, and more reliable results making the inclusion of these outside taxa possible. The additional outgroups (Appendix 1) included two taxa from the subfamily Barnadesioideae (*Barnadesia* and *Dasyphyllum*), the basal branch in the family, along with two species of African Mutisieae (*Gerbera*), and two species of Cardueae (thistles; *Carthamus* and *Cirsium*). Data from *ndhF* and *trnL-F* were collected for all six taxa and ITS data from five (minus *Dasyphyllum*) for a total of 17 additional sequences. All of these taxa are located relatively close to the base of the family. The *trnL-F* and the *ndhF* of these six additional taxa aligned relatively easily, but the ITS sequences were aligned with less confidence. Fortunately, the ambiguous areas were confined to two places in the data matrix, and the data could be analyzed with and without these two areas.

Figure 11 shows one of two trees produced by the combined dataset (*trnL-F*, *ndhF* and ITS) of all taxa

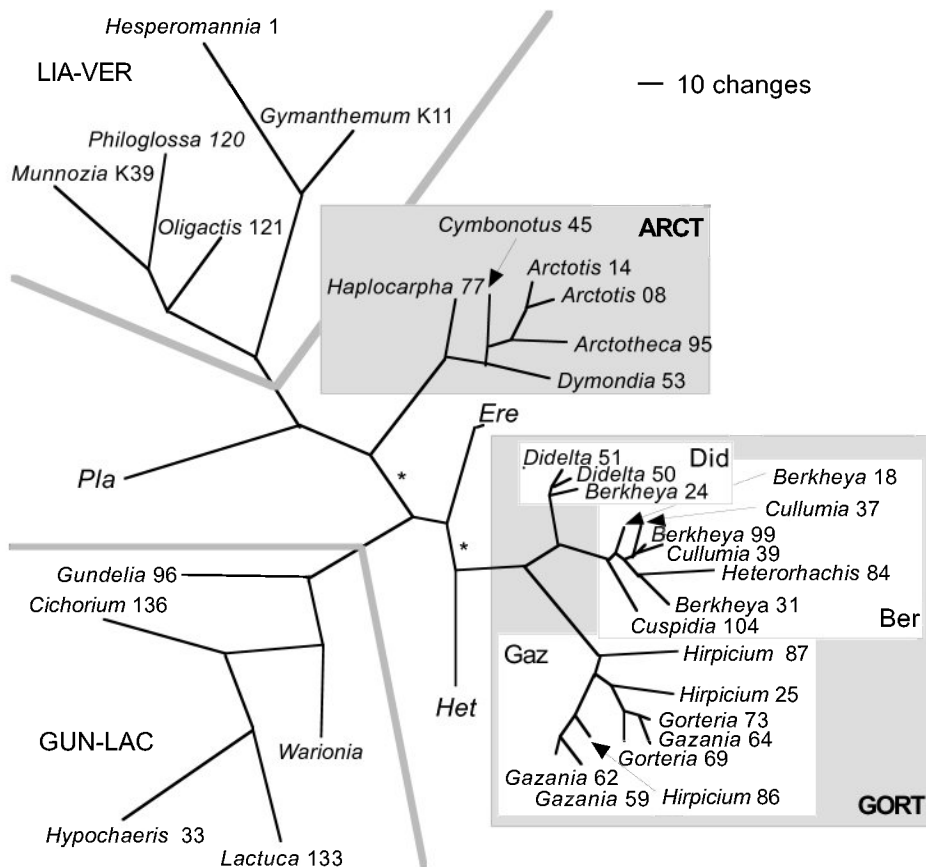


Fig. 10. The upper diagram is an unrooted phylogram from the combined data set (*trnL-F*, *ndhF*, ITS), with problem taxa and outgroup clades. The * indicates branches that collapse in the strict consensus tree (8 trees, L = 1850, pi = 441, CI = 0.530, RI = 0.665). The lower diagram is a bootstrap consensus tree reduced to show the main groups.

including the second set of outgroups (L = 2345, pi = 516, CI = 498, RI = 0.638). The two trees differed only in a minor change in the position of one species of *Cullumia*. The Gundelieae-Lactuceae clade is more distant from Arctoteae than the Liabeae-Vernonieae clade. *Platycarpha* is basal to the Liabeae-Vernonieae clade and cannot be considered part of Arctoteae. Within monophyletic Arctoteae there are several clades: the subtribe Arctotinae, the genus *Heterolepis*, the tribe Eremothamneae, and the subtribe Gorteriinae. Furthermore, Gorteriinae can be broken up into three clades: the *Gazania-Gorteria-Hirpicium* clade, the *Didelta-Berkheya* clade and the *Berkheya-Cullumia* clade. The same result was also obtained using just Mutisieae and Cardueae as outgroups or Cardueae or Mutisieae alone. The bootstrap values are listed on the branches; note that although there is good support for nearly all the branches (all but two critical nodes have support of 72% or higher), the support for Arctoteae is less than 50%. Likewise there is little support for the grouping of Eremothamneae and *Heterolepis* or for these two taxa grouping with the subtribe Gorteriinae. If the

data are analyzed without the three problem taxa, the support for monophyly of Arctoteae is 72%.

