

Phylogenetic relationships of *Tolpis* (Asteraceae: Lactuceae) based on *ndhF* sequence data

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Abstract. *Tolpis* includes approximately 12–20 species distributed primarily in Europe, Middle East, and Africa with most species (10) confined to the Macaronesian archipelagos of the Azores, Madeira, Canary Islands, and Cape Verde Islands. Although there has been considerable effort to resolve generic circumscriptions and relationships among genera of the tribe Lactuceae, much controversy still remains regarding these taxonomic issues for *Tolpis*. Phylogenetic analyses of the chloroplast encoded gene *ndhF* were performed using 36 species from 28 genera of Lactuceae and outgroup taxa from the five other tribes of Cichorioideae. The sampling included most previously suggested generic relatives of *Tolpis* and seven species of *Tolpis* from Africa, Europe, and Macaronesia. The *ndhF* phylogeny indicates that *Tolpis* is not monophyletic because two continental species, *T. capensis* from south-central Africa and Madagascar and *T. stacticifolia* from central and southeast Europe, do not occur in the strongly supported core *Tolpis* clade. This result is in agreement with recent suggestions based on macromorphology and palynology that these two species should be excluded from *Tolpis*. *Tolpis capensis* is sister to *Taraxacum* and *T. stacticifolia* is nested within the genus *Crepis*. The *ndhF* tree also indicates that the core *Tolpis* clade is an isolated

lineage that is not related to any of the previously suggested genera of Lactuceae. Relationships among the five examined core *Tolpis* species are fully resolved in the *ndhF* tree. The three examined island species occur in two different clades suggesting either multiple colonizations or a single origin and subsequent recolonization of the continent. Resolution of the origin of the island endemics will require additional taxon sampling and the use of more variable molecular markers.

Key words: *Tolpis*, Asteraceae, Macaronesia, Lactuceae, Islands.

The number of species placed in *Tolpis* Adans. (Asteraceae; Lactuceae) ranges from 12 (Jarvis 1980) to 20 (Tomb 1977, Bremer 1994). The genus is distributed primarily in Europe, Turkey, the Middle East, and Africa with 10 species endemic to the Macaronesian archipelagos of the Azores, Madeira, Canary Islands, and Cape Verde Islands (Jarvis 1980). *Tolpis* is characterized by a suite of morphological characters, including usually annual or perennial herbaceous habit, mostly rosulate leaves, capitula arranged in corymbs or panicles, yellow florets, styles short, cypselas ribbed

and glabrous, and a pappus of few to many long scabrid bristles interspersed with minute scales.

Although the Lactuceae has been the focus of several morphological (Stebbins 1953; Jeffrey 1966; Tomb 1975, 1977; Baagøe 1980; Blackmore 1981; Bremer 1994) and molecular (Whitton et al. 1995) studies, considerable controversy still exists regarding the generic circumscription and subtribal placement of *Tolpis*. Three major classification systems have been proposed for the Lactuceae by Stebbins (1953), Jeffrey (1966), and Bremer (1994). In each system the generic affinities of *Tolpis* were different (Table 1). Stebbins (1953) placed the genus in the subtribe Cichoriinae with the genera *Arnosseris* Gaertn., *Catananche* L., *Cichorium* L., *Hispidella* Barnad. ex Lam., *Hymenonema* Cass., *Hyoseris* L., and *Koelpinia* Pall. Jeffrey (1966) erected an informal intra-tribal classification which grouped *Tolpis* with *Andryala* L., *Arnosseris*, *Hieracium* L., *Hispidella*, *Koelpinia*, and *Pilosella* Hill in the *Tolpis* group. The most recent classification based on a morphological cladistic analysis (Bremer 1994) considered *Tolpis* to be a member of the subtribe Hieraciinae with *Andryala*, *Arnosseris*, *Hieracium*, *Hispidella*, *Hololeion* Kitam., and *Pilosella*. The only molecular phylogenetic analysis of the Lactuceae did not include *Tolpis* (Whitton et al. 1995). Although this study included 39 of the 98 recognized genera,

none of the subtribes with more than one genus in the three major classifications systems were monophyletic.

