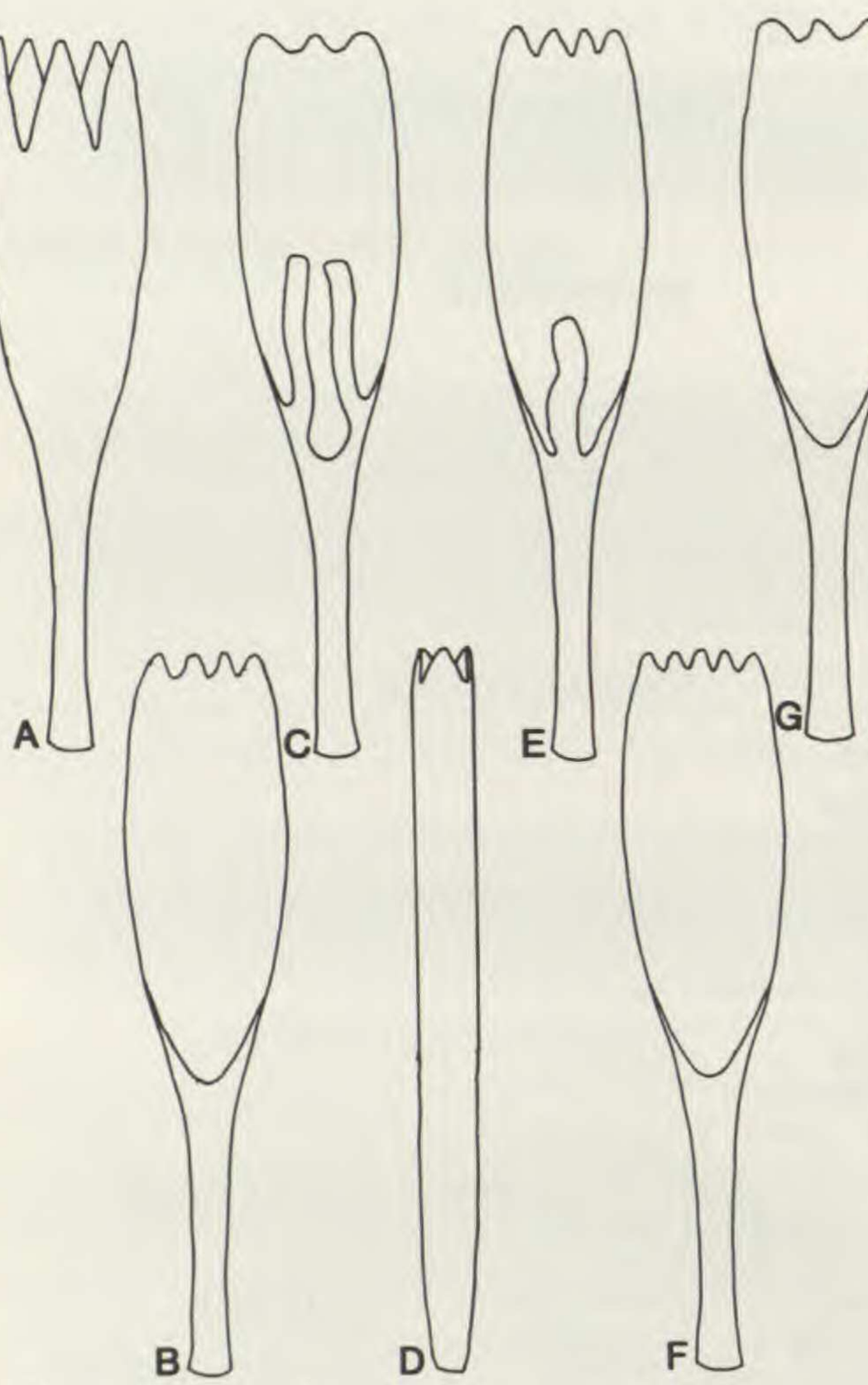


FIGURE 2. Corollas in Asteraceae.—A. Actinomorphic.—B. Gorteriinae ray.—C. Bilabiate.—D. Tubular.—E. Pseudobilabiate.—F. Ligule.—G. True ray.

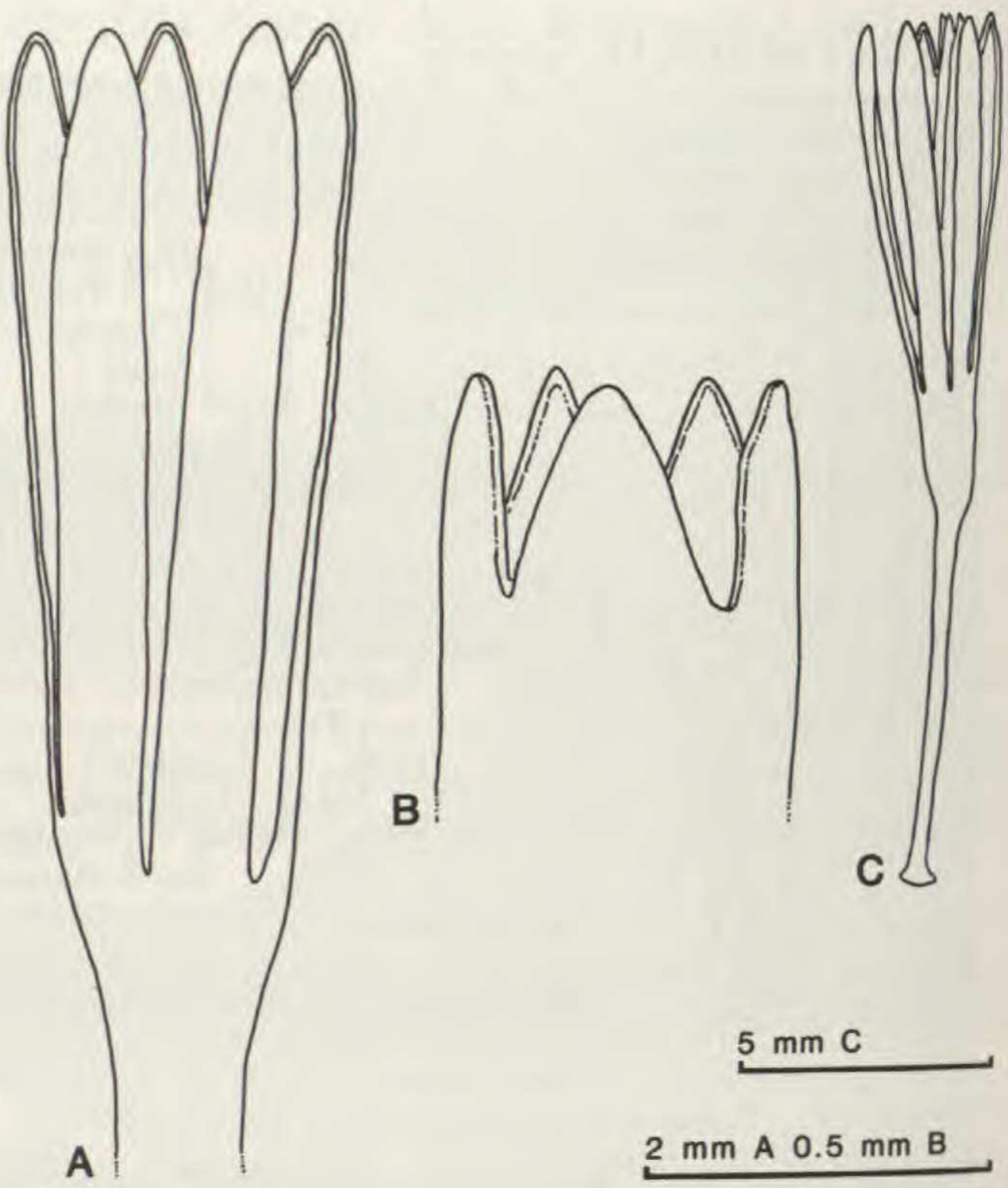


FIGURE 3. Corolla characters.—A. Actinomorphic, deeply 5-lobed, typical for most Mutisieae and Cardueae.—B. Shortly lobed actinomorphic, typical for most Asteroideae.—C. Corolla with a long slender tube typical for Cardueae. (A, *Dicoma galpinii, Germishuizen 294*; B, *Helichrysum auriceps, Ross 2099*; C, *Carduus argyroa, Segelberg 16460/3.*)

deae similar to that from Bremer's analysis. The status of the Cichorioideae thus requires further investigation.

Our aim with this study is to clarify the relationships in the basal part of the family. This is important because these relationships were not resolved with certainty by Bremer (1987). Further, the relationships based on molecular data (Jansen et al., 1990) seem unstable.

Relationships of the tribe Mutisieae (excluding the Mutisieae–Barnadesiinae) are crucial for understanding the basal diversification of the family (Bremer, 1987).

## MATERIAL AND METHODS

The study is based on herbarium material, but we also gathered a large amount of information (used as characters, Appendix II) from the literature. Our emphasis is on morphological features, and especially large is the number of characters gained from florets, including many microcharacters. We investigated numerous genera of Mutisieae with selected genera from other cichorioid tribes and selected Asteroideae representatives.

