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PHYLOGENETIC IMPLICATIONS OF POLLEN MORPHOLOGY AND ULTRASTRUCTURE IN THE BARNADESIOIDEAE (ASTERACEAE)

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Abstract: The subfamily Barnadesioideae of the Asteraceae consists of nine genera and approximately 90 species. Both molecular and morphological phylogenies indicate that this subfamily is sister to the rest of the family. We have used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to study pollen of 41 species from all genera of the Barnadesioideae. Three general pollen types are described in the subfamily: *Barnadesia*-type (*Barnadesia, Huarpea*), *Chuquiraga*-type (*Chuquiraga, Doniophyton, Duseniella, Fulcaldea*) and *Dasyphyllum*-type (*Dasyphyllum* and *Schlechtendalia*). A fourth type, *Arnaldoa*-type, consisting solely of *Arnaldoa,* is intermediate between the *Chuquiraga*- and *Dasyphyllum*-types. These types parallel and confirm findings from previous studies. Psilolophate grains are found only in the *Barnadesia*-type. Pollen with a cavity (cavea) between pollen wall units in each of the three interapertural regions is present in *Barnadesia* (*Barnadesia*-type), *Dasyphyllum* (*Dasyphyllum*-type) and *Arnaldoa* (*Arnaldoa*-type). The *Chuquiraga*-type does not have cavate pollen. Intercolpar concavities occur only in the *Dasyphyllum*- and *Arnaldoa*-types. In the latter, intercolpar regions are accompanied by pairs of indentations flanking the colpi. The presence of intercolpar concavities in *Dasyphyllum* and *Schlechtendalia,* often cited as a synapomorphy for the Barnadesioideae and Calyceraceae, has apparently evolved independently within the subfamily. *Chuquiraga* pollen exhibits the least derived palynological features in the subfamily. Palynological characters, when placed in the context of current phylogenies for the Barnadesioideae, suggest additional phylogenetic analyses are needed to re-evaluate intergeneric relationships within the subfamily.

Keywords: pollen, Barnadesioideae, Asteraceae, Calyceraceae, cavea, exine, intercolpar concavities.

Recent phylogenetic studies of the Asteraceae have resolved many systematic issues at higher taxonomic levels in the family (Jansen and Palmer 1987; Jansen et al. 1991; Jansen and Kim 1996; Kim and Jansen 1995; Kim et al. 1992; Karis et al. 1992; Bremer and Jansen 1992; Bremer 1987, 1994). The subfamily Barnadesioideae is now widely regarded as sister to the remaining members of the Asteraceae. Although there have been many efforts to resolve the higher level phylogeny of the family, relationships between the Barnadesioideae and other proposed basal lineages of Asteraceae remain unresolved. In particular, relationships among genera within the paraphyletic tribe Mutisieae (Cichorioideae) and the subfamily Barnadesioideae are still problematic.

Past classifications of the tribe Mutisieae have included members of the subfamily Barnadesioideae. Bentham (1873) and later Cabrera (1965, 1977) recognized the subfamily as one of the four or five subtribes of the Mutisieae (Barnadesiinae, Gerberiinae, Gochnatiinae, Mutisiinae, and Nassauviinae). Cladistic analyses of morphological and molecular data prompted Bremer and Jansen (1992) to elevate the subtribe Barnadesiinae to subfamilial rank

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as the Barnadesioideae. The subfamily is defined by a number of morphological synapomorphies (e.g., axillary spines, "barnadesioid" trichomes present on corollas, achenes, and pappus) and absence of a large chloroplast DNA inversion which is present in all other members of the Asteraceae (Jansen and Palmer 1987). Bremer (1994) recognized one tribe within the Barnadesioideae (Barnadesieae) consisting of nine genera (*Arnaldoa, Barnadesia, Chuquiraga, Dasyphyllum, Doniophyton, Duseniella, Fulcaldea, Huarpea,* and *Schlechtendalia*) and approximately 90 species.