Generic circumscription of *Tolpis* has also been controversial, especially with respect to the placement of two species, *T. capensis* (L.) Sch.-Bip. from south-central Africa and Madagascar and *T. staticifolia* (All.) Sch.-Bip. from central and southeast Europe. Although both species have been traditionally included in *Tolpis*, macromorphological (Jarvis 1980) and palynological (Blackmore and Jarvis 1986) evidence was used to exclude these two species from the genus. *Tolpis capensis* was placed in *Hieracium* and *T. staticifolia* was considered to be the only member of *Chlorocephalis* Monn.

One of the most interesting features of *Tolpis* is that most of the species are endemic to the Macaronesian archipelagos. This region comprises 32 Atlantic Ocean islands in the Azores, Madeira, Selvagens, Canaries and Cape Verde Islands situated between 15° and 40°N latitude. The islands are 0.8 to 21 million years old (Mitchell-Thomé 1985, Carracedo 1994) and they exhibit a wide range of distances from the mainland with the closest island, Fuerteventura, only 100 km from the coast of Africa and the most distant island in the Azores 1600 km from the continent. The Macaronesian flora is distinct because it has been suggested to contain relicts of an older Tertiary flora from southern Europe and northern Africa (Bramwell 1985, Sunding 1979, Cronk 1992). Much of the evidence for the relictual nature of the flora comes from the fossil flora of southern Europe (Saporta 1865, Depape 1922, Sunding 1979). These fossil deposits contain many subtropical genera that are now present in Macaronesia but not in the Mediterranean, including such genera as *Clethra* L. (Clethraceae), *Dracaena* Vand. ex L. (Dracaenaceae), *Ocotea* Aublet. and *Persea* Miller (Lauraceae). These groups were eliminated from southern Europe by climatic changes at the end of the Tertiary and in the Pleistocene (Koeniguer 1993). Several recent molecular phylogenetic comparisons of Macaronesian groups have not confirmed the relic-

Table 1. Summary of subtribal placement of *Tolpis* in the classification systems of Stebbins (1953), Jeffrey (1966) and Bremer (1994)

Stebbins (1953)	Jeffrey (1966)	Bremer (1994)
Cichoriinae	<i>Tolpis</i> subgroup	Hieraciinae
<i>Arnosseris</i>	<i>Andryala</i>	<i>Andryala</i>
<i>Catananche</i>	<i>Arnosseris</i>	<i>Arnosseris</i>
<i>Cichorium</i>	<i>Hieracium</i>	<i>Hieracium</i>
<i>Hispidella</i>	<i>Hispidella</i>	<i>Hispidella</i>
<i>Hymenonema</i>	<i>Koelpinia</i>	<i>Hololeion</i>
<i>Hyoseris</i>	<i>Tolpis</i>	<i>Pilosella</i>
<i>Koelpinia</i>		<i>Tolpis</i>
<i>Tolpis</i>		

tual nature of many of the endemics because these taxa occurred in a derived position relative to their continental ancestors (Böhle et al. 1996; Kim et al. 1996a; Francisco-Ortega et al. 1997, 1999a, b; Caujape-Castells et al. 1999; Helfgott et al. 2000).

Tolpis is particularly interesting from a biogeographic perspective because it has species in four of the five Macaronesian archipelagos. The Canary Islands harbor six species that occur on the five western islands of El Hierro, La Palma, La Gomera, Tenerife, and Gran Canaria. The Azores contain two species that occur on eight islands (Lack 1981) and one of these is also distributed on the four islands of the Madeiran archipelago. One species is endemic to Madeira and one restricted to the Cape Verde Islands (Kilian 1988). Thus *Tolpis* is an ideal genus to determine if multiple colonization events may have occurred in a single endemic Macaronesian group.

In this study we perform phylogenetic analyses of *Tolpis* and putative generic relatives in the Lactuceae using the chloroplast encoded gene *ndhF*. The three objectives are to: (1) assess the monophyly of the genus, especially in view of previous suggestions (Jarvis 1980, Blackmore and Jarvis 1986) that *T. capensis* and *T. staticifolia* should be excluded from *Tolpis*; (2) examine the phylogenetic relationships of *Tolpis* to other genera of Lactuceae; and (3) examine the origin of the Macaronesian island endemics.