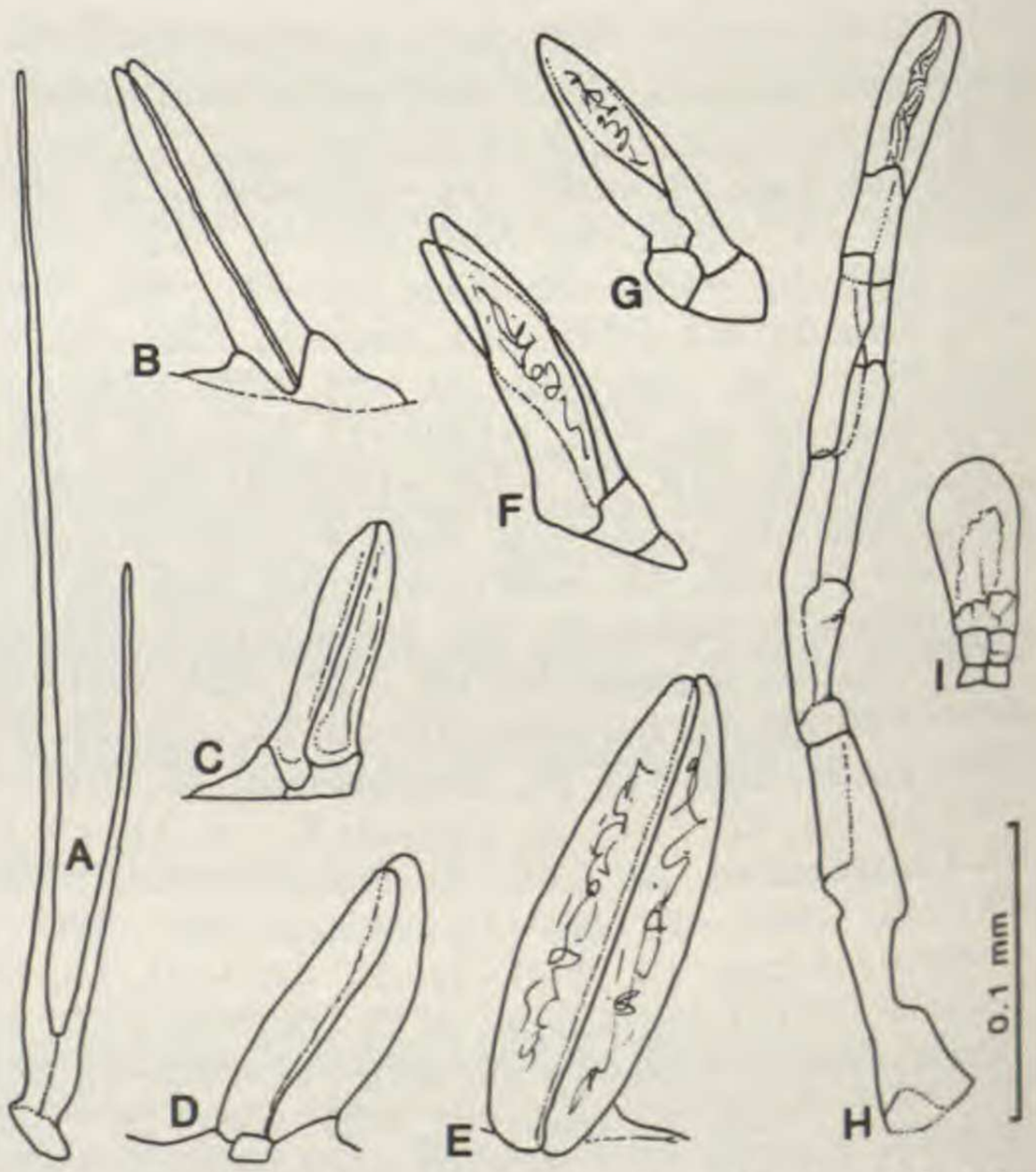


FIGURE 4. Twin trichomes.—A. Deeply cleft twin hair.—B. Short ovoid twin hair, nonmyxogenic.—C–G. Short ovoid twin hairs, myxogenic.—H. Long glandular hair.—I. Short glandular hair. (A, *Erythrocephalum zambesiacum, Faulkner 110*; B, *Acourtia glomeriflora, Asplund 148*; C, *Trixis brasiliensis, Malme 1124*; D, *Trixis cacalioides, Asplund 10832*; E, *Trixis californica, Dillon et al. 997*; F, G, *Jungia axillaris, Chavez 3260*; H, I. *Munnozia hastifolia, Asplund 12510.*)

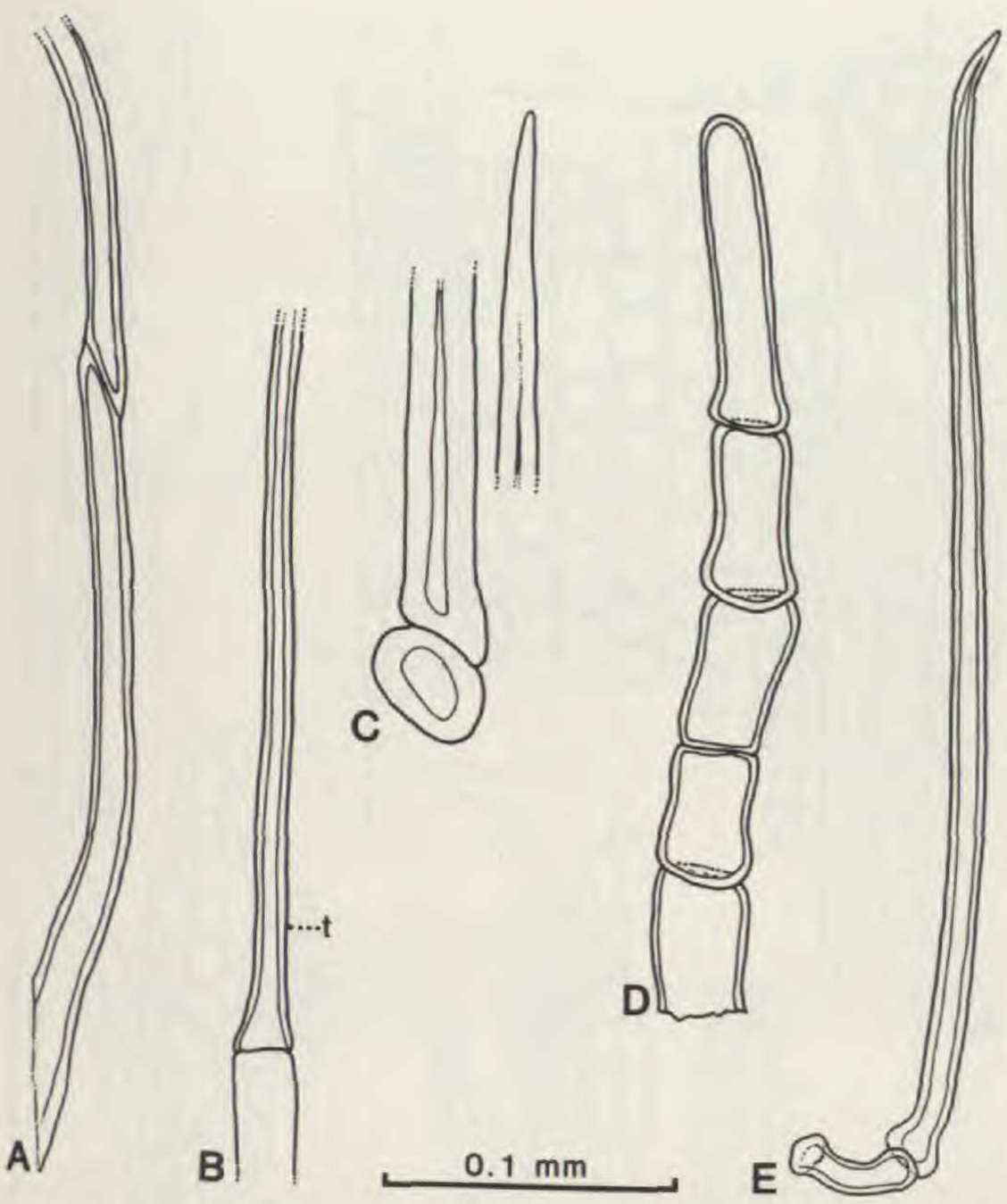


FIGURE 5. Simple corolla hairs.—A. Cell walls oblique.—B. Walls in ultimate cell much thicker than in the cell below, marked t.—C. With basal cell.—D, E. Walls straight. (A. *Mutisia clematis, Killip & Smith 19600*; B, *Scolymus hispanicus, Starbäck 119*; C, *Dasyphyllum reticulatum, Hatschbach 27243*; D, *Pluchea camphorata, Jansson s.n.*; E, *Pluchea lanceolata, Meebold 11036*.)

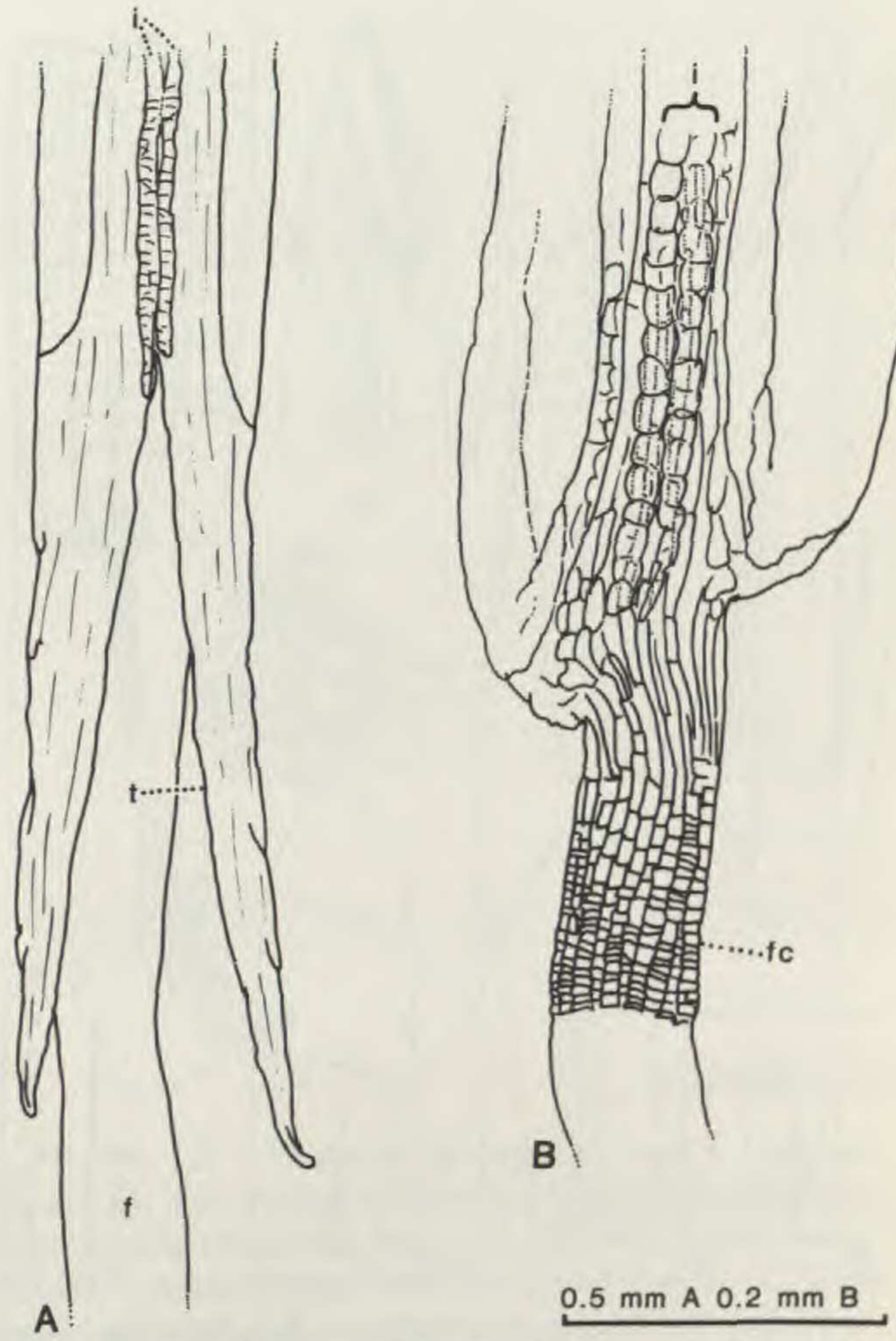


FIGURE 6. Features of anther bases.—A. Calcarate, caudate, i = inner pollen sacs, opened, oriented toward the observer, t = sterile tail, f = filament.—B. Ecalcarate, ecaudate, i = inner pollen sacs, oriented toward the observer, fc = filamental collar. (A, *Lycoseris latifolia, Breteler 4414*; B, *Geissolepis suadaefolia, Pringle 3762*.)

Some poorly understood genera were included to refine hypotheses about their placement.

Genera used as a basis for coding terminal taxa are given in Appendix I. A list of specimens examined is available from P. O. Karis. All material cited in the figure legends is housed in S, except for a specimen of *Inula inuloides*, which is housed in K.

Wagner parsimony analyses were performed using HENNIG 86 (Farris, 1988). In all analyses, options mhennig* and bb* were applied to provide the most extensive heuristic option available. All multistate characters are treated as unordered. Some multistate characters include states that may be correlated. The details of the analyses are discussed further below. Polarization was determined by the outgroup comparison method (Stevens, 1980; Maddison et al., 1984).