The maximum likelihood tree of the ingroup rooted on *Platycarpha* has monophyletic Arctotinae and monophyletic Gorteriinae with Eremothamneae and *Heterolepis* between the two subtribes (score 8781.5000). The maximum likelihood tree of the ingroup with the Liabeae-Vernonieae clade as the outgroup gave the same tree except that, because the tree was rooted with the Liabeae-Vernonieae clade, *Platycarpha* was basal to this clade; Fig. 11 (score 11513.36510).

Table 3 gives the size of the aligned matrices, the number of gaps added in order to align the sequences, the number of informative characters, and the percent of sequence divergence (calculated using PAUP 4.0*) for all three regions. When applicable, the figures were calculated for both the ingroup alone and for the ingroup plus the outgroup. This table shows that the percent sequence divergence is low in the cpDNA (2.9–4.5% for *trnL-F* and 5.6–7.7% for *ndhF*) and higher in the ITS sequences (19.9–28.3%). However, none of the divergence percentages were alarmingly high. Of more con-

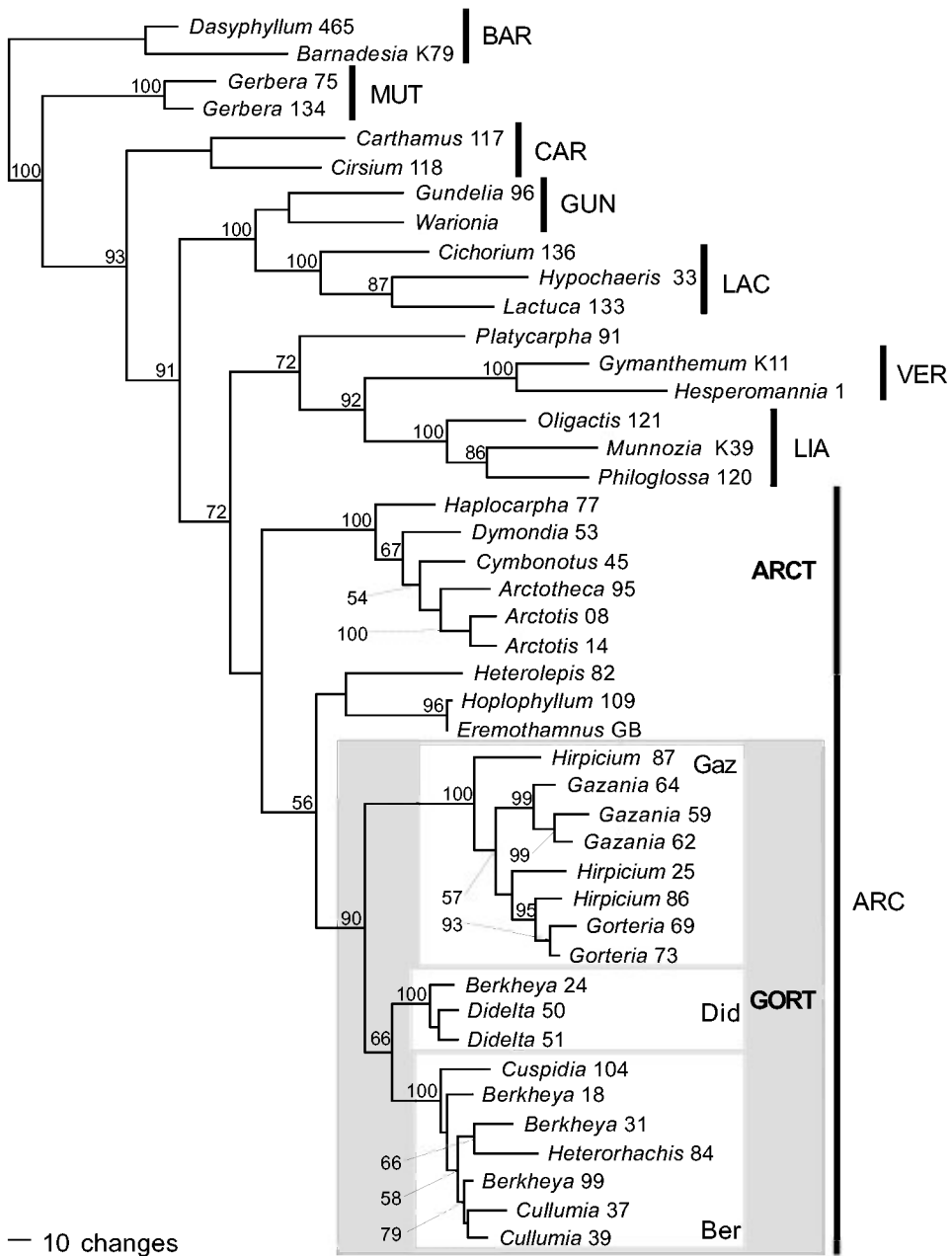


Fig. 11. Phylogram with all taxa from the combined data set (*trnL-F*, *ndhF*, ITS). The tribe Arctoteae is monophyletic, the subtribes Arctotineae and Gorteriinae are monophyletic; two problem taxa, the genus *Heterolepis* and the tribe Eremothamneae, are weakly grouped together and are sister to Gorteriinae. The closest outgroup is the Liabaeo-Vernonieae clade and the problem genus *Platycarpha*. The bootstrap values are listed on the branches; note that although there is good support for nearly all the branches, the support for Arctoteae is less than 50%.

cern is the number of informative characters, which reaches 50% for ITS data in the combined analysis (with all outgroups). The cpDNA scores reach as high as 8.6% for the *trnL-F* and as high as 15% for the *ndhF* (combined analysis using all outgroups). The alignment problems in the ITS were confined to two areas, positions 39–165 (ITS 1) and positions 457–484 (ITS 2). As an

experiment, these two areas were replaced with question marks for all of the outgroups and the three problem taxa. All of the analyses were rerun, including the addition and subtraction of all outgroups and problem taxa. The only real difference between the final figure (combined analyses) for this altered dataset and Fig. 11 is a slight change in the bootstrap values for some nodes and the fact that

Table 3. Size of aligned matrices, number of gaps added, number of informative characters, and percent sequence divergence for *trnL-F*, *ndhF*, and ITS. Figures usually given for both “only the ingroup” and for “ingroup plus all of the outgroups”; single outliers have been removed.