Materials and methods

Plant sampling. Our sampling strategy involved selecting 36 species from 28 genera of Lactuceae and outgroup taxa from the five other tribes (Arctoteae, Cardueae, Liabeae, Mutisieae, and Vernoniae) of the subfamily Cichorioideae (sensu Jansen and Kim 1996, Table 2). Outgroup selection was based on several previous phylogenetic comparisons of Asteraceae using both morphological (Bremer 1987, Karis et al. 1992) and molecular (Jansen et al. 1991, Kim and Jansen 1995, Jansen and Kim 1996) data. The generic level sampling of Lactuceae included all genera (except *Hololeion*

from Asia and *Hispidella* from southwest Europe) that have been suggested as close relatives of *Tolpis* in the classification systems of Stebbins (1953), Jeffrey (1966), and Bremer (1994), as well as the monograph of *Tolpis* by Jarvis (1980). We examined seven species of *Tolpis*, including the two species, *T. capensis* and *T. staticifolia*, which have been excluded from the genus by some previous workers (Jarvis 1980, Blackmore and Jarvis 1986). We also included representative species from three of the Macaronesian archipelagos and the Mediterranean region.

DNA isolation, PCR amplification, and sequencing. Total genomic DNAs were isolated from both living or silica dried leaves and herbarium specimens. Fresh material was obtained from field collected plants or those cultivated in the greenhouses at the University of Texas at Austin. The CTAB method of Doyle and Doyle (1987) was used to isolate DNA from fresh and silica dried material and DNAs were further purified by ultracentrifugation in cesium chloride/ethidium bromide gradients (Sambrook et al. 1989). Small-scale DNA isolations of herbarium specimens were performed as described in Loockerman and Jansen (1996).

The 3' region of *ndhF* was amplified by polymerase chain reaction (PCR) in 50 µl reactions using the primers 1201 and +607 (Jansen 1992, Kim and Jansen 1995) and the following protocol: one cycle of 3 min denaturation at 94 °C, 1 min annealing at 50 °C, and 1 min 20 sec extension at 72 °C; and 29 cycles in which the denaturation time was reduced to 1 min and the extension step was increased by 3 sec per cycle. This was followed by a final extension cycle of 7 min at 72 °C. In some cases, standard PCR did not generate sufficient DNA for sequencing, especially for *T. farinulosa*. In these instances, hotstart PCR (Delidow et al. 1993) was employed in which an initial denaturation for 5 min at 95 °C replaced cycle 1. The temperature was then reduced to 72 °C and the polymerase was added.

PCR products were purified using QIA-quick PCR purification kit (Qiagen Ltd) and automated sequencing was performed as described in Francisco-Ortega et al. (1999a). Sequencing primers included the two amplifying primers (1201 and +607) and one additional forward primer (1600) (Jansen 1992, Kim and Jansen 1995).

Sequence alignment and phylogenetic analysis. Sequences were edited using Sequencher (Genecodes). CLUSTAL X (Thompson et al. 1994) was

Table 2. Plant material included in this study with information about the source and the voucher. Collection or accession numbers follow the name of collector or botanic garden. Standard herbarium acronyms that follow collection numbers indicate location of voucher. Botanical gardens are: Basel, Botanical Garden, University of Basel; Beal, Beal Botanical Garden, Michigan State University; Belgium, National Botanical Garden of Belgium; Berlin, Botanischer Garten Berlin-Dahlem; Faial, Botanic Garden of Faial, Azores, Portugal; Göttingen, Botanischer Garten und Neuer Botanischer Garten der Universität Göttingen; Kew, Kew Botanical Garden