## Data Analysis and Results

Running our entire initial matrix causes a computer memory overflow because of numerous equally parsimonious solutions. Therefore, we have performed a series of more than 100 analyses with reductions of taxa, in order to explore the phylogenetic information in the matrix (similar to those of Doyle & Donoghue, 1986). Using this approach (detailed below), we hypothesize relationships of the Cichorioideae tribes, although generic relationships remain uncertain.

Numerous equally parsimonious trees are a major problem with analyses of large data matrices. Strict consensus trees of these equally parsimonious trees may be uninformative. A single unstable taxon may cause the strict consensus tree to collapse. Adams's (1973) consensus trees are less sensitive to unstable taxa, but conceptually more difficult to evaluate in a phylogenetic context. For example, an Adams consensus tree may contain branching sequences that are not present in any of the equally parsimonious cladograms.

Initially, eight genera of Mutisieae–Barnadesiinae were included as the outgroup, but various equally parsimonious arrangements among these genera multiplied the number of ingroup solutions. The outgroup was replaced subsequently with a hypothetical ancestor based on these Barnadesiinae. In cases of variation among the outgroup genera, coding followed the states of Calyceraceae, Goodeniaceae, Campanulaceae, and Lobeli-

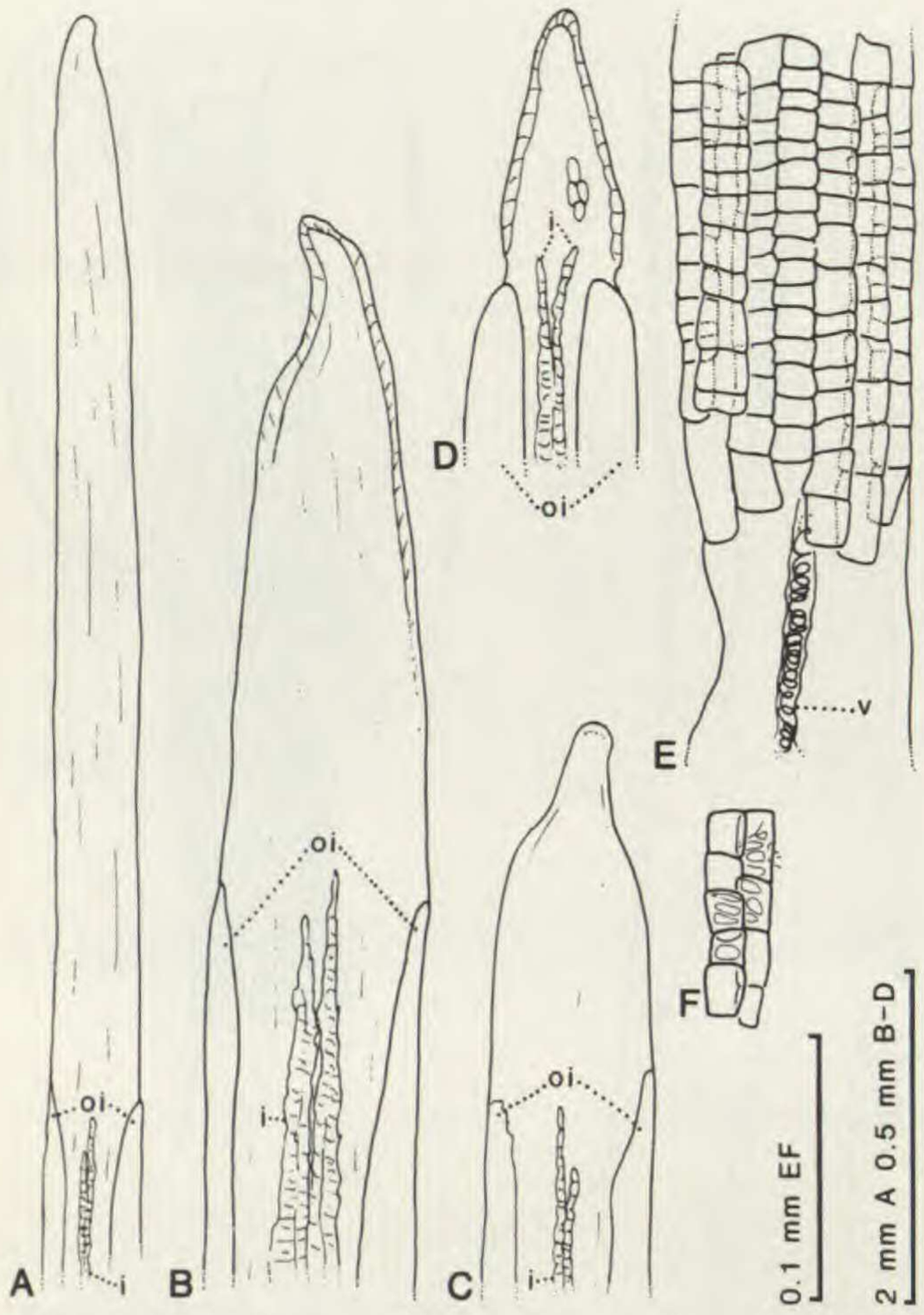


FIGURE 7. Features of anther appendages and collar, i = inner pollen sacs, opened and faced toward the observer, oi = outer and inner pollen sacs, without a distinct limit in between.—A, B. Long appendage.—C. Medium appendage.—D. Short appendage.—E. Conspicuous collar, v = vein.—F. Collar cells with transverse thickenings. (A, *Mutisia acuminata, Hutchison 1044*; B, *Dicoma picta, Dinter 8253*; C, *Gochnatia rupestris, Malme 2417*; D, *Aster simplex, Jones 32876*; E, *Inula helenium, Wall s.n.*; F, *Lactuca muralis, Karis & Källersjö s.n.*)

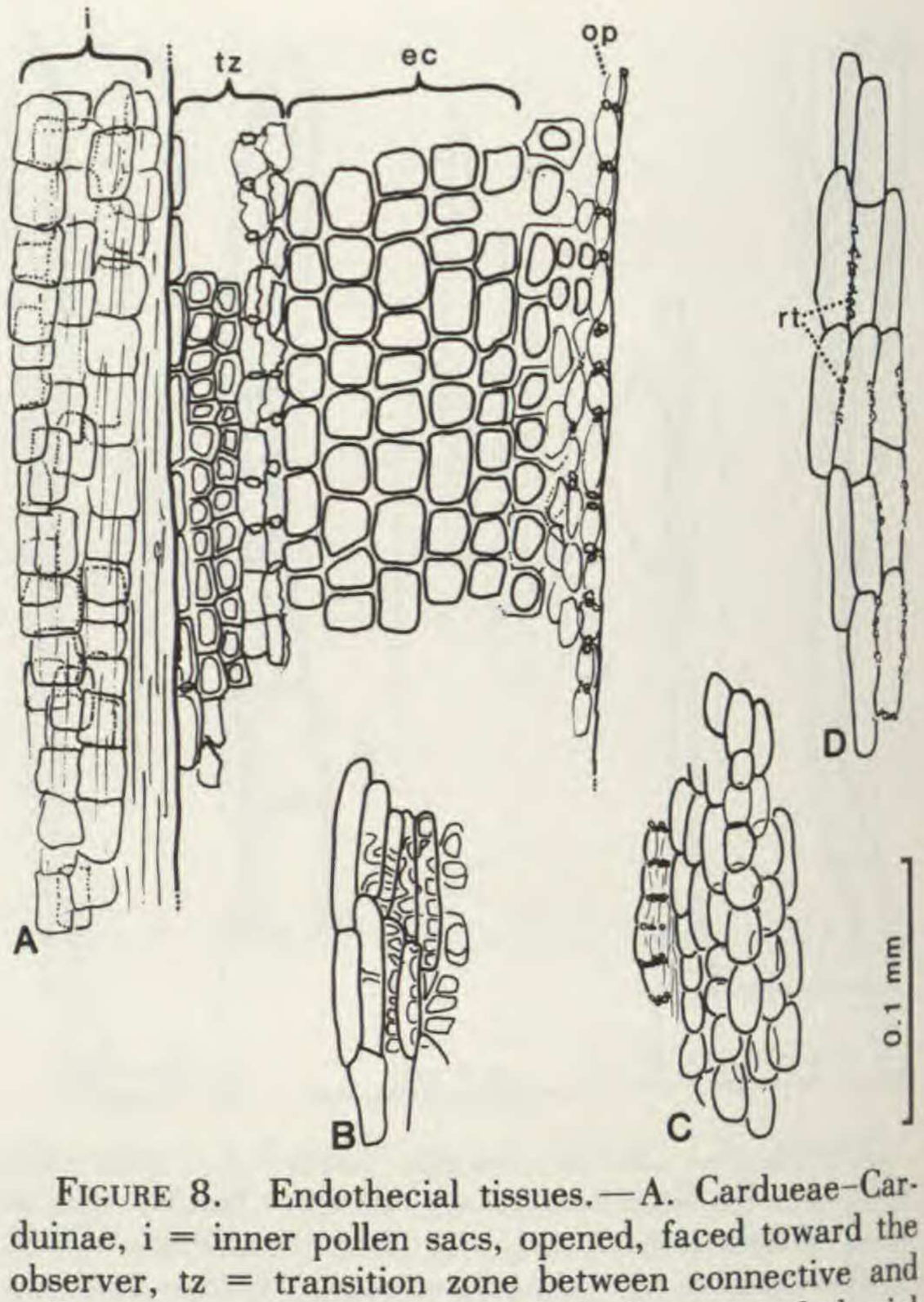


FIGURE 8. Endothecial tissues.—A. Cardueae–Carduinae, i = inner pollen sacs, opened, faced toward the observer, tz = transition zone between connective and pollen sacs, partly seemingly polarized, ec = endothecial cells typical for the Cardueae–Carduinae, op = outer pollen sac, polarized at the edge.—B. Lactuceae, transversal bands.—C. Mutisieae and Cardueae sens. lato, with polarized thickenings at the edges of the pollen sacs.—D. Barnadesiinae, rt = radial thickenings. (A, *Carduus nutans, Wall s.n.*; B, *Lactuca muralis, Karis & Källersjö s.n.*; C, *Gochnatia rupestris, Malme 2417*; D, *Dasyphyllum candolleanum, Irwin, Souza & dos Santos 7960.*)

aceae. When families outside Asteraceae could not be used to decide among coding variants for the hypothetical ancestor, character states were coded as "inapplicable" in the outgroup. Analysis using the hypothetical ancestor continued to result in large numbers of trees in which taxa moved within the same subclade of the different cladograms.

We ascertained stable configurations of taxa and unstable genera that were subsequently excluded. Finally, some genera, e.g., *Senecio*, *Aster*, *Geissolepis*, and *Cirsium*, were added in order to stabilize the Asteroideae and the Cardueae; in previous analyses the position of clades such as Asteroideae and Cardueae (sens. str.) was influenced by the choice and number of genera included.

Following experimental taxon deletion and addition, the data matrix consisted of 53 genera and 72 characters (with taxon reduction some characters became uninformative and were deleted). Two equally parsimonious cladograms (one shown in Fig. 1), 419 steps long, resulted from this matrix. The two cladograms differ only in the position of *Onoseris*, which is placed as the sister group of the taxa above node 17 in the alternative. The consistency index is 0.24 and the retention index 0.57.