Regions	Size of aligned matrices	# of gaps added (ingroup/outgroup)	# of informative characters (ingroup/outgroup)	% sequence divergence (ingroup/outgroup)
<i>trnL-F</i>	897	47–49 /50–65	49/77	0.02965/0.04505
<i>ndhF</i>	699	22–26/27–42	47/103	0.05578/0.07716
ITS	679	39–42/34–64	196/346	0.19897/0.28353
Total	2275	ca. 113/ ca. 141	292/526	n/a

Heterolepis moves to a position as the sister taxon to subtribe Arctotinae. Since the position of *Heterolepis* in Fig. 11 or in the new analyses is not supported by either high bootstrap values or the maximum likelihood analysis, this shifting of positions is not surprising.

DISCUSSION

Molecular data. — The final phylogeny (Fig. 11) supports three out of four of the previously described subtribes of Arctoteae: Arctotinae, Gorteriinae, and Eremothamninae; the remaining subtribe, Gundeliinae, was previously elevated to tribal level and is here confirmed as the sister group of Lactuceae. It is clear that *Haplocarpha* and *Cymbonotus* belong in Arctotinae, and *Heterorhachis*, *Cuspidia*, and *Didelta* in Gorteriinae. However, to achieve monophyly in Arctoteae, *Platycarpha* must be removed from the tribe. Over the years this genus has been in the tribes Mutisieae and Cardueae as well as Arctoteae, and in the most recent classification it was “unassigned to tribe” (Bremer, 1994). Of any existing tribes it may be the closest to the Liabeae-Vernonieae clade. However, final placement of this genus must await the addition of several taxa which may be near the base of the Liabeae-Vernonieae clade.

The phylogeny from the combined analysis (Fig. 11) is a major step forward in understanding relationships among taxa within Arctoteae, specifically the composition of the subtribes, and within subfamily Cichorioideae s.s., particularly the sistergroup of Arctoteae, and placement of Eremothamninae. The extensive analyses have also provided insights into affinities of taxa of Arctoteae that are not expressed in the final figure. For instance, when any of the three DNA regions were examined alone or in combination, but without any of the problem taxa, the results were similar: the two main subtribes were monophyletic, the two outgroup clades were monophyletic, and either Arctoteae was monophyletic or the relationship among the four clades (two outgroups and two subtribes) was unresolved. The clades always have the same composition, however, and there is little if any conflict; it is just a problem of resolution. However, when the problem taxa were added, changes occurred. In