Extensive pollen studies of the Mutisieae (including the subtribe Barnadesiinae) using light microscopy have been carried out on selected genera by Wodehouse (1928, 1929a, b), Carlquist (1957), Stix (1960), and Parra and Marticorena (1972). Wodehouse (1928, 1929a, b) observed the lophate grains of *Barnadesia* and stated that "this genus bears little or no relationship to the Mutisieae but that its affinities are closer to the Vernonieae". Wodehouse (1929b) also concluded that Mutisieae were polyphyletic. Carlquist (1957) surveyed pollen morphology of enigmatic taxa of the Mutisieae from the Guyana Highlands. Stix (1960) found six types of internal exine patterns in the six genera she studied: *Dicoma, Erythrocephalum, Mutisia, Ameghinoa, Oxyphyllum,* and *Trixis.* Parra and Marticorena (1972) recognized eight exine patterns among Chilean genera of Mutisieae (*Chuquiraga, Dasyphyllum, Chaetanthera, Mutisia, Gochnatia, Proustia, Trixis,* and *Leucheria*-types).

Later studies utilizing electron microscopy by Skvarla et al. (1977) and Hansen (1991a, b) showed that the most diverse assemblage of pollen grains within the Asteraceae were found in the tribe Mutisieae. These authors noted that *Barnadesia* and related genera currently placed in the subfamily Barnadesioideae exhibited ultrastructural exine features that were strikingly similar to those found in the Calyceraceae. In particular, they noted a strong resemblance of the wall ultrastructure of *Dasyphyllum* to *Nastanthus* (Calyceraceae).

Urtubey (1997) used SEM to distinguish two symmetry patterns in *Barnadesia* pollen: radiosymmetric (*B. corymbosa, B. glomerata, B. odorata, B. parviflora, B. spinosa*) and radioasymmetric (*B. aculeata, B. arborea, B. blackeana, B. carypophylla, B. dombeyana, B. horrida, B. jelskii, B. lehmannii, B. macbrideana, B. macrocephala, B. polycantha, B. pycnophylla, B. reticulata*). Most recently, Urtubey and Telleria (1998) examined pollen morphology of 59 species of Barnadesioideae with light microscopy (LM) and SEM. They recognized three pollen types, constructed a key for their identification, and discussed their phylogenetic significance.

In the current study we survey pollen grain morphology of 41 species of all nine genera of the subfamily Barnadesioideae, including representatives from all subgenera and sections of the three larger genera *Barnadesia, Chuquiraga,* and *Dasyphyllum.* Also included for purposes of discussion and comparison is *Gamocarpha alpina* pollen of the Calyceraceae. Our study extends previous contemporary SEM palynological investigations of this subfamily (Urtubey 1997; Urtubey and Telleria 1998) by incorporating data from freeze fracture SEM and TEM. We use these data in combination with previously published data to examine the similarity between intercolpar concavities and exine types in the subfamily Barnadesioideae and the Calyceraceae. Additionally, we examined the distribution of pollen data on two recently published morphological phylogenies of the Barnadesioideae (Bremer 1994; Stuessy et al. 1996).

MATERIALS AND METHODS

POLLEN SAMPLING. Pollen was obtained from herbarium sheets (Table 1) for 41 species representing all nine genera of the Barnadesioideae and one genus of the Calyceraceae (*Gamocarpha*). There is strong evidence based on molecular (Jansen and

TABLE 1. Taxon sampling for pollen comparisons. Herbarium acronyms follow Holmgren et al. (1990). Taxonomic circumscriptions and nomenclature follow recent treatments of *Arnoldoa* (Stuessy and Sagastegui, 1993), *Barnadesia* (Urtubey, 1999), *Chuquiraga* (Ezcurra, 1985), *Dasyphyllum* (Cabrera, 1959), *Doniophyton* (Katinas and Stuessy, 1997) and *Huarpea* (Cabrera, 1951).