Our cladogram (Fig. 1) is obviously partly in conflict with earlier schemes. Alternative topologies consistent with prior classifications and earlier cladistic analyses (e.g., Bremer, 1987; Jansen et al., 1990) were investigated using the xx-command in HENNIG 86. More complicated rearrangements, such as forcing monophyly of Mutisieae (excluding the Barnadesiinae) and the entire Cichorioideae, were achieved using heavily weighted dummy characters to produce the desired topology (the steps added by the dummy character were deleted in computing tree length).

Forcing monophyly of the entire subfamily Ci-

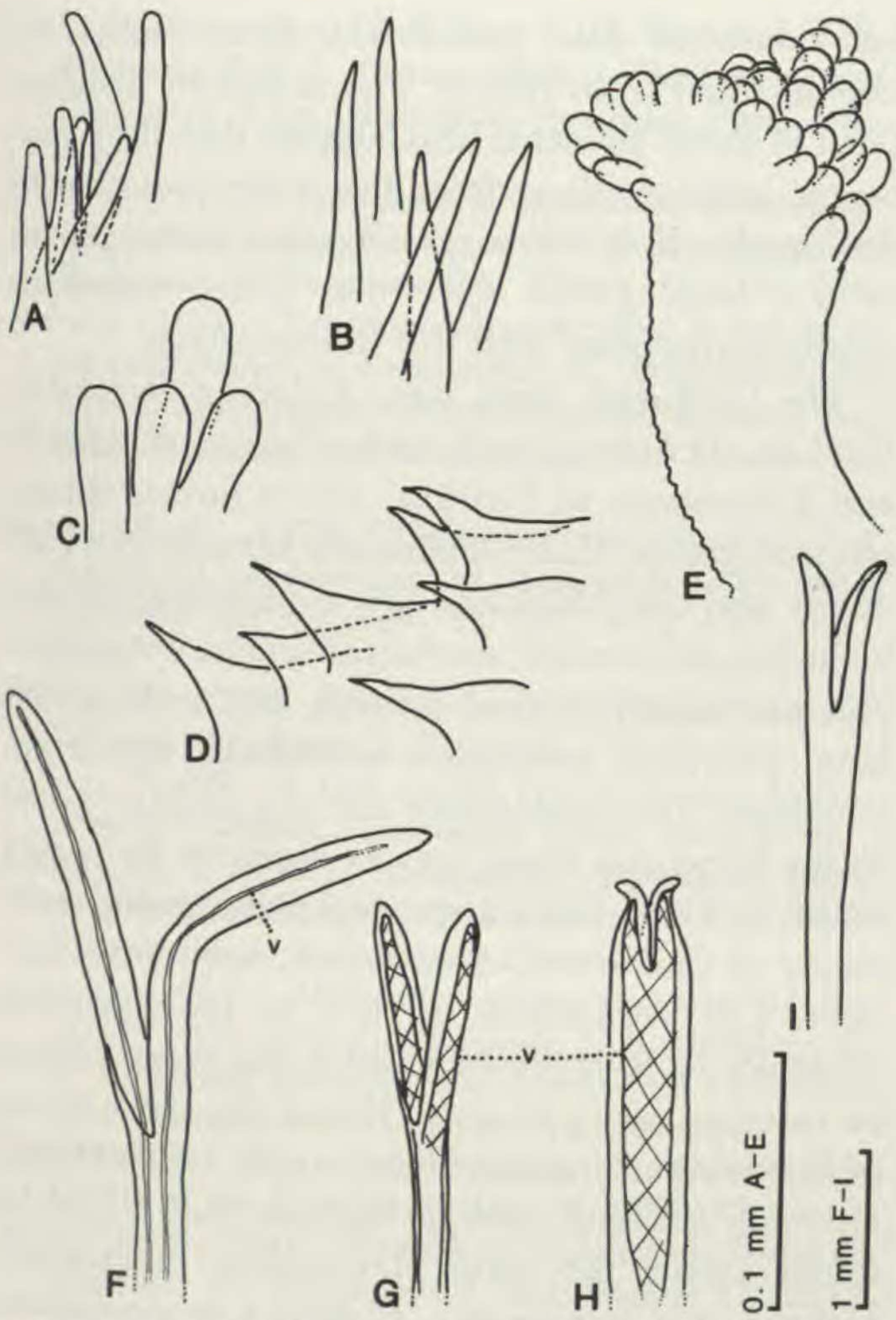


FIGURE 9. Features of styles and nature of sweeping hairs, v = vein.—A. Narrow obtuse sweeping hairs.—B. Narrow acute sweeping hairs.—C. Broad obtuse sweeping hairs.—D. Broad acute sweeping hairs.—E. Anthemoid style, with sweeping hairs only apically, typical for Nassauviinae and many Asteroideae.—F. Style with long, gradually tapering branches with narrow veins.—G. Style with long branches with thick veins (hatched).—H. Style with short branches with thick veins (hatched).—I. Style with short branches. (A, I, *Lulia nervosa, Hatschbach 23205*; B, *Chaetanthera elegans, Eyerdam 10725*; C, F, *Distephanus aurantiacus, La Croix 3024*; D, *Scolymus hispanicus, Starbäck 119*; E, *Helichrysum aureolum, Hilliard & Burtt 14369*; G, *Inula inuloides, Davis 24353* [K]; H, *Dicoma burmannii, Hafström & Acocks 2284*.)

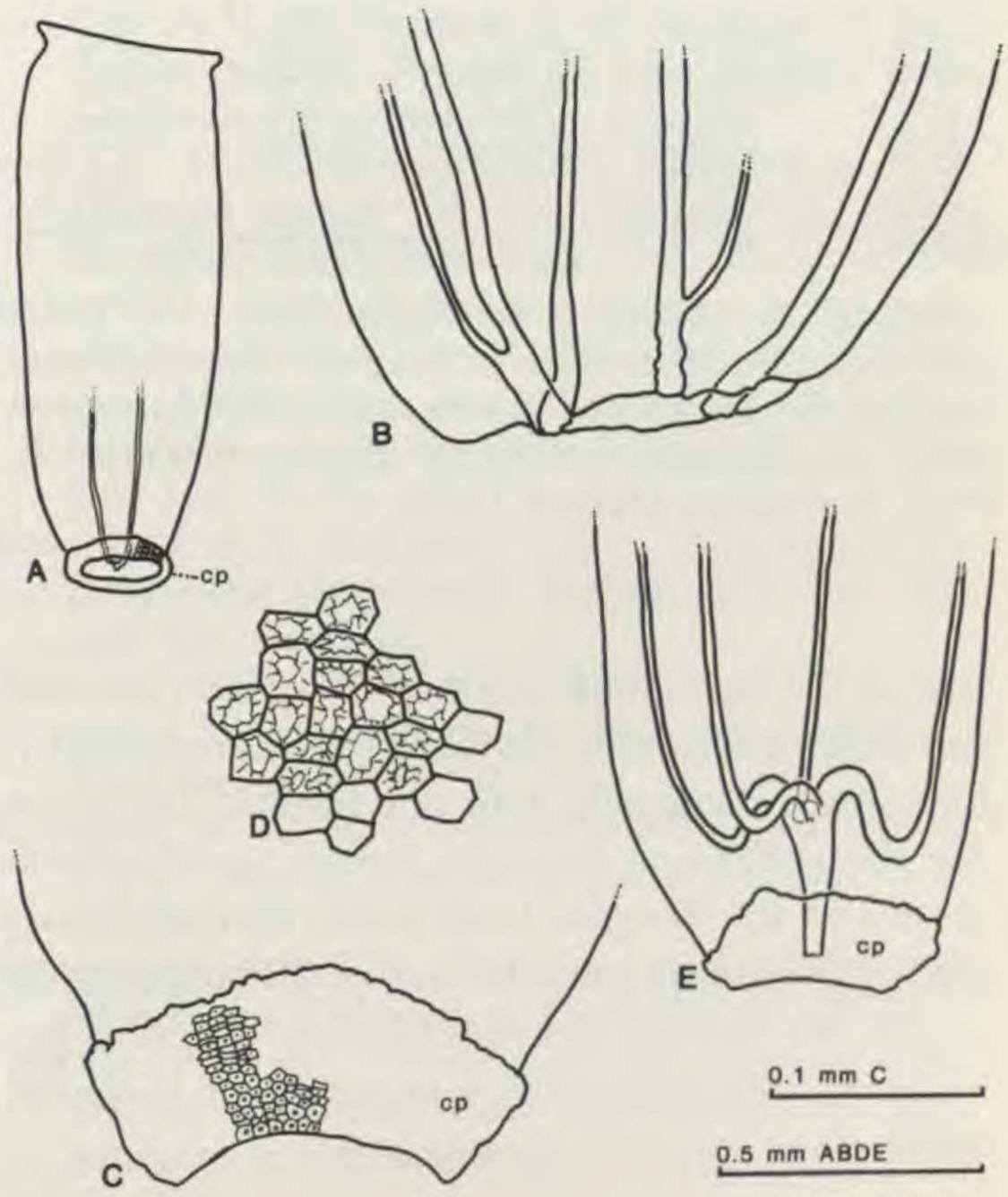


FIGURE 10. Features of cypselas, cp = carpopodium.—A. Two-veined cypsela with a small carpopodium, veins uniting within the base.—B. Cypsela without a carpopodium, veins uniting below the cypsela base.—C. Well-developed carpopodium consisting of sclerified cells.—D. Magnification of cells in C.—E. 5-veined cypsela, veins uniting within the base, carpopodium well developed. (A, *Helichrysum arenarium, Rechinger 666*; B, *Dasyphyllum candolleanum, Irwin, Souza & dos Santos 7960*; C, D, *Pertya scandens, Furuse s.n.*; E, *Vernonia novaboracensis, Wilkens 4255*.)

chorioideae, in agreement with Jansen et al.'s (1990) results, requires 12 extra steps. Making the tribe Mutisieae monophyletic, excluding *Pertya* and *Warionia*, requires nine extra steps. Moving *Warionia* or *Pertya* to the main Mutisieae branch, below *Brachylaena* in Figure 1, requires ten and five extra steps, respectively. Moving *Arctotis* to the Cardueae sensu lato, in agreement with Bremer's (1987) results, requires five extra steps, and moving *Berkheya* to *Arctotis*, making the Arctotideae monophyletic, requires four extra steps.