Taxon	Source	Genbank Accession Number
Lactuceae		
<i>Andryala integrifolia</i> L.	Belgium 2495	AF218338
<i>Arnoseris minima</i> (L.) Schweigger & Koerte	Belgium 2527	AF218347
<i>Catananche caerulea</i> L.	Kew 076-82-00460	AF218349
<i>Cichorium intybus</i> L.	Jansen 1110 (TEX)	L39390
<i>Crepis biennis</i> Bieb.	Berlin s.n.	AF218345
<i>Crepis pyrenaica</i> (L.) Greuter	Univ. of Reading Bot. Gard. 200	AF218339
<i>Crepis tectorum</i> (L.) W. Greuter	Belgium s.n.	AF218348
<i>Dendroseris marginata</i> (Bert ex Decne) Hook. & Arn.	Stuessy et al. 6515 (OS)	AF218340
<i>Hieracium longipilum</i> Torr.	Jansen 810 (TEX)	AF218351
<i>Hyoseris radiata</i> L.	Jansen 1106 (TEX)	AF218344
<i>Hypochaeris uniflora</i> Vill.	Kew 050-87-00486	AF218333
<i>Koelpinia linearis</i> Pallas	S. Park s.n. (TEX)	AF218341
<i>Lactuca sativa</i> L.	Grocery Store	L39389
<i>Leontodon saxatilis</i> Lam.	Jansen 1120 (TEX)	AF218330
<i>Malacothrix saxatilis</i> (Nutt.) Torrey & Gray	UC 831385	AF218335
<i>Picris pauciflora</i> Willd.	Belgium s.n.	AF218343
<i>Pilosella aurantiaca</i> Sch.-Bip.	Jansen 801 (TEX)	AF218336
<i>Reichardia tingitana</i> (L.) Roth	Kew 223-70-02090	AF218350
<i>Rhagadiolus stellatus</i> (L.) Gaertn.	Belgium s.n.	AF218331
<i>Scolymus hispanicus</i> L.	Basel 1174	AF218332
<i>Taraxacum officinale</i> L.	Jansen 1107 (TEX)	AF218346
<i>Tolpis azorica</i> (Nutt.) P. Silva	Faial, Index Seminum 12 (ORT)	AF218327
<i>Tolpis barbata</i> (L.) Gaertn.	Basel 222	AF218326
<i>Tolpis capensis</i> (L.) Sch.-Bip.	G. Germishuizen 5790 (J)	AF218342
<i>Tolpis coronopifolia</i> (Desf.) Biv.	A. Santos-Guerra & J. Francisco-Ortega s.n. (ORT)	AF218329
<i>Tolpis farinulosa</i> (Webb) J. A. Schmidt	T. Leyens s.n. 11-19-98	AF218328
<i>Tolpis staticifolia</i> (All.) Sch.-Bip.	Göttingen 507 (TEX)	AF218337
<i>Tolpis virgata</i> (Desf.) Bertol.	Vogt 15533 (Berlin)	AF218325
<i>Tragopogon porrifolius</i> L.	K.-J. Kim 13028 (SNU)	L39391
<i>Uropappus lindleyi</i> (DC.) Nutt.	Chambers 5278 (OSC)	AF218334
<i>Urospermum dalechampii</i> (L.) Scop. ex F. W. Schmidt	Kew 193-77-01532	AF218352
Outgroups		
<i>Arctotis stoechidifolia</i> P. Bergius	Jansen 920 (MICH)	L39425
<i>Centaurea americana</i> Nutt.	K.-J. Kim 13030 (SNU)	L39416
<i>Leibnitzia anandria</i> (L.) Turcz.	K.-J. Kim 13564 (SNU)	L39403
<i>Liabum glabrum</i> Hemsley	Panero 2437 (TEX)	L39421
<i>Vernonia mespilifolia</i> Less.	Jansen 995 (MICH)	L39427

used for multiple alignment of DNA sequences followed by minor manual adjustments. Parsimony analyses were conducted using PAUP 4.0b2b (Swofford 1998). Heuristic searches with 100 random entries were performed using ACCTRAN, MULPARS, and TBR options. Gaps were treated as missing data and all characters were accorded equal weight. The amount of support for monophyletic groups was evaluated by 100 bootstrap replicates (Felsenstein 1985) with the ACCTRAN, MULPRAS, and TBR options and a single random entry of taxa. Parsimony Jackknifing (Farris et al. 1996) using 1000 replications was also performed to assess support for monophyletic groups. The consistency index (CI, Kluge and Farris 1969), retention index (RI, Farris 1989) and the *gl* statistic (Hillis and Huelsenbeck 1992) were computed by PAUP. The latter was obtained after computing the treelength distribution of 100,000 random trees by means of the random trees command.