Taxon	Locality	Collector	Herbarium
Barnadesioideae			
Arnaldoa macbrideana Ferreyra	Peru	Ferreyra & Wurdack 14415	MO
A. weberbaueri (Muschl.) Ferreyra	Peru	Smith & Sanchez 4323	US
Barnadesia aculeata (Benth.) I. C. Chung	Ecuador	Panero 2959	TEX
B. arborea Kunth in H. B. K.	Ecuador	Stuessy 12288	OS
B. caryophylla (Vell.) S. F. Blake	Brazil	Irwin 17486	TEX
B. dombeyana Less.	Peru	Stuessy 12470	OS
B. horrida Muschl.	Peru	Grifo 1065	TEX
B. jelskii Hieron. ex Sod.	Peru	Stuessy 12567	OS
B. lehmannii Hieron.	Peru	Stuessy 12699	OS
B. odorata Griseb.	Bolivia	Solomon 10342	TEX
B. parviflora Spruce ex Benth. & Hook. f.	Ecuador	Stuessy 12364	OS
B. pycnophylla Muschl.	Bolivia	Solomon 8341	TEX
B. spinosa L. f.	Ecuador	Grimes & Todzia 2492	TEX
Chuquiraga aurea Skottsb.	Argentina	Stuessy 12911, 12919	OS
C. avellanedae Lorentz	Argentina	Stuessy 12920, 12929, 12938	OS
C. erinacea D. Don	Argentina	Stuessy 12882, 12977, 12979	OS
C. jussieui J. F. Gmel.	Ecuador	Asplund 17727	TEX
C. morenonis (O. Kuntze) C. Ezcurra	Argentina	Stuessy 12940	OS
C. oblongifolia A. Sagastegui Alva & I. Sánchez Vega	Peru	Stuessy 12625	OS
C. oppositifolia D. Don	Bolivia	Nee 31311	TEX
C. rotundifolia Wedd.	Argéntina	Stuessy 12988	OS
C. spinosa D. Don	Peru	Saunders 609	TEX
C. ulicina Hook.	Chile	Stuessy 12777, 12799	OS
C. weberbaueri Tovar	Peru	Stuessy 12496	OS
Dasyphyllum argenteum H. B. K.	Ecuador	Webster & Kim 23022	TEX
D. brasiliense (Spreng.) Cabrera	Bolivia	Nee 35087, 40455	TEX
D. candolleanum (Gardner) Cabrera	Brazil	Mori & Silva 16888	TEX
D. excelsum (D. Don) Cabrera	Chile	Hellwig 1186	FH-LS
D. ferox (Wedd.) Cabrera	Bolivia	Balls 5883	TEX
D. horridum (Muschl.) Cabrera	Peru	Smith & Buddensiek 10850	TEX
D. inerme (Rusby) Cabrera	Bolivia	Nee 40722	TEX
D. popayanense (Hieron.) Cabrera	Ecuador	Stuessy 12289	OS
D. sprengelianum (Gardn.) Cabrera	Brazil	Webster 25187	TEX
D. velutinum (Baker) Cabrera	Guyana	Williams 8022	TEX
Doniophyton anomalum Kuntze	Argentina	Stuessy 12780, 12853, 12857	OS
D. weddellii Katinas & Stuessy	Argentina	Paci 289	TEX
Duseniella patagonia K. Schum.	Argentina	Correa 4119	UC
Fulcaldea laurifolia Poir.	Peru	Stuessy 12701	OS
Huarpea andina Cabrera	Argentina	Kiesling 4555	K
Schlechtendalia luzulaefolia Less.	Brazil	Hatschbach 47339	US
Calyceraceae			
Gamocarpha alpina (Poepp. Ex Less.) H. V. Hansen	Chile	DeVore 1250	SHST

Kim 1996), morphological (DeVore 1994; DeVore and Stuessy 1995; Pesecreta et al. 1994; Carlquist and DeVore 1998) and phytochemical (Bohm and Stuessy 1995; Bohm et al. 1995) data that the Calyceraceae is sister to the Asteraceae. Furthermore, *Gamocarpha* possesses pollen features typical for basal members of the family (M. DeVore, Z. Zhao, J. Skvarla, and R. Jansen, unpublished).