Most of Jansen et al.'s Wagner trees are similar in outline to our cladogram, with the Cichorioideae paraphyletic and Mutisieae and Cardueae sensu

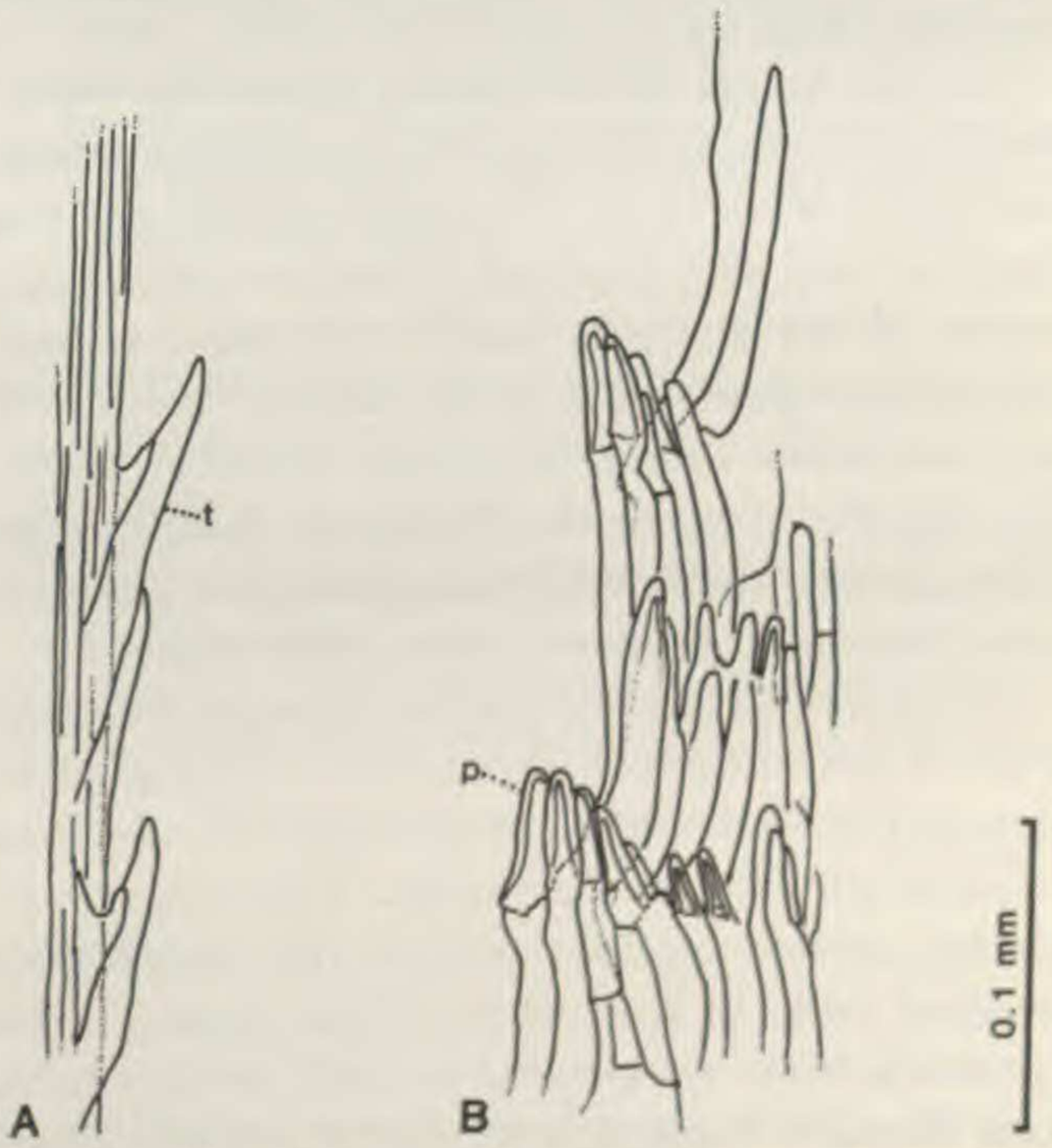


FIGURE 11.—A. Pappus bristle teeth consisting of the top of one cell together with the base of the adjacent marginal cell, t = tooth.—B. Epicarp with finlike arranged papillae, p = papillae. (A, *Actinoseris radiata, Hatschbach 27447*; B, *Hypochoeris glabra, Klackenberg & Lundin 253*.)

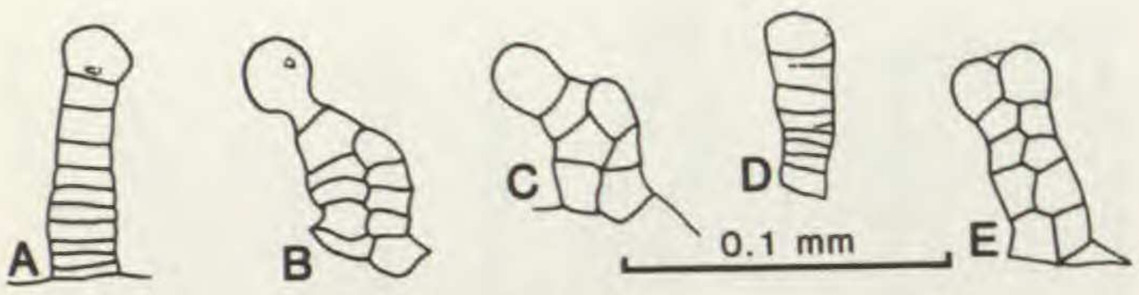


FIGURE 12. Glands of the Nassauviinae.—A. *Trixis divaricata* (*Mexia 8110*).—B. *Jungia axillaris* (*Chavez 3260*).—C. *Leucheria leontopodioides* (*Santesson 469*).—D. *Acourtia vanillosma* (*Ekman 43034*).—E. *Trixis brasiliensis* (*Malme 1124*).

lato at the base. Eight of their Wagner trees and the Dollo trees, with the Cichorioideae monophyletic, are incongruent with our results. The cause for the difference between Jansen et al.'s Dollo trees and the Wagner trees based on morphology and cpDNA data (i.e., 12 out of 20) remains obscure. We find no morphological or chemical features to corroborate the monophyly of Cichorioideae.

## CONCLUSIONS

Our analyses support the placement of Mutisieae as a basal assemblage of the family, but the tribe is paraphyletic even without the Barnadesiinae. A monophyletic Mutisieae is an unparsimonious solution using morphological data. A large part of the Mutisieae, including the Nassauviinae, most of the Mutisiinae, and part of the Gochnatiinae, form a monophyletic group, but its exact circumscription requires study.

The placement of the genera cannot be established with certainty because the most parsimonious cladogram differs from other alternatives only few steps longer. However, all Mutisieae genera included in the analyses consistently form a basal assemblage; hence there is no basis for transfer of any Mutisieae except *Warionia* to other tribes. Moving *Warionia* to the Mutisieae branch in the cladogram is highly unparsimonious, but it cannot immediately be assigned to any other tribe.

The subtribe Nassauviinae is monophyletic, and part of the Mutisiinae also forms a monophyletic group. The Gochnatiinae are paraphyletic and form a basal complex in the family. This complex includes several isolated genera that presumably evolved early in the history of the family. These genera include *Stenopadus* and discoid derivatives (the bilabiate *Gongylolepis* and its satellite genera may or may not be immediately related) from the Guyana Highlands, *Hesperomannia* from Hawaii, *Wunderlichia* from Brazil, *Ainsliaea* from East Asia, the presumably artificial genus *Gochnatia* (cf. Appendix I) from Central and South America and Southeast Asia, and *Brachylaena* (with *Tarchonanthus*) from Africa. The scattered distributions of these elements also suggest that they represent ancient relicts from the early evolution of the family. It is not surprising that these genera form a basal grade, since they share numerous symplesiomorphies with the Barnadesiinae.

The Cardueae sensu lato, including *Berardia*, *Carlina*, *Echinops*, and their relatives (Carlineae and Echinopeae of Dittrich, 1977) form a monophyletic group. Many similarities between the Cardueae and the Mutisieae are symplesiomorphies, including pluriseriate involucral bracts, actinomorphic and deeply 5-lobed corollas, and anthers with long, sclerified, sometimes acuminate, apical appendages. The Arctotideae are not closely related to the Cardueae sensu lato as assumed by earlier authors. They form a monophyletic group consisting of four tribes, Arctotideae, Lactuceae, Liabeae, and Vernonieae, as well as the subfamily Asteroideae. Relationships within this group cannot be resolved with certainty. Hence the sister group of the subfamily Asteroideae cannot be identified, although our data strongly indicate that it is to be found among the tribes Arctotideae, Lactuceae, Liabeae, and Vernonieae. Placing a monophyletic Cichorioideae as the sister group of Asteroideae is highly unparsimonious for morphological data.

The Liabeae and the Vernonieae do not form a clade in this study, but this may result from sampling problems and the choice not to code "liaboid pollen" for these taxa (as was done by Bremer, 1987, referring to Skvarla et al., 1977).

Significantly, our results show the basal position and the paraphyly of Mutisieae, the position of the Cardueae, and the presence of the monophyletic group mentioned above, consisting of the tribes Arctotideae, Lactuceae, Liabeae, and Vernonieae, as well as the subfamily Asteroideae. Our analyses did not focus on the sister group relationship of the Asteroideae but did indicate that it is not to be found within the Mutisieae or the Cardueae. We consider a new classification based on this analysis premature because tribal relationships above the basal Mutisieae–Cardueae remain uncertain.

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Appendix I. Genera used as terminal taxa in the analysis and species used as the basis for coding. Generic types are marked with an asterisk (*).

Mutisieae–Barnadesiinae.

*Barnadesia* Mutis in L. f.: *B. dombeyana* Less., *B. horrida* Muschler, *B. parviflora* Spruce ex Benth. & Hook. f., *B. rosea* Lindl.

*Chuquiraga* Juss.: *C. aurea* Skottsberg, **C. jussieui* J. F. Gmel., *C. kingii* Ball, *C. macrocephala* Baker, *C. rotundifolia* Wedd.

*Dasyphyllum* HBK: *D. candolleanum* (Gardn.) Cabr., *D. ferox* (Wedd.) Cabr., *D. leptacanthum* (Gardn.) Cabr., *D. reticulatum* (DC.) Cabr., *D. velutinum* (Baker) Cabr.

Mutisieae–Gochnatiinae.

*Actinoseris* (Endl.) Cabr.: *A. angustifolia* (Gardn.) Cabr., **A. radiata* (Vell.) Cabr.

*Ainsliaea* DC.: *A. acerifolia* Sch.-Bip., *A. pteropoda* DC.

*Cnicothamnus* Griseb.: **C. lorentzii* Griseb.

*Dicoma* Cass.: *D. anomala* Sond., *D. antunesii* O. Hoffm., *D. argyrophylla* Oliv., *D. burmannii* Less., *D. capensis* Less., *D. galpinii* F. C. Wilson, *D. picta* (Thunb.) Druce, *D.* (*Hochstetteria*) *schimperi* (DC.) Baill., **D. tomentosa* Cass.

*Erythrocephalum* Benth. in Benth. & Hook. f.: **E. zambesiacum* Oliv. & Hiern.

*Gochnatia* HBK: *G. argentina* Cabr., *G. argyrea* (Dusén ex Malme) Cabr., *G. avicenniifolia* (DC.) Cabr., *G. barrosoii* Cabr., *G. cordata* Less., *G. cowellii* (Britt.) Jervis & Alain, *G. discoidea* (Less.) Cabr., *G. microcephala* (Griseb.) Jervis & Alain, *G. paniculata* (Less.) Cabr., *G. picardae* (Urb.) Jiménez.

*Gongylolepis* R. H. Schomb.: **G. benthamiana* R. H. Schomb., *G. paniculata* Maguire & Phelps.

*Hesperomannia* A. Gray.: **H. arborescens* A. Gray.

*Oldenburgia* Less.: *O. grandis* (Thunb.) Baill., **O. paradoxa* Less., *O. papionum* DC.

*Onoseris* Willd.: *O. gnaphalioides* Muschler, *O. odorata* H. & A., *O. onoseroides* B. L. Robinson, *O. speciosa* HBK.

*Pertya* Sch.-Bip.: *P. glabrescens* Sch.-Bip., *P. phylicoides* J. F. Jeffrey, **P. scandens* Sch.-Bip.

*Plazia* Ruiz & Pavon: *P. daphnoides* Wedd.

*Pleiotaxis* Steetz: **P. pulcherrima* Steetz, *P. racemosa* O. Hoffm., *P. rugosa* O. Hoffm.