Results

Sequence variation. The 36 aligned *ndhF* sequences are 980 bp long, with 691 invariant and 289 variable positions, including 140 parsimony-informative sites. The number of insertions/deletions (indels) relative to the outgroups *Leibnitzia* and *Centaurea* is nine and they range in size from 3 to 9 bp. Only two of these indels were shared by more than one taxon. The first is a 9 bp deletion in all taxa except the outgroup. The second is a 6 bp insertion in *Dendroseris*, *Hyoseris*, *Reichardia*, and *Urospermum*. The insertions are associated with short direct repeats suggesting that they are caused by slipped-strand mispairing during DNA replication. This phenomenon was suggested for *ndhF* by Kim and Jansen (1995) in the Asteraceae and Olmstead and Sweere (1994) in the Solanaceae. The G+C content ranges from 12.3% to 39.9%. Base average is A: 28.2%, G: 13.2%, C: 14.8%, T: 36.8%. Transition (Ts)/Transversion (Tv) ratio is 1:1.25.

Phylogenetic analyses. Phylogenetic analyses produce 57 equally parsimonious trees of 554 steps with a consistency index (CI) of

0.687 (excluding autapomorphies), a retention index (RI) of 0.674, and *gl* value of -0.876 (100,000 random trees). The seven examined species of *Tolpis* occur in different clades in the *ndhF* tree (Fig. 1). *Tolpis capensis* from south-central Africa and Madagascar is sister to *Taraxacum*, although support for this relationship is not strong with four characters and 28% and 62% bootstrap and jackknife values, respectively. These two taxa are in the same clade as *Urospermum* but again this position is weakly supported. *Tolpis staticifolia* is nested within the genus *Crepis* and is sister to *C. pyrenaica*. The monophyly of the clade that includes the three examined species of *Crepis* and *Tolpis staticifolia* is strongly supported by 15 characters and bootstrap and jackknife values of 100%. The remaining five species of *Tolpis* form a strongly supported clade (18 characters and a 100% bootstrap and jackknife values). This core *Tolpis* group is sister to a clade of 11 genera from five different subtribes (sensu Bremer 1994), including two genera, *Crepis* and *Hyoseris*, which have been previously suggested to be closely allied to *Tolpis* (Stebbins 1953). Support for this relationship is moderate with 3 changes and 67% and 99% bootstrap and jackknife values, respectively.

Interspecific relationships within the core *Tolpis* clade are fully resolved (Fig. 1). The species endemic to the Azores (*T. azorica*) is sister to the remaining four species, which are split into two clades. The first includes the two continental taxa, *T. barbata* and *T. virgata*, and is supported by only one character and bootstrap and jackknife values of 66% and 90%, respectively. The second clade includes the two remaining island endemics, *T. coronopifolia* (Canary Islands) and *T. farinulosa* (Cape Verde Islands), which is supported by one character change and bootstrap and jackknife values of 56% and 91%, respectively.

Discussion

Generic circumscription. The *ndhF* tree (Fig. 1) clearly indicates that *Tolpis* in the traditional