results of the analysis using chloroplast data, *Platycarpha* was the sister group of Gorteriinae, Eremothamninae was the sister group of Arctotinae and *Heterolepis* was near the Liabeae-Vernonieae clade, but its exact position was unresolved (Figs. 5, 6). In ITS results, *Platycarpha* was closer to the Liabeae-Vernonieae clade, which in turn was closer to Arctotinae while *Heterolepis* and Eremothamninae were inside Gorteriinae (Figs. 8, 9). This presents a nearly complete reversal of their previous positions. The combined analysis produced results that were somewhere in the middle, with *Platycarpha* sister to Liabeae-Vernonieae (as in the ITS tree), Arctoteae and its two main subtribes (Arctotinae and Gorteriinae) monophyletic; *Heterolepis* and Eremothamninae were between the two subtribes and the sister group of Gorteriinae. It is not unusual for there to be some discordance of chloroplast and nuclear DNA (e.g., Yoo & al., 2002), and it is usually attributed to the possibility of hybridization. Following that reasoning, the problem taxa could have such a history. Chromosome numbers might be useful in searching for hybrids but they are not available for most taxa in this analysis. For seed plants it has been shown that inadequate taxon sampling can be responsible for conflicting results (Rydin & Källersjö, 2002), but that is most likely not the problem with this study because the taxa removed from the analysis were very similar in their molecular characters to ones that were retained in the study. For now it seems best to keep *Heterolepis* and Eremothamninae in Arctoteae but out of either of the main subtribes.

Taxa within each of the two subtribes are closely related, as evidenced by the short branch lengths that are usually found within them. However, the problem taxa and outgroups have much longer branches and a few of the branches have weak or no support from bootstrap analysis (Fig. 11). It is possible that there has been a relatively recent radiation within the two subtribes and that the outgroups and problem taxa represent old lineages that have been separated for a long time.

Morphology. — Based on the literature (Cassini, 1816, 1828; Robinson & Brettell, 1973a; Norlindh, 1977; Robinson, 1992, 1994; Bremer, 1994; Herman & al., 2000) and personal observation, the morphology can be examined in light of the phylogeny (Fig. 11).