LIGHT MICROSCOPY. Acetolyzed pollen grains were stained and mounted on glass slides according to Nair (1970) and then examined with transmitted light using a Leitz Wetzlar microscope. The slides are housed in the reference collection at the University of Texas at Austin (TEX).

SEM. Pollen was placed in tapered test tubes, acetolyzed according to the method of Erdtman (1960), screened with fine wire mesh to remove undigested coarse plant fragments (Skvarla 1966), and placed on sucrose pads to remove finer particles (Chissoe and Skvarla 1974).

For whole grain pollen mounts, dehydration was accomplished using 5 min washes in graded ethanol (EtOH) solutions, three 5 min washes in absolute EtOH, and two 5 min treatments in 100% hexamethyldisilazane (Chissoe et al. 1994). After dehydration, all samples received a sputter-coating with gold for 4–5 min. Finally, pollen grains were examined with a JEOL JSM 880 scanning electron microscope.

Freeze-sectioned pollen grains for structural study were prepared following the method described by Vezey et al. (1994). A drop of Optimal Cutting Temperature (OCT) compound (Tissue-Tek embedding medium) was placed on an IEC Microtome-Cryostat specimen mount (precooled to −20°C). Within seconds the OCT drop was frozen and a drop of concentrated pollen/water was immediately placed on the frozen OCT drop. The resulting pollen/ice mixture was sectioned with a razor blade into 8–15 μm sections and placed on a precooled boat-shaped specimen mount. Mounts were then warmed to room temperature and placed in a desiccator for several hours to evaporate thoroughly the melted ice. Sections were then sputter-coated with gold and examined with a JEOL JSM 880 scanning electron microscope.

TEM. TEM sample preparation followed the method described by Skvarla (1966, 1973). Examination and photography were performed with a Philips 200 TEM.

RESULTS AND DISCUSSION

POLLEN TYPES IN THE BARNADESIOIDEAE. The distribution of pollen characters for the 41 examined taxa is summarized in Table 2 and in representative photomicrographs (Figs. 1, 2). Accordingly, four pollen types could be distinguished. They are outlined in Table 3 and discussed below. It is evident from these tables that the most useful characters for determining pollen types are the presence or absence of lophate surfaces, intercolpar concavities, and microspines.

Barnadesia-type. Psilolophate pollen of *Barnadesia* exemplifies the *Barnadesia*-type and is characterized by having many lacunae (Fig. 1I). Most species have irregular (radioasymmetrical) lacunae, however, radiosymmetrical lacunae are found in three taxa: *B. odorata, B. parviflora,* and *B. spinosa.* Urtubey (1997) previously reported that *Barnadesia* can be divided into two groups based on this character. All examined radiosymmetrical pollen grains have 32 lacunae even though their patterns may not be the same. For example, *B. parviflora* has an equatorial lacuna with four neighboring lacunae, whereas *B. spinosa* has an equatorial lacuna with six neighboring lacunae. Pollen of *Huarpea* is smaller than most *Barnadesia* pollen (Fig. 1B, Table 2), although *B. aculeata* has the smallest grains in this group.

Dasyphyllum-type. This type is distinctive in the possession of intercolpar concavities between aperture furrows (Figs. 1D, 1H). The feature was first recognized by