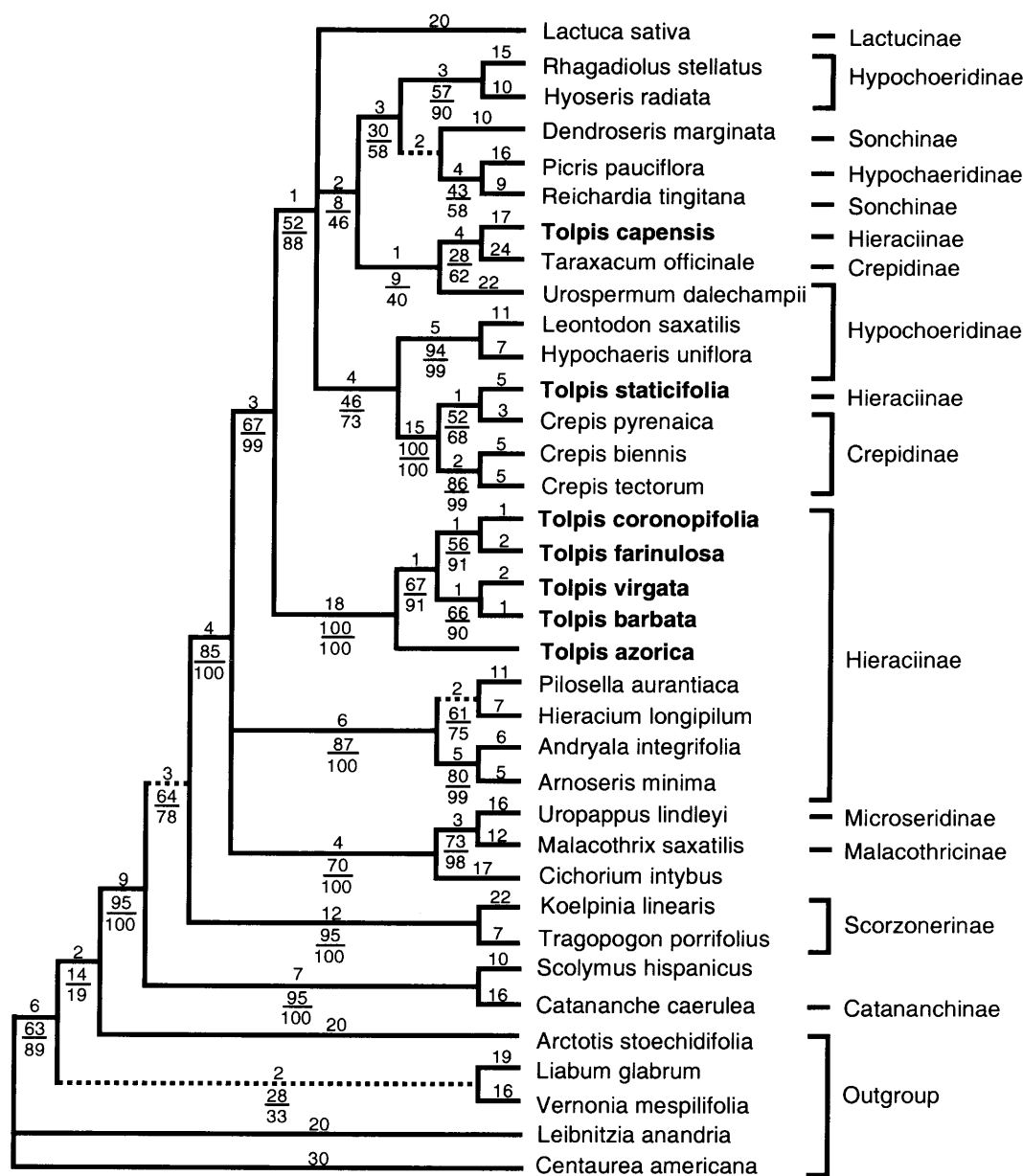


Fig. 1. One of the 57 most parsimonious trees (554 steps, CI = 0.687, RI = 0.674) based on 140 phylogenetically informative changes in *ndhF* sequences of 36 taxa. The number of changes is indicated above branches and the bootstrap/jackknife values are found below branches. Dashed lines indicate nodes that collapse in the strict consensus tree. Vertical bars indicate subtribes of Lactuceae following Bremer (1994)

sense is not monophyletic because two species, *T. capensis* and *T. staticifolia*, are not grouped with the other five examined species. The lack of monophyly of the genus is not surprising in view of previous suggestions that these two species should be excluded from the genus

(Jarvis 1980, Blackmore and Jarvis 1986). Jarvis (1980) used both crossing data and morphology to exclude these species from *Tolpis*. In the case of *T. capensis*, Jarvis (1980) argued that both gross morphology and cypsela characters suggest affinities of this

species with *Hieracium*. The *ndhF* tree does not confirm a relationship with *Hieracium* but instead groups *T. capensis* with *Taraxacum*, although this relationship is weakly supported.

Tolpis staticifolia is nested within the genus *Crepis* in the *ndhF* tree, and specifically is sister to *C. pyrenaica*. Support for the inclusion of *T. staticifolia* in *Crepis* is very strong. Jarvis (1980) excluded this species from *Tolpis* and suggested that it belongs in *Chlorocrepis*, a genus which has not been recognized in any recent classification systems of the Lactuceae (Stebbins 1953, Jeffrey 1966, Bremer 1994). Our *ndhF* data suggest that *T. staticifolia* should be transferred into *Crepis*, a large and morphologically diverse genus with approximately 200 species (Babcock 1947). Expanded sampling and additional molecular and morphological comparisons are needed to determine the most accurate placement of *T. staticifolia* among the 27 recognized sections of *Crepis* (Babcock 1947).