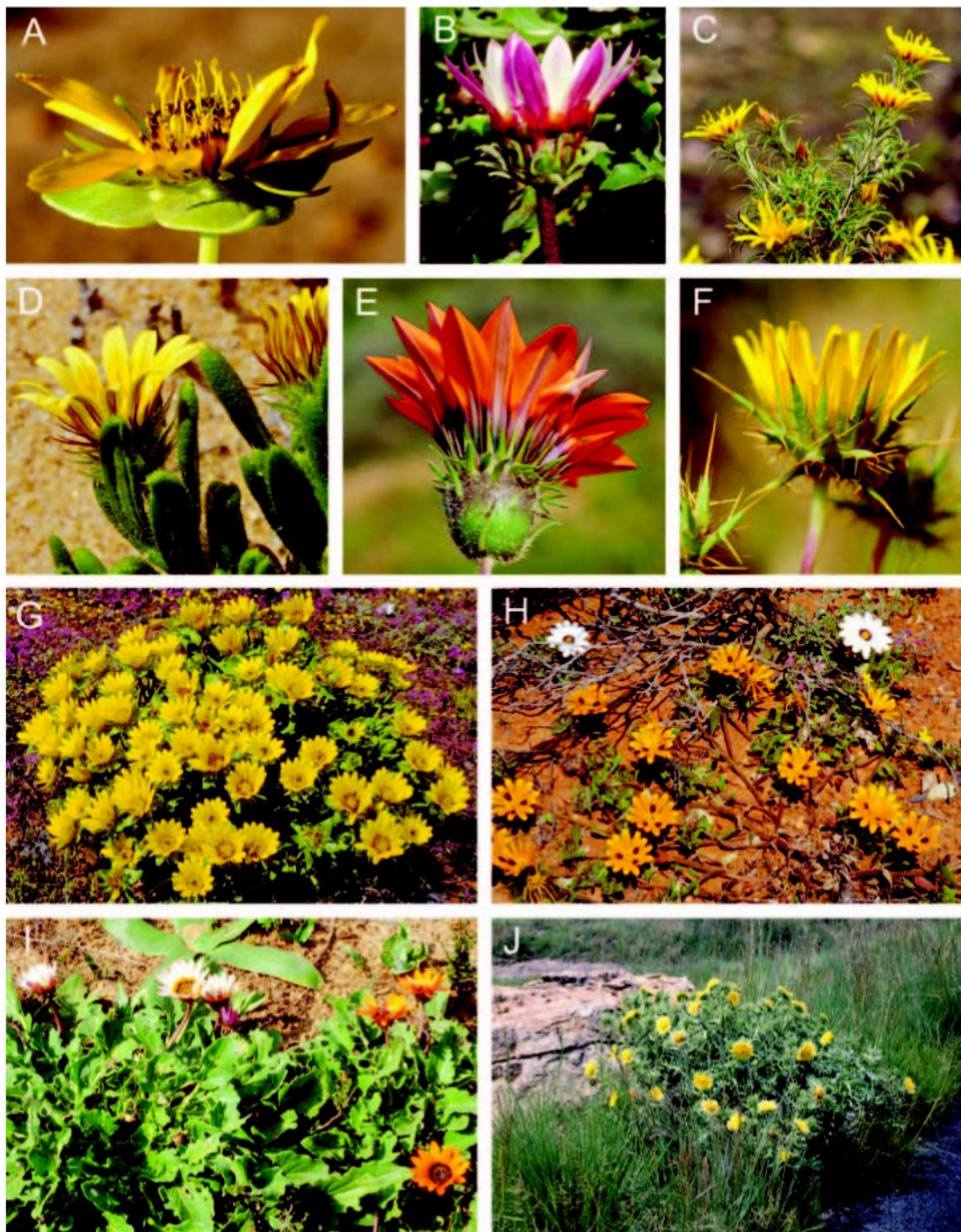


Fig. 12. Photographs of several heads and habits of Arctoteae. A, *Didelta*; B, *Arctotis*; C, *Cullumia*; D, *Hirpicium*; E, *Gazania*; F, *Berkheya*; G, *Didelta*; H, *Arctotis*; I, *Gorteria*; J, *Berkheya*. All photos by V. Funk.

Photographs of several members of the tribe are found in Figs. 1 and 12.

The members of the subtribe Arctotinae (*Arctotis*, *Arctotheca*, *Cymbonotus*, *Dymondia* and *Haplocarpha*; Figs. 1B, C, 12B, H) can be characterized as follows: they do not produce latex; their involucral bracts are not fused (one species of *Arctotheca* and two of

Haplocarpha have a tendency toward fusion), the outermost bracts are foliaceous and have a scarios apical lamina and the receptacle is smooth or shallowly areolate-alveolate; the ligulate florets have 4 veins and 3-lobes when lobes are present (similar to the tribe Liabeae and the subfamily Asteroideae), and they are fertile (except for *Arctotheca*); the central florets have shallow-

ly lobed corollas, the anthers are not tailed (except for *Arctotis* the largest genus of the subtribe), and the style has a swollen portion below the branch point sometimes with a ring of hairs; the achenes have 3–5 dorsal, well-developed ribs or wings. Many of the members of this subtribe are rosette-forming, perennial herbs (Fig. 12H). Most of these characters would be considered plesiomorphic except for the swollen portion of the style with the ring of sweeping hairs (also found in some thistles) and the 3–5 well-developed ribs or wings on the achenes. The lack of latex is most likely apomorphic, but it comes and goes so often in the subfamily that it is difficult to use at this level.

The members of the subtribe Gorteriinae (*Berkheya*, *Cullumia*, *Cuspidia*, *Didelta*, *Gazania*, *Gorteria*, *Heterorhachis*, and *Hirpicium*; Figs. 1A, D, E, 12A, C–G, I, J) are better defined as follows: they have laticifers (the cells that normally contain latex), and latex is present in *Gazania*, some *Berkheya* and possibly others; involucre bracts are connate at least at the base (Fig. 12E), or in various degrees upwards, forming a cup; receptacles are more or less deeply alveolate and enclosing at least the base of the achenes, the apex of the ray florets often have 5 veins and 4 lobes when lobes are present, but it can vary from 2–5 lobes in some taxa; the rays are sterile, the central florets are deeply lobed (Figs. 1A and D), the anthers are sagittate but not tailed, and the previously mentioned conspicuous swollen portion of styles is either not present or not well-developed; the achenes are without well-developed ribs or wings. Figure 11 shows the three clades of this subtribe: the *Gazania-Hirpicium-Gorteria* clade comprises annual or perennial herbs with strongly fused involucre bracts (Fig. 12E), the *Berkheya-Cullumia* clade comprises a mixture of subshrubs, shrubs, and annual and perennial herbs that are usually spiny in some manner (Figs. 1D, 12C, F, J), and the *Didelta-Berkheya* clade consists of subshrubs or shrubs with the *Didelta* members having heads that break apart (Figs. 1A, 12A, G). Members of both the *Berkheya-Cullumia* and the *Didelta-Berkheya* clades have partially fused involucre bracts. The apomorphic characters for this subtribe include the connate involucre, deeply alveolate receptacles, and 4-lobed and 5-veined sterile rays.

All three problem taxa have sets of characters that make it difficult to place them in either of the subtribes. The genus *Platycarpha* (three species) is a perennial herb confined to southern Africa. The heads are discoid and have only a few florets, which are purple, and these small heads are aggregated into a secondary head that is large and sessile, lying mostly flat on the ground with the leaves spread out like the spokes of a wheel. The plant is either acaulescent, without branches, or with subterranean branches. The receptacle has pales (bracts), the

anthers either have tails (or are sagittate), and the style has a swelling and a ring of sweeping hairs. The achenes are only faintly ribbed and the pappus is made up of scales. While *Platycarpha* has the swollen style and the ring of sweeping hairs of Arctotinae (and some Cardueae), it does not have the other potential apomorphies of either subtribe, and it has purple flowers and pales which they do not.