*Stenopadus* Blake: *S. campestris* Maguire & Wurdack, *S. chimantensis* Maguire, Steyermark & Wurdack.

*Stifftia* Mikan: **S. chrysantha* Mikan.

*Wunderlichia* Riedel: *W. crulziana* Taub., *W. tomentosa* Glaziou.

Mutisieae–Mutisiinae.

*Chaetanthera* Ruiz & Pavon: *C. acerosa* (Remy.) Benth. & Hook., *C. elegans* Phil., *C. euphrasioides* Meigen, *C. glabrata* (DC.) Meigen, *C. pentacaenoides* Hauman, **C. villosa* D. Don.

*Gerbera* L.: *G. ambigua* (Cass.) Sch.-Bip., *G. jamesonii* Bolus ex Adlam, **G. linnaei* Cass.

*Hyaloseris* Griseb.: *H. camataquensis* Hieron. ex Koster, *H. cinerea* (Griseb.) Griseb.

*Lulia* Zardini: **L. nervosa* (Less.) Zardini.

*Mutisia* L. f.: *M. acerosa* Poepp. ex Less., *M. acuminata* Ruiz & Pavon, *M. clematis* L. f., *M. ledifolia* Decne ex Wedd.

*Trichocline* Cass.: *T. auriculata* (Wedd.) Hieron.

Mutisieae–Nassauviinae.

*Jungia* L. f.: *J. axillaris* (DC.) Sprgl, *J. floribunda* Less., *J. paniculata* (DC.) Gray.

*Leucheria* Lag.: *L. cerberoana* Remy, *L. floribunda* DC., *L. leontopodioides* (O. Kuntze) K. Schumann.

*Lophopappus* Rusby: *L. blakei* Cabr., **L. foliosus* Rusby.

*Macrachaenium* Hook. f.: **M. gracile* Hook. f.

*Nassauvia* Comm. ex Juss.: *N. abbreviata* Dusén, *N. acerosa* (Meyen) Wedd., *N. darwinii* O. Hoffm. & Dusén, *N. lagascae* Meigen, **N. magellanica* Gmel.

*Trixis* P. Browne: *T. auriculata* Hook., *T. brasiliensis* DC., *T. cacalioides* (HBK) D. Don, *T. californica* Kellogg.

Arctotideae.

*Arctotis* L.: **A. angustifolia* L., *A. hirsuta* (Harv.) Beauv., *A. venusta* T. Norl.

*Berkheya* Ehrh.: *Berkheya* represents the Arctotideae–Gorteriinae (Roessler, 1959). *B. armata* (Vahl) Druce, *B. bipinnatifida* (Harv.) Roessler ssp. *bipinnatifida*, *B. canescens* DC., *B. heterophylla* (Thunb.) O. Hoffm.

Cardueae.

*Carlina* L.: *C. acaulis* L., *C. corymbosa* L., *C. salicifolia* Cav., **C. vulgaris* L.

*Carduus* L.: *C. argyroa* Biv., *C. carpetanus* Boiss. & Reuter, *C. crispus* L., **C. nutans* L.

*Cirsium* L.: *C. pitcheri* Torr. & Gray, *C. vulgare* L.

*Echinops* L.: *E. angustilobus* S. Moore, *E. longifolius* A. Rich., **E. sphaerocephalus* L., *E. strigosus* L.

*Saussurea* DC.: **S. alpina* L., *S. amabilis* Kitamura, *S. chondrilloides* C. Winkl.

Vernonieae.

*Distephanus* Cass.: *D. divaricatus* (Steetz) H. Robinson & H. Kahn.

*Vernonia* Schreb. sens. str.: *V. glabra* (Steetz) Vatke, **V. nova-boracensis* (L.) Willd.

Liabeae.

*Liabum* Adans.: *L. igniarium* (HBK) Less., *L. solidagineum* (HBK) Less.

*Munnozia* Ruiz & Pavon: *M. hastifolia* (Poepp. & Endl.) H. Robinson & R. D. Brettell, *M. lanceolata* Ruiz & Pavon, *M. senecionidis* Benth.

Lactuceae.

*Hypochoeris* L.: *H. glabra* L., *H. maculata* L.

*Lactuca* L.: *L. canadensis* L., *L. muralis* L., **L. sativa* L.

*Scolymus* L.: *S. grandiflorus* Desf., *S. hispanicus* L., **S. maculatus* L.

*Sonchus* L.: *S. arvensis* L., *S. congestus* Willd.

Inuleae.

*Inula* L.: **I. helenium* L.

Gnaphalieae.

*Helichrysum* Mill.: *H. arenarium* (L.) Moench, *H. areolum* Hilliard, *H. auriceps* Hilliard, **H. orientale* (L.) Gaertn.

Plucheeae.

*Pluchea* Cass.: *P. camphorata* DC., *P. lanceolata* Oliv. & Hiern, *P. odorata* Cass.

Astereae.

*Aster* L.: *A. simplex* Willd., *A. tripolium* L.
*Geissolepis* B. L. Robinson: **G. suaedaefolia* B. L. Robinson.

Senecioneae.

*Senecio* L.: **S. vulgaris* L.

Taxa of uncertain position.

*Berardia* Vill.: **B. subacaulis* Vill.
*Brachylaena* R. Br.: **B. neriifolia* (L.) Less.
*Eremothamnus* O. Hoffm.: **E. marlothianus* O. Hoffm.
*Warionia* Benth. & Coss.: **W. saharae* Benth. & Coss.

APPENDIX II. Characters. The plesiomorphic states are coded as 0, apomorphic states as 1, 2, etc.

1. Habit: woody (0), perennial/annual herbs (1).
2. Leaf position: alternate (0), opposite (1), rosulate (2).
3. Leaves: not spiny (0), spiny (1). The spinulose leaf margins in many Lactuceae are not coded as spines.
4. Leaf trichomes: without woolly hairs (0), with woolly hairs ("type B" of Drury & Watson, 1966) (1).
5. Leaf margin: entire (0), serrate to dentate or pinnatisect (1).
6. Leaf texture: herbaceous (0), coriaceous, glossy (1).
7. Leaf shape: ovate to lanceolate (0), broadly obovate, obtuse-cuneate (1).
8. Leaf veins: even with leaf surface (0), submerged (1).
9. Involucral bracts: pluriseriate (0), 1–3 seriate (1).
10. Involucral bract margins: not scarious (0), scarious (1).
11. Involucral bract morphology: homogeneous (0), divided into a basal stereome and an apical lamina (1).
12. Involucral bract apex: acute (0), rounded, obtuse and ± broad (1).
13. Involucral bract texture/venation: coriaceous to herbaceous, thick, venation inconspicuous (0), thin, with several parallel veins (1).
14. Receptacle: epaleate (0), paleate (1).
15. Floret dimorphism: absent (0), present (1). We interpret the occurrence of two kinds of florets in the same head as a synapomorphy, regardless whether the florets are rays, actinomorphic, bilabiate, etc. Since the Barnadesiinae vary in this instance, the condition in adjacent outgroups was taken into consideration (Watrous & Wheeler, 1981; Maddison et al., 1984). It is important to remember that the Asteraceae heads are inflorescences, and as such are comparable to other inflorescences. The different kinds of florets are distinguished in the multistate character 16.
16. Marginal/central floret organization: only actinomorphic (0), bilabiate/actinomorphic (Fig. 2C/A) (1), bilabiate/bilabiate (Fig. 2C/C) (2), true rays/actinomorphic (Fig. 2G/A) (3), ligules (Fig. 2F) (4), Gorteriinae-rays/actinomorphic (Fig. 2B/A) (5), tubular/actinomorphic (Fig. 2D/A) (6). This multistate character is formulated in such a way as to separate nondiscoid heads into distinct groups. Actinomorphic deeply 5-lobed corollas (Fig. 3A, C) are interpreted as plesiomorphic, following Bremer's (1987) argument. Gorteriinae-rays (Fig. 2B) are an autapomorphy for the Arctotideae–Gorteriinae (Bremer, 1988).
17. Floret morphology: bilabiate (Fig. 2C) or true rays (Fig. 2G) absent (0), bilabiate or true rays present (1). Rays are often thought to have evolved from bilabiate corollas, where the two ventral lobes are reduced (Jeffrey, 1977; Bremer, 1987, 1988), but ligules presumably have evolved directly from actinomorphic corollas.
18. Marginal floret sex: hermaphroditic (0), female (1), neuter (2).
19. Disc corolla lobes: long, narrowly triangular (Fig. 3A, C) (0), short, ± deltoid (Fig. 3B) (1).
20. Disc corolla tube: short, thick (0), long, slender (Fig. 3C, Dittrich, 1977) (1).
21. Disc corolla vein bifurcation: well below the lobes (0), adjacent to the lobes (1).
22. Disc corolla lobe venation: without a midvein (0), with a midvein (1).
23. Disc corolla lobe venation: without thick-bundled apical veins (0), with thick-bundled apical veins (1).
24. Long corolla glandular hairs (Fig. 4H, cf. 4I): absent (0), present (1).
25. Corolla hairs: absent (0), simple multicellular with oblique walls (Fig. 5A) (1), simple multicellular with straight walls (Fig. 5D, E) (2), simple multicellular, ultimate cell with much thicker walls (Fig. 5B) (3).
26. Zygomorphic marginal floret (rays, bilabiate, ligules) epidermal cell outline (Baagøe, 1977a, b, 1978): narrowly oblong (0), tabular (1), rounded (2).
27. Zygomorphic marginal floret (rays, bilabiate, ligules) epidermal cell cuticle ornamentation (Baagøe, 1977a, b, 1978): none (0), transversely striate (1), longitudinally striate (2), intestinelike (H. V. Hansen, Copenhagen, unpublished) (3).
28. Zygomorphic marginal floret (rays, bilabiate, ligules) epidermal cell surface (Baagøe, 1977a, b, 1978): flat (0), crested (1), papillose (2).
29. Apical anther appendage length: at least three times as long as wide (Fig. 7A, B) (0), at least twice as long as wide (Fig. 7C) (1), up to twice as long as wide (Fig. 7D) (2).
30. Apical anther appendage outline: rounded to acute (0), acuminate to apiculate (1).
31. Filament collar cell wall thickenings (King & Robinson, 1987; Fig. 7F): absent (0), present (1).
32. Apical anther appendage texture: sclerified (0), nonsclerified (1), soft (2).
33. Endothecial cell wall thickening organization: on the lateral walls (radial, Fig. 8D; Dormer, 1962; Nordenstam, 1978, Vincent & Getliffe, 1988; Thiele, 1988) or with transverse bands (Fig. 8B) (0); with thickenings on the cell ends (polarized, with or without vertical bands, Robinson, 1977; Fig. 8A, C, at the edge of the pollen sacs) (1), without thickenings (2), outer pollen sacs with large rectangular cells with semicircular to elliptic inner walls (Fig. 8A) (3).
34. Endothecial cell wall thickening organization: polarized (0), with vertical bands (1).
35. Endothecial cell wall thickening organization: radial (0), with transverse bands (1).
36. Anther thecae base morphology (Robinson, 1983; Bremer, 1987): calcarate (0), ecalcarate (Fig. 6B) (1).
37. Anther thecae base morphology (Robinson, 1983; Bremer, 1987): ecaudate (0), caudate (1).
38. Filament collar: inconspicuous or absent (0), conspicuous (Figs. 6B, 7E) (1), swollen (2).
39. Filament surface: smooth (0), papillose or hairy (1).