Phylogenetic position of *Tolpis* within the Lactuceae. Generic affinities of *Tolpis* have been examined by a number of workers in the Asteraceae. The three most recent subtribal classifications of the Lactuceae provided differing opinions on relationships of the genus to other members of the tribe (Table 1). Stebbins (1953) placed *Tolpis* in the subtribe Cichoriinae with seven other genera. He suggested that *Tolpis* was most closely allied to *Arnoseris* and *Hispidella*. Jeffrey (1966) placed *Tolpis* in his informal *Tolpis* subgroup and allied it to *Arnoseris* based on several morphological characters, including styles with short arms and collecting hairs, corolla tube covered externally with crisped hairs, and a pappus of setae and small scales. Bremer's (1994) subtribal treatment, which was based on a morphological cladistic analysis of 22 genera representing the major monophyletic groups of Lactuceae, classified *Tolpis* in the Hieraciinae with six other genera. His morphological cladogram did not support or refute the monophyly of this subtribe because the two representatives, *Hieracium* and *Tolpis*, were in an unresolved tetrachotomy (Fig. 2). Overall

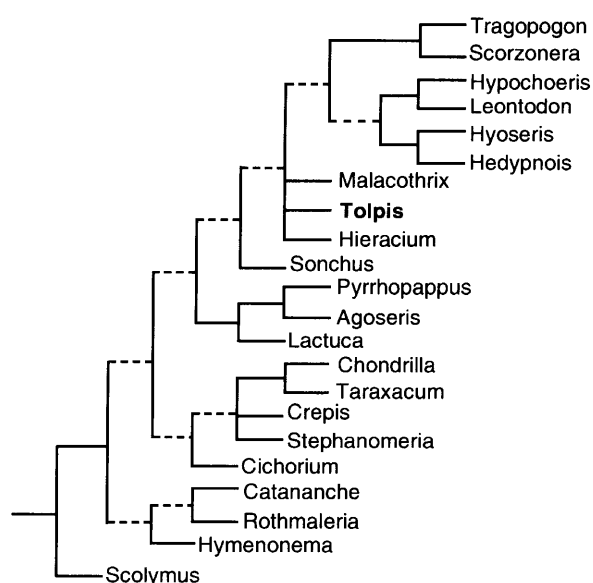


Fig. 2. One of 93 equally parsimonious cladograms (65 steps, CI = 0.53) based on 23 morphological and chromosomal characters and 22 genera (adapted from Bremer 1994). Dashed lines indicate nodes that collapse in the strict consensus tree

the morphological cladogram does not resolve intergeneric relationships in the Lactuceae because all but five nodes collapse in the strict consensus tree (see dashed lines in Fig. 2).

Two additional studies discussed the generic affinities of *Tolpis*. Pollen investigations of Blackmore (1981) suggested that the small pollen grains with double rows of spines on the equatorial ridges support a close relationship between *Arnoseris*, *Hispidella*, and *Tolpis*. Jarvis (1980) suggested that morphological similarities of the pappus and cypselas and chromosome number suggest a close relationship between *Tolpis* and *Hieracium*.

The *ndhF* tree (Fig. 1) agrees with earlier cpDNA restriction site comparisons (Whitton et al. 1995) indicating that none of the currently recognized subtribes of Lactuceae are monophyletic. The core *Tolpis* clade (excluding *T. capensis* and *T. staticifolia*) is an isolated lineage that is sister to a group of 11 genera from several different subtribes (sensu Bremer 1994). These 11 genera do not include any of those commonly suggested as close relatives of

Tolpis (Table 1). Most previous workers have suggested a close relationship with the five genera *Arnosericis*, *Andryala*, *Hieracium*, *Hispidella*, and *Koelipinia* (Table 1). Bremer (1994) excluded *Koelipinia* from this list of putative relatives primarily because pollen studies (Blackmore 1981) clearly indicated a close relationship with *Tragopogon*. The *ndhF* tree (Fig. 1) strongly supports a sister group relationship between these two genera. The *ndhF* tree places *Arnosericis*, *Andryala*, and *Hieracium* (also *Pilosella*, which is often considered congeneric with *Hieracium*) into a very strongly supported monophyletic group. Thus it appears that *Tolpis* is not closely allied with any of these genera. Unfortunately we did not have material of the monotypic genus *Hispidella*, the fourth genus frequently considered a close relative of *Tolpis* (Table 1).