Heterolepis (three species) is found only in the Western Cape of South Africa. It is a small shrub with involucre bracts somewhat connate in 1–3 rows with the outer ones sometimes herbaceous and the inner with membranous margins. They have tailed anthers and the heads have fertile rays each with 3 lobes and 4 veins. The styles are slender with short branches, the apical portion either is not or is only slightly thickened, and it has a ring of sweeping hairs. There are no ribs on the achenes and the pappus is made up of slender “bristle-like” scales. *Heterolepis* has somewhat connate involucre bracts but none of the other apomorphies of Gorteriinae. The margins of its involucre bracts and style are somewhat similar to Arctotinae.

The tribe Eremothamneae (*Eremothamnus*, one species from Namibia; *Hoplophyllum*, two species from Northwest Cape Province, South Africa) contains shrubs that are radiate or discoid (respectively). When present, the rays are female and fertile with three apical lobes. The anthers have short tails and the styles are long and slender (similar to Vernoniaceae and some Liabaceae), they have unusual bi- and tri-cellular sweeping hairs but not the ring of sweeping hairs found in Arctotinae. There are no distinct ribs on the achenes, and the pappus is capillary. It has none of the apomorphies of either subtribe although its members have spines or thorns on the leaves as do many members of Gorteriinae.

It is heartening that all three of the problem taxa (based on molecular data) have morphological characters that have traditionally made it difficult to place them in either of the two main subtribes of Arctoteae. *Platycarpha* has a swollen area on the shaft of the style, but it also has discoid heads with purple florets, a paleaceous receptacle, and tailed anthers, characters that are unlike either of the subtribes. *Heterolepis* has tailed anthers, unlike the two subtribes, connate involucre bracts as in Gorteriinae but without the deeply alveolate receptacle, the common type of ray floret as in Arctotinae, and an unusual pappus. Finally, members of Eremothamneae have unique hairs on long, slender styles, and, unlike members of the two subtribes, they have a capillary pappus and tailed anthers, and *Hoplophyllum* has a discoid head. None of them have the connate involucre bracts with the deeply alveolate receptacle and the 4-lobed sterile rays of Gorteriinae, nor do they have the prominently winged or ribbed achenes. *Platycarpha* and

Eremothamneae do not have the scarious-margined bracts of Arctotinae.

Given the morphological and molecular data, it seems that we can be certain that the two main subtribes, Arctotinae and Gorteriinae, are monophyletic (as we have defined them) with robust support. The three problem taxa do not belong in either of these two subtribes nor are they closely related to one another. Our data indicate that Eremothamneae and possibly *Heterolepis* most likely belong in Arctoteae but that *Platycarpha* does not. Of course, with the inclusion of both *Heterolepis* and the tribe Eremothamneae (as a subtribe it would be Eremothamninae) the few morphological characters that existed for the tribe Arctoteae have been lost; the scaly pappus, lack of tailed anthers, usually radiate heads, and typical styles are not found in one or both of these groups. A separate morphological analysis is underway (Karis & Funk, pers. comm.) that will hopefully shed light on this problem. The weak support for the tribe is supported by repeated bootstrap analyses, with and without problem taxa, which have failed to find strong support for monophyletic Arctoteae. For now, our conclusion must be that there are little data (either from molecules or morphology) to support the monophyly of Arctoteae, and molecular work currently underway in both Lactuceae and Vernoniae may help in making this determination.

There are several important lessons learned in this study. First, the problem taxa are of critical importance. It is clear that in the subfamily Cichorioideae s.s. unusual and morphologically hard to place taxa must be included in any molecular analysis. For instance, in Fig. 2 (all problem taxa and all outgroups removed), the resulting tree shows that the two subtribes could easily be monophyletic, and if only one of the two outgroup clades were used it would present a perfect picture of a monophyletic Arctoteae. If any of the problem taxa (*Heterolepis*, Eremothamneae, or *Platycarpha*) or any of the outgroups are added, they attach between the two subtribes, and when used one at a time or in certain combinations of two, they produce a clear result. Combinations of two or more problem taxa in conjunction with both main outgroups generates problems with monophyly of the tribe. All previous studies involving a few taxa from the core subtribes did not reveal the problems in the tribe. Second, multiple outgroups must be evaluated in an exhaustive process to determine how the outgroups are behaving and what effect they are having on the ingroup. This is probably one of the most underrated steps in cladistic analyses, and it has great impact on the outcome. One or two token representatives will not suffice unless a thorough analysis has been done previous to the study in question. Finally, unrooted phylograms and unrooted strict consensus trees give very use-

ful results for analyzing data by freeing the investigator from preconceived notions about relationship.