40. Filament vein arrangement: running throughout, uninterrupted (0), in distinct groups without connection in between (1).
41. Pollen exine (Leins, 1971; Skvarla et al., 1977; Bolick, 1978): ecaveate (0), caveate (1).
42. Pollen surface: smooth (0), granular (1), spiny (2).
43. Pollen surface sculpturing: smooth to echinate (0), lophate to echinolophate (1).
44. Pollen exine columellae anatomy (Skvarla et al., 1977): unperforated (0), with internal foramina (1).
45. Style branch length: short (Fig. 9H, I) (0), long (Fig. 9F, G) (1). Some genera in the Cardueae have clearly secondarily fused style branches and have been coded as having long branches.
46. Style apex outline: rounded (0), tapering gradually toward the top (Fig. 9F) (1), truncate (Fig. 9E) (2).
47. Stigmatic surface: entire (0), in two apically confluent lines (1), in two separate lines (2).
48. Stylar sweeping hair shape: styles papillose or without sweeping hairs (0), broad, obtuse (Fig. 9C, E) (1), broad, acute (Fig. 9D) (2), narrow, acute (Fig. 9B) (3), narrow, obtuse (Fig. 9A) (4).
49. Stylar sweeping hair position: without sweeping hairs (0), reaching below the bifurcation (1), ± covering the abaxial surface of style branches, not reaching below the bifurcation (2), in a subapical tuft of style branches (3), in an apical tuft (4), in a ring below the bifurcation (5).
50. Style branch veins: very thick (Fig. 9G, H) (0), narrow (9F) (1).
51. Style base: not swollen (0), swollen (1).
52. Style apex: as wide as the rest of the style (0), conspicuously thickened (1).
53. Style apical appendage: absent (0), present (1).
54. Cypsela shape: terete to prismatic (0), compressed (1).
55. Cypsela carpopodium (Fig. 10A, D, E): absent (0), present (1).
56. Cypsela pericarp: not rugose (0), rugose (1).
57. Cypsela epicarp papillae arrangement: papillae absent or individually arranged (0), finlike (Fig. 11B) (1).
58. Cypsela twin hairs: absent (0), ± long (1), deeply cleft (Fig. 4A) (2), short, narrowly ovoid (myxogenic) (Fig. 4C–G) (3).
59. Cypsela glands: biseriate with a collapsing head (0), compact, persistent, uni- or biseriate, generally with a spherical ultimate cell (Fig. 12) (1).
60. Cypsela vein union: below the base (Fig. 10B) (0), at the base (Fig. 10A, E) (1).
61. Cypsela vein number: 5–10 (Fig. 10B, E) (0), 2–3 (Fig. 10A) (1).
62. Cypsela epicarp crystals (Anderberg, 1982, 1989): absent (0), present (1).
63. Testa (Grau, 1980; Dittrich 1977): persistent, often reinforced (0), collapsed (1).
64. Testal epidermis strengthening pattern (Grau, 1980): none or diffuse (0), stalagmitelike (1).
65. Pappus: terete bristles with unicellular hairs (0), true bristles, or bristlelike scales (1). The Barnadesiinae pappus consists of terete bristles with unicellular hairs with a basal cell, exactly like those on florets and fruits as well. They therefore have a superficial resemblance to plumose bristles that are found in various tribes in the family. The Barnadesiinae bristles also seem to be of quite another texture than the bristles in the rest of the family. It is possible that they are of another origin than the pappus bristles outside the Barnadesiinae, and the conclusion is that true bristles is an evolutionary novelty for the ingroup. Most of the pappus characters are therefore scored as inapplicable in the outgroup.
66. Pappus bristle margins: scabrid to barbellate (0), plumose (1).
67. Pappus base fusion: present (0), absent (1).
68. Pappus setae cell thickenings (Karis, 1989): absent (0), present (1).
69. Pappus bristle teeth organization (Karis, 1989, 1990): of one cell (0), of the top of one cell together with the base of the adjacent marginal cell (Fig. 11A) (1).
70. Pappus element dimorphism: absent (0), present (1).
71. Laticiferous tissue (Col, 1899–1901): absent (0), present (1), with latex-resin (2).
72. Chemistry: benzofurans and benzopyrans absent (0), present (Proksch, 1985; Proksch & Rodrigues, 1983) (1).

Appendix III. Datamatrix. Inapplicable or unknown character states are coded -

| | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
|---|---|---|---|---|---|---|---|---|
| **OUTGROUP** | 00000 | 00000 00000 | 00000 00000 | 00000 ——00 | 000–0 | 00000 00000 | 00000 00000 | 00000 ——0 00 |
| MUTISIEAE-GOCHNATIINAE | | | | | | | | |
| **Actinoseris** | 12001 | 00000 00001 | 11000 00001 | 01111 | 0010– | 01000 –00–1 | 00001 10000 | 00101 00–01 01110 00 |
| **Ainsliaea** | 100–1 | 00000 00000 | 00100 00000 | 01111 | 0010– | 01100 –00–0 | 00000 00001 | 00101 00001 10100 00 |
| **Brachylaena** | 00011 | 00000 00001 | 00–00 00000 | 13011 | 0010– | 01000 00000 | 00001 10001 | 00101 00001 00000 00 |
| **Cnicothamnus** | 00001 | 00000 00001 | 11000 00000 | 01101 | 0010– | 11000 –10–0 | 00001 00000 | 00101 00001 00100 00 |
| **Dicoma** | 10011 | 00000 –0001 | 11100 10100 | 03001 | 0010– | 01100 –20–0 | 00320 10000 | 00101 00011 00110 00 |
| **Erythrocephalum** | 10011 | 00100 00011 | 11001 10100 | 03011 | 0110– | 01100 02001 | 10321 00000 | 00201 00011 11100 00 |
| **Gochnatia** | 00011 | 00000 00000 | 00000 10000 | 01111 | 0010– | 01000 01000 | 00000 00001 | 00101 00001 001–0 00 |
| **Hesperomannia** | 00001 | 00000 00000 | 00000 00000 | 00100 | 012— | 01100 01001 | 10110 00000 | 00100 00001 00100 00 |
| **Oldenburgia** | 00010 | 01000 00001 | 11000 10100 | 01101 | 0010– | 01100 –00–0 | 00321 00001 | 00101 00–01 00100 00 |
| **Onoseris** | –0011 | 00000 00001 | 11110 10010 | 01100 | 0010– | 01111 01001 | 00321 10001 | 00101 01001 00000 00 |
| **Pertya** | 00011 | 00000 00000 | 00000 10000 | 01111 | 0010– | 01100 –10–1 | 10410 10001 | 00101 00001 00000 00 |
| **Plazia** | 000–0 | 00000 00001 | 11000 11100 | 01101 | 0010– | 01100 01001 | 00330 10000 | 00101 00001 00100 00 |
| **Pleiotaxis** | 10011 | 00101 00000 | 00001 00000 | 03001 | 0010– | 11100 –20–0 | 00330 00001 | 00101 00011 00000 00 |
| **Stenopadus** | 00000 | 11000 010–0 | 00000 –1000 | 00100 | 0010– | 01000 –00–0 | 00000 10001 | 00000 00001 00000 00 |
| **Stifftia** | 00000 | 00010 01000 | 00000 10000 | 01101 | 0010– | 01–00 –00–1 | 10000 10001 | 00001 00001 00010 00 |
| **Warionia** | 00001 | 00000 00000 | 00000 10010 | 03010 | 0010– | 01101 –20–1 | 10310 10000 | 00101 01–01 00000 10 |
| **Wunderlichia** | 00010 | 00000 00000 | 00000 01001 | 00101 | 0010– | 01100 –10–0 | 00000 00000 | 00101 00001 00100 00 |
| MUTISIEAE-MUTISIINAE | | | | | | | | |
| **Chaetanthera** | 10010 | 00001 –0001 | 211–0 ——00 | 01100 | 0010– | 01100 00000 | 00330 00000 | 00301 00001 00000 00 |
| **Gerbera** | 12011 | 00000 00001 | 211–0 ——00 | 01110 | 0010– | 01100 00000 | 10321 00000 | 00300 00001 01000 00 |
| **Gongylolepis** | 00000 | 11000 01000 | 210–0 ——00 | 0—01 | 0010– | 01000 –10–1 | 00001 10001 | 00001 00001 00110 00 |
| **Hyaloseris** | 00010 | 00001 00000 | 400–0 ——00 | 01100 | 0010– | 01000 –10–1 | 10001 00001 | 00201 00001 00100 00 |
| **Lulia** | 10010 | 00000 00001 | 211–0 ——10 | 01101 | 0010– | 00010 –00–0 | 10421 00000 | 00001 10–01 00100 00 |
| **Mutisia** | 00010 | 00000 00001 | 210–0 ——01 | 01101 | 0010– | 01101 00001 | 10420 00000 | 00000 00001 10100 00 |
| **Trichocline** | 02010 | 00000 00001 | 211–0 ——00 | 01100 | 0010– | 01111 00000 | 00420 00000 | 00300 00001 00000 00 |
| MUTISIEAE-NASSAUVIINAE | | | | | | | | |
| **Jungia** | –0011 | 00010 00010 | 210–0 ——00 | 01100 | 0110– | 01000 00001 | 20141 10001 | 00311 00001 10100 00 |
| **Leucheria** | 10011 | 00010 00001 | 210–0 ——02 | 01100 | 0010– | 01200 00001 | 20141 10001 | 00311 00001 10100 00 |
| **Lophopappus** | 00110 | 00010 00000 | 21000 –0000 | 01100 | 0010– | 01000 00000 | 00120 10001 | 00111 00001 –0100 00 |
| **Macrachaenium** | 12011 | 00010 00000 | 210–0 ——00 | 0—10 | 0110– | 11000 00001 | 10121 10001 | 0000– 00–01 10100 00 |
| **Nassauvia** | 10101 | 00010 00000 | 210–0 ——00 | 01110 | 0010– | 01200 00001 | 20141 10000 | 00001 –0001 01000 00 |
| **Trixis** | 10011 | 00010 00001 | 210–0 ——00 | 01100 | 0–10– | 01000 00001 | 20141 10001 | 00311 00001 00100 00 |
| ARCTOTIDEAE | | | | | | | | |
| **Arctotis** | 10011 | 00001 00001 | 31110 00000 | 02120 | 020–0 | 00000 11001 | 10351 11000 | 00100 00–01 –1000 00 |
| **Berkheya** | 10111 | 00000 00001 | 50200 10010 | 01120 | 1111– | 01100 12001 | 10211 10001 | 00101 00–01 01100 00 |
| **Eremothamnus** | 00111 | 00000 00001 | 31100 11000 | 10010 | 120–1 | 01100 120–1 | 10311 00000 | 00100 00–01 00000 00 |
| CARDUEAE sensu lato | | | | | | | | |
| **Berardia** | 12011 | 00000 000–0 | 00011 00000 | 12011 | 0010– | 01100 –10–0 | 00311 1000– | 00000 00001 01000 10 |
| **Carduus** | 10101 | 00000 00000 | 00001 10000 | 00011 | 003— | 01110 –20–1 | 10350 11000 | 10000 01001 00000 20 |
| **Carlina** | 10101 | 00000 10010 | 00010 11100 | ——11 | 0010– | 01100 –10–0 | 00350 10001 | 00100 00001 10000 20 |
| **Cirsium** | 10111 | 00000 00000 | 00001 10000 | ——01 | 003— | 11110 01001 | 10351 10000 | 00000 00001 11100 00 |
| **Echinops** | 10111 | 00000 0000– | 00–01 10010 | ——11 | 0010– | 01000 –10–1 | 10351 11000 | 00000 00001 00000 00 |
| **Saussurea** | 10011 | 00000 00000 | 00001 10000 | ——11 | 0010– | 01100 –10–1 | 10351 10000 | 10100 10001 –0001 00 |
| VERNONIEAE | | | | | | | | |
| **Distephanus** | 00000 | 00000 00000 | 00000 10000 | ——20 | 0111– | 01000 –20–1 | 10111 10001 | 00101 00–01 00000 00 |
| **Vernonia** | 10001 | 00000 00000 | 00000 11000 | ——10 | 0111– | 00100 02001 | 10311 10001 | 00101 00–01 10101 00 |
| LACTUCEAE | | | | | | | | |
| **Hypochoeris** | 12001 | 00001 00110 | 400–0 ——03 | ——20 | 120–1 | 01100 –21–1 | 10211 00001 | 01001 00–01 11001 10 |
| **Lactuca** | 10001 | 00011 00100 | 400–0 ——01 | ——20 | 120–1 | 01100 –21–1 | 10211 00011 | 01001 –0101 010–0 10 |
| **Scolymus** | 10101 | 00001 00110 | 400–0 ——13 | ——20 | 0210– | 00100 –21–1 | 10211 00010 | 00101 00001 —100 10 |
| **Sonchus** | 10001 | 00011 00100 | 400–0 ——13 | ——20 | 120–1 | 01100 –21–1 | 10211 00010 | 00001 00001 01011 10 |
| LIABEAE | | | | | | | | |
| **Liabum** | 11011 | 00010 00001 | 31100 11010 | 10020 | 0110– | 00100 02001 | 10211 00001 | 00101 00–01 01101 00 |
| **Munnozia** | 11011 | 00000 00001 | 31100 10010 | 12020 | 0110– | 00100 –20–1 | 10211 00000 | 0000– 00–01 01100 10 |
| ASTEROIDEAE | | | | | | | | |
| **Helichrysum** | 10010 | 00000 10001 | 60110 10000 | ——20 | 0110– | 11100 12011 | 22140 10001 | 00000 10101 01100 01 |
| **Inula** | 10001 | 00000 00001 | 311-0 00002 | 11020 | 000–0 | 11100 12011 | 11330 10001 | 00000 01101 00100 01 |
| **Pluchea** | 10000 | 00000 00001 | 60110 10002 | ——20 | 000–0 | 01100 120–1 | 10111 10001 | 00101 00001 00100 01 |
| **Aster** | 10001 | 00001 00001 | 31110 10000 | 11220 | 010–0 | 00100 12011 | 12121 10101 | 00101 10001 01000 01 |
| **Geissolepis** | 10001 | 00001 00001 | 31110 10000 | 11220 | 010–0 | 10100 12011 | 12120 1010– | 00101 00001 01000 01 |
| **Senecio** | 10000 | 00010 00001 | 31110 10000 | 11020 | 010–0 | 10200 12011 | 22140 10001 | 00101 00–01 01000 01 |