Our *ndhF* phylogenies, combined with previous cpDNA restriction site (Whitton et al. 1995) and morphological (Bremer 1994) cladograms, indicate that the subtribal classification of the Lactuceae needs to be re-evaluated. Additional taxon sampling for all three of these data sets is needed to generate a phylogenetically-based classification for the tribe.

Biogeography of island endemics. The Macaronesian archipelagos are distinctive from other oceanic island systems in two biogeographic features. First, they exhibit a wide range of distances from continental source areas, ranging from 100 km for Fuerteventura of the Canary Islands to 1600 km for the islands of the Azores. Second, there is a wide geographic separation of approximately 3000 km between the northernmost (Azores) and southernmost (Cape Verde) archipelagos. Both of these features, especially the close proximity of some of the islands to the continent, make it possible that some plant groups with endemic species in more than one archipelago of Macaronesia may have experienced multiple colonizations from the mainland. Also, the close proximity of some of the islands to continental region and the fact that some members of the flora may be relictual

opens the possibility for colonization of continental regions from the islands. Although we have only examined three of the ten island endemics, the *ndhF* phylogeny indicates that the island species do not form a monophyletic group. There are two equally parsimonious biogeographic scenarios that could account for the relationships depicted in the *ndhF* tree (Fig. 1): (1) There were multiple colonizations of the Macaronesian islands, one to the Azores and a second to the Canary and Cape Verde Islands. If this scenario is correct, the basal position of *T. azorica* would suggest that the Azores were colonized first with a second introduction into the Canary and Cape Verde Islands. (2) There was a single origin of island species of *Tolpis* followed by colonization of the continent.

Multiple colonizations of the same endemic plant group to oceanic archipelagos is not common, primarily because of the distant geographical isolation of the islands. In the Pacific Rim only two examples are known: *Rubus* (Rosaceae) in Hawaii (Howarth et al. 1997) and *Gossypium* (Malvaceae) in the Galápagos (Wendel and Percival 1990, Wendel et al. 1995). The only previously documented cases of multiple colonizations of Macaronesia are in *Hedera* (Vargas et al. 1999), *Ilex* (Cuénoud et al. 2000), and *Lavatera* (Malvaceae; Ray 1995, J. Fuertes, J. Francisco-Ortega, M. Ray, A. Santos-Guerra, and R. Jansen, unpubl). Multiple colonizations of Macaronesia in these genera are not consistent with the pattern of a single introduction that has been demonstrated for many other endemic Macaronesian plant groups, including *Argyranthemum* (Asteraceae, Francisco-Ortega et al. 1996, 1997), *Echium* (Boraginaceae, Böhle et al. 1996), *Sonchus* alliance (Asteraceae, Kim et al. 1996a, b), *Aeonium* (Crassulaceae, Mes et al. 1996), *Crambe* (Brassicaceae, Francisco-Ortega et al. 1999b), *Bencomia* alliance (Rosaceae, Helfgott et al. 2000), *Pericallis* (Asteraceae, Panero et al. 1999), and *Sideritis* (Barber et al. 2000).

There is only one example of the derivation of continental taxa from Macaronesian island

endemics. Molecular phylogenies of *Aeonium* (Mes et al. 1996) indicated that the east African species, *A. leucoblepharum*, is nested within a derived clade of Macaronesian island species.

Expanded taxon sampling and the use of more variable molecular markers should enable the resolution of which of these two alternative biogeographic scenarios is correct for *Tolpis*. We are currently gathering both cpDNA restriction site data and DNA sequences of the external transcribed spacer region of nuclear ribosomal DNA for all continental and island species to resolve the biogeography of this genus.

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