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Appendix. Sources of sequence data and GenBank numbers.

Label	Tribe	Genus	Species	Authority	Collector & No.	Locality	Herb.	ITS	trnL	ndhF
Ingroup										
<i>Arctotheca</i> 95	Arctoteae	<i>Arctotheca</i>	<i>calendula</i>	(L.) Levyns	Funk 12266	South Africa	US	AY504703	AY504785	AY504745
<i>Arctotis</i> 08	Arctoteae	<i>Arctotis</i>	<i>bellidifolia</i>	Berg.	Koekemoer & Funk 1926	South Africa	PRE	AY504704	AY504786	AY504746
<i>Arctotis</i> 14	Arctoteae	<i>Arctotis</i>	<i>fastuosa</i>	Jacq.	Trinder-Smith 238	South Africa	US	AY504705	AY504787	AY504747
<i>Berkheya</i> 18	Arctoteae	<i>Berkheya</i>	<i>carlinopsis</i>	Welw. ex O. Hoffm.	Bourrel et al. 2689	South Africa	MO	AY504709	AY504791	AY504751
<i>Berkheya</i> 99	Arctoteae	<i>Berkheya</i>	<i>cruciata</i>	Willd.	Koekemoer 2002	South Africa	PRE	AY504712	AY504794	AY504754
<i>Berkheya</i> 31	Arctoteae	<i>Berkheya</i>	<i>cuneata</i>	Willd.	Funk 12275	South Africa	PRE	AY504711	AY504793	AY504753
<i>Berkheya</i> 24	Arctoteae	<i>Berkheya</i>	<i>spinosisima</i>	Willd.	Koekemoer & Funk 1962	South Africa	PRE	AY504710	AY504792	AY504752
<i>Cullumia</i> 37	Arctoteae	<i>Cullumia</i>	<i>bisulca</i>	(Thunb.) Less.	Koekemoer & Funk 1935	South Africa	PRE	AY504713	AY504795	AY504755
<i>Cullumia</i> 39	Arctoteae	<i>Cullumia</i>	<i>rigida</i>	DC.	Trinder-Smith 182	South Africa	US	AY504714	AY504796	AY504756
<i>Cuspidia</i> 104	Arctoteae	<i>Cuspidia</i>	<i>cernua</i>	(L.f.) B.L. Burt	Koekemoer 1986	South Africa	PRE	AY504715	AY504797	AY504757
<i>Cymbonotus</i> 145	Arctoteae	<i>Cymbonotus</i>	<i>lawsonianus</i>	Gaudich.	Holland & Fechner 1336	Australia	US	AY504706	AY504788	AY504748
<i>Didelta</i> 50	Arctoteae	<i>Didelta</i>	<i>carcosa</i>	Ait.	Koekemoer & Funk 1943	South Africa	PRE	AY504716	AY504798	AY504758
<i>Didelta</i> 51	Arctoteae	<i>Didelta</i>	<i>spinosa</i>	(L.f.) Ait.	Trinder-Smith 142	South Africa	US	AY504717	AY504799	AY504759
<i>Dymondia</i> 53	Arctoteae	<i>Dymondia</i>	<i>margaretae</i>	Compton	Trinder-Smith 197	South Africa	US	AY504707	AY504789	AY504749
<i>Eremothamnus</i> GB	Eremothamneae	<i>Eremothamnus</i>	<i>marlothianus</i>	O.Hoffm.		Namibia		-	-	L39424
<i>Gazania</i> 59	Arctoteae	<i>Gazania</i>	sp.		Koekemoer & Funk 1929	South Africa	PRE	AY504718	AY504800	AY504760
<i>Gazania</i> 62	Arctoteae	<i>Gazania</i>	<i>krebsiana</i>	Less.	Koekemoer & Funk 1969	South Africa	PRE	AY504719	AY504801	AY504761
<i>Gazania</i> 64	Arctoteae	<i>Gazania</i>	<i>tenuifolia</i>	Less.	Trinder-Smith 64	South Africa	US	AY504720	AY504802	AY504762
<i>Gorteria</i> 73	Arctoteae	<i>Gorteria</i>	<i>diffusa</i>	Thunb.	Trinder-Smith 103	South Africa	US	AY504722	AY504804	AY504763
<i>Gorteria</i> 69	Arctoteae	<i>Gorteria</i>	<i>personata</i>	L.	Dodd 289	South Africa	US	AY504721	AY504803	-
<i>Haplocarpha</i> 77	Arctoteae	<i>Haplocarpha</i>	<i>scaposa</i>	Harv.	Trinder-Smith 191	South Africa	US	AY504708	AY504790	AY504750