# PHYLOGENETIC IMPLICATIONS OF *rbc*L SEQUENCE VARIATION IN THE ASTERACEAE[1]

*Ki-Joong Kim,*[2,3] *Robert K. Jansen,*[2,3] *Robert S. Wallace,*[2,4] *Helen J. Michaels,*[5] *and Jeffrey D. Palmer*[6]

## Abstract

Complete nucleotide sequences of the *rbc*L gene were obtained for 25 species of Asteraceae representing 15 of the currently recognized tribes and three outgroup families. A total of 345 variable nucleotide positions was identified, 170 of which were phylogenetically informative. Phylogenetic analyses of the *rbc*L data generated eight equally parsimonious trees with a consistency index of 0.47. Three major monophyletic clades that correspond to the subfamilies Barnadesioideae, Cichorioideae, and Asteroideae were identified in the most parsimonious cladograms; however, support for these groups was not strong. Relationships among tribes were not supported strongly except for the close affinity of the Tageteae, Coreopsideae, Heliantheae, and Eupatorieae. These results are congruent with chloroplast DNA restriction site comparisons with respect to the subfamilial circumscription in the Asteraceae and the sister group relationship of the Heliantheae and Eupatorieae. In contrast, morphological cladograms indicated the paraphyly of the Cichorioideae and a closer relationship of the Eupatorieae to the Astereae. Parsimony analyses were also performed on data sets combining *rbc*L and cpDNA restriction site mutations and DNA and morphological characters. These phylogenies provide moderate to strong support for the monophyly of the Asteroideae and Cichorioideae and the sister-group relationship of the Eupatorieae and Heliantheae. Comparisons of trees generated from restriction site and sequence data also indicate that cpDNA restriction site data from the entire chloroplast genome are more useful for phylogenetic studies in the Asteraceae than sequences from the highly conserved *rbc*L gene. This is due to the sampling of more sequence variation in the restriction site comparisons and the higher incidence of homoplasy in the sequence data.

Recent studies of phylogenetic relationships of the Asteraceae at higher taxonomic levels using both chloroplast DNA (cpDNA) (Jansen & Palmer, 1987a, 1988; Jansen et al., 1990, 1991a, b; Keeley & Jansen, 1991; Watson et al., 1991) and morphological (Bremer, 1987; Harris, 1991; Karis et al., 1992) data have clarified many controversial systematic issues involving this large angiosperm family. Both *rbc*L sequence data (H. Michaels et al., unpublished; Jansen et al., 1991b) and morphology (Harris, 1991) provided strong evidence that the Calyceraceae and Goodeniaceae are sister taxa of the Asteraceae. This finding appears to resolve a phylogenetic controversy that has puzzled synantherologists for over 150 years. Within the Asteraceae phylogenetic trees generated from cpDNA restriction site and morphological data are congruent in three areas: (1) the Barnadesioideae are the sister group to the rest of the family; (2) the Asteroideae are monophyletic; and (3) 14 of the 17 currently recognized tribes (Jansen et al., 1991b) are monophyletic. The primary incongruence between morphology and cpDNA concerns the placement of the tribe Eupatorieae and the monophyly of the Cichorioideae. Morphological trees (Bremer, 1987) placed the Eupatorieae closest to the Astereae and indicated that the Cichorioideae are paraphyletic. In contrast, cpDNA restriction site studies (Jansen et al., 1990, 199la; K.-J. Kim, B. Turner and R. Jansen, unpublished)

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[2] Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269, U.S.A.
[3] Present address: Department of Botany, University of Texas, Austin, Texas 78713, U.S.A.
[4] Present address: Department of Botany, Iowa State University, Ames, Iowa 50011, U.S.A.
[5] Department of Biology, Bowling Green University, Bowling Green, Ohio 43403, U.S.A.
[6] Department of Biology, Indiana University, Bloomington, Indiana 47405, U.S.A.

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indicated that the Eupatorieae are most closely allied to the Heliantheae and that there is moderate support for the monophyly of the Cichorioideae.

In this paper we present the results of phylogenetic analyses of sequences of the chloroplast gene encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) from 25 species of Asteraceae and three outgroup families. This gene is currently being used to examine evolutionary relationships in a wide diversity of plant groups. Several studies have demonstrated the potential of *rbc*L for resolving phylogenetic relationships at the interfamilial level (Olmstead et al., 1992; Donoghue et al., 1992), but its utility at the intrafamilial level has only been examined in the Poaceae and Saxifragaceae (Doebley et al., 1990; Soltis et al., 1990). In the study of grasses (Doebley et al., 1990), insufficient sequence variation was encountered to decide reliably among alternative tree topologies. The goals of our study were: (1) to examine *rbc*L sequences in the Asteraceae to provide additional characters for resolving incongruencies between phylogenies generated in previous morphological and cpDNA restriction site studies: (2) to perform phylogenetic comparisons of combined DNA and morphological characters to provide a comprehensive and broadly based assessment of evolutionary relationships in the Asteraceae; and (3) to evaluate the utility of cpDNA restriction site and *rbc*L sequence data for phylogenetic comparisons at the intrafamilial level.

## Materials and Methods

We obtained complete sequences of *rbc*L for 24 species of Asteraceae and two closely related outgroup families (Appendix 1). Data for two taxa (*Nicotiana tabacum* and *Flaveria trinervia*) come from previously published reports (Shinozaki et al., 1986; Hudson et al., 1990). Species names and voucher information for all taxa examined except *Cichorium intybus* L. are provided in Jansen & Palmer (1987a) and Jansen et al. (1990, 1991a). The new sequences have been submitted to Genbank and may also be obtained directly from R. Jansen.

A cloning strategy was employed for generating sequence data. Chloroplast DNAs were isolated from each species using the sucrose gradient method of Palmer (1986). Previous restriction site comparisons within the Asteraceae identified two conserved sites for BamHI and SacI (Jansen & Palmer, 1987b; R. Jansen, unpublished data). These enzymes produced a 2.0–2.6 kilobase (kb) fragment that contained the entire *rbc*L gene and associated noncoding regions at the 3′ and 5′ end of the gene (Fig. 1). Restriction fragments containing *rbc*L were excised from 1.2% Sea-Plaque low melting temperature agarose (FMC) and ligated directly to BamHI/SacI- digested bacteriophage M13mp18 DNA or the phagemid pBlueScript II (Stratagene). The entire cloned fragment was sequenced by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase (US Biochemicals) and a series of 14 overlapping synthetic primers and universal primers for M13mp18 DNA and pBlueScript II (Fig. 1). Nine of the synthetic primers (provided by G. Zurawski, DNAX) were based on the *rbc*L sequences of maize and spinach, and the others were constructed at the University of Connecticut Biotechnology Center using sequences from the Asteraceae. The M13mp18 clones were sequenced from single-stranded DNA, and pBlueScript II clones were sequenced from double-stranded template. Although sequences were obtained for a single strand only, the sequence data are accurate because we used cloned single or double stranded template and because the 14 sequencing primers (Fig. 1) have considerable overlap.

Sequences were aligned manually and only the coding region up to 1428 basepairs (bp) was used in phylogenetic analyses because of length mutations at the 3′ end of *rbc*L and in the noncoding regions (K.-J. Kim, R. Jansen & R. Wallace, unpublished). Phylogenies were constructed using parsimony, and the most parsimonious trees were searched for on a MacIntosh IIcx using PAUP version 3.0q (developed by D. Swofford) with the Tree Bisection Reconnection (TBR) and mulpars options. One hundred random entries of the data were performed for all phylogenetic analyses in an attempt to locate all equally parsimonious trees (Maddison, 1991).

The bootstrap method (Felsenstein, 1985) was employed to evaluate the reliability of phylogenetic estimates. One hundred replicates were performed using parsimony and the TBR option (without mulpars) of PAUP. The topological constraints option of PAUP was used to determine the number of additional steps required to break up the monophyly of selected groups.

All *rbc*L trees were rooted using multiple outgroups from the families Solanaceae, Campanulaceae, and Goodeniaceae. The close phylogenetic relationship of the latter family is supported by recent comparisons of *rbc*L sequences (Jansen et al., 1991b; H. Michael et al., unpublished) and features of inflorescence and floral development (Harris, 1991).

The *rbc*L sequence data were combined with