<i>Heterorhachis</i> 84	Arctoteae	<i>Heterorhachis</i>	<i>aliena</i>	(L.f.) Druce	Trinder-Smith 188	South Africa	US	AY504700	AY504782	AY504742
<i>Hirpicium</i> 25	Arctoteae	<i>Hirpicium</i>	<i>aculeata</i>	(Burm.f.) Roessler	Oliver 3867	South Africa	PRE	AY504723	AY504805	-
<i>Hirpicium</i> 87	Arctoteae	<i>Hirpicium</i>	<i>echinus</i>	(Less.)	Koekemoer & Funk 1966	South Africa	PRE	AY504724	AY504806	AY504764
<i>Hirpicium</i> 86	Arctoteae	<i>Hirpicium</i>	<i>gazanoides</i>	(Harv.) Roessler	Long & Rae 734	Namibia	MO	AY504726	AY504808	AY504766
<i>Hoplophyllum</i> 109	Eremothamneae	<i>Hoplophyllum</i>	<i>integrifolium</i>	Less.	Koekemoer & Funk 1956	South Africa	PRE	AY504725	AY504807	AY504765
<i>Platycarpha</i> 91	Arctoteae	<i>Platycarpha</i>	<i>spinosum</i>	DC.	Koekemoer 2045	South Africa	PRE	AY190607	AY504784	AY504744
			<i>carlinoides</i>	Oliver & Hiern.	Germishuizen 2613	South Africa	PRE	AY504701	AY504783	AY504743
Outgroup										
<i>Cichorium</i> 36	Lactuceae	<i>Cichorium</i>	<i>intybus</i>	L.	Kellogg & Funk 1415	USA	US	AY504694	AY504776	AY504736
<i>Gundelia</i> 96	Gundelieae	<i>Gundelia</i>	<i>turnerfortii</i>	L.	al-Hosseini s.n.	Iran	US	AY504691	AY504773	AY504733
<i>Gymnanthemum</i> K11	Vernonieae	<i>Gymnanthemum</i>	<i>arabodolina</i>	Delile	Kew 318-86-02802 s.n.	Africa (cultivated)	K	AY504695	AY504777	AY504737
<i>Hysseromannia</i> 1	Vernonieae	<i>Hysseromannia</i>	<i>arborescens</i>	A. Gray	Ching 420	Hawaii (cultivated)	PTBG	AY504696	AY504778	AY504738
<i>Hypochoeris</i> 33	Lactuceae	<i>Hypochoeris</i>	<i>glabra</i>	L.	Funk 12217	Australia	US	AY504692	AY504774	AY504734
<i>Lactuca</i> 133	Lactuceae	<i>Lactuca</i>	<i>sativa</i>	L.	Funk s.n.	cultivated	none	AY504693	AY504775	AY504735
<i>Munozia</i> K39	Liabeae	<i>Munozia</i>	<i>gigantea</i>	(Rusby) Rusby	Dillon s.n.	Peru	F	AY504697	AY504779	AY504739
<i>Oligactis</i> 121	Liabeae	<i>Oligactis</i>	<i>volubilis</i>	(Kunth) Cass.	Funk 12042	Colombia	US	AY504698	AY504780	AY504740
<i>Philoglossa</i> 120	Liabeae	<i>Philoglossa</i>	<i>mimuloides</i>	(Hieron.) H. Rob. & Cuatr.	Funk 11453	Ecuador	US	AY504699	AY504781	AY504741
<i>Warionia</i> s.n.	Gundelieae	<i>Warionia</i>	<i>saharae</i>	Benth. & Cross	Lippert 25346	Morocco	US	AY190608	AY702089/	AY702088
								AY702090		
<i>Barnadesia</i> K79	Barnadesieae	<i>Barnadesia</i>	<i>caryophylla</i>	S.F. Blake	Matthei Bot. Gard s.n.	Peru (cultivated)	MICH	AY504686	AY504768	AY504728
<i>Carthamus</i> 117	Cardueae	<i>Carthamus</i>	<i>oxycantha</i>	Bieb.	al-Hosseini s.n.	Iran	US	AY504689	AY504771	AY504731
<i>Cirsium</i> 118	Cardueae	<i>Cirsium</i>	<i>drumundii</i>	Torr. & Gray	Funk 12302	U.S.A.	US	AY504690	AY504772	AY504732
<i>Dasyphyllum</i> 465	Barnadesieae	<i>Dasyphyllum</i>	<i>reticulatum</i>	(DC.) Cabrera	Roque, Funk & Kim 485	Brazil	US	-	AY504767	AY504727
<i>Gerbera</i> 75	Mutisieae	<i>Gerbera</i>	<i>crocea</i>	Kuntze	Koekemoer & Funk 1924	South Africa	PRE	AY504687	AY504769	AY504729
<i>Gerbera</i> 134	Mutisieae	<i>Gerbera</i>	sp.		Funk s.n.	Africa (cultivated)	none	AY504688	AY504770	AY504730