TABLE 2. Summary of 10 pollen characters in the Barnadesioideae and Calyceraceae

Taxon	Shape	Lophate Pollen	Size (μm)	Inter-colpar con-cavity
Barnadesioideae				
Arnaldoa macbrideana	subspheroidal w/depressions	absent	34.1 × 31.2	present
A. weberbaueri	subspheroidal w/depressions	absent	45.7 × 40.0	present
Barnadesia aculeata	spheroidal w/lacunae	present	31.5 × 31.5	present
B. arborea	spheroidal w/lacunae	present	40.0 × 40.0	present
B. caryophylla	spheroidal w/lacunae	present	48.0 × 48.0	present
B. dombeyana	spheroidal w/lacunae	present	45.0 × 45.0	present
B. horrida	spheroidal w/lacunae	present	43.0 × 43.0	present
B. jelskii	spheroidal w/lacunae	present	40.0 × 40.0	present
B. lehmannii	spheroidal w/lacunae	present	40.0 × 40.0	present
B. odorata	spheroidal w/lacunae	present	42.0 × 42.0	present
B. parviflora	spheroidal w/lacunae	present	44.0 × 44.0	present
B. pycnophylla	spheroidal w/lacunae	present	45.0 × 45.0	present
B. spinosa	spheroidal w/lacunae	present	43.0 × 43.0	present
Chuquiraga aurea	subspheroidal	absent	26.8 × 22.0	absent
C. avellanedae	subspheroidal	absent	22.4 × 19.2	absent
C. erinacea	subspheroidal	absent	26.5 × 21.3	absent
C. jussieui	subspheroidal	absent	27.5 × 26.5	absent
C. morenonis	subspheroidal	absent	35.9 × 27.1	absent
C. oblongifolia	subspheroidal	absent	30.0 × 28.0	absent
C. oppositifolia	subspheroidal	absent	22.0 × 17.3	absent
C. rotundifolia	subspheroidal	absent	29.2 × 25.5	absent
C. spinosa	subspheroidal	absent	27.5 × 22.0	absent
C. ulicina	subspheroidal	absent	29.5 × 26.5	absent
C. weberbaueri	subspheroidal	absent	35.5 × 26.5	absent
Dasyphyllum argenteum	subspheroidal w/depressions	absent	27.2 × 26.5	present
D. brasiliense	subspheroidal w/depressions	absent	24.0 × 22.0	present
D. candolleanum	subspheroidal w/depressions	absent	35.0 × 34.0	present
D. excelsum	subspheroidal w/depressions	absent	33.0 × 31.0	present
D. ferox	subspheroidal w/depressions	absent	27.5 × 26.5	present
D. horridum	subspheroidal w/depressions	absent	26.0 × 22.8	present
D. inerme	subspheroidal w/depressions	absent	22.0 × 23.0	present
D. popayanense	subspheroidal w/depressions	absent	30.7 × 27.0	present
D. sprengelianum	subspheroidal w/depressions	absent	35.0 × 29.0	present
D. velutinum	subspheroidal w/depressions	absent	29.5 × 25.8	present
Doniophyton anomalum	subspheroidal	absent	39.0 × 30.0	absent
D. weddellii	subspheroidal	absent	35.3 × 31.3	absent
Duseniella patagonia	subspheroidal	absent	33.3 × 25.5	absent
Fulcaldea laurifolia	subspheroidal	absent	33.3 × 26.6	absent
Huarpea andina	spheroidal w/lacunae	present	36.0 × 36.0	present
Schlechtendalia luzulaefolia	subspheroidal w/depressions	absent	28.2 × 26.0	present
Calyceraceae				
Gamocarpha alpina	spheroidal	absent	20.0 × 18.5	absent

TABLE 2. Extended.

Tem Exine	Foot Layer Thickness (μm)	Furrow End	Endocolpi Shape and Length to Width Ratio (μm)	Cavus	Micro-spine (μm)
N/A	0.4	pointed	lalongate—6.6 × 12.0	absent	0.15
one layer	0.4	pointed	lalongate—4.0 × 8.8	absent	0.18
N/A	0.8	N/A	lolongate—14.0 × 8.0	present	absent
N/A	0.9	N/A	lolongate—18.0 × 10.0	present	absent
N/A	0.9	N/A	lolongate—20.0 × 10.0	present	absent
N/A	0.9	N/A	lolongate—17.0 × 8.0	present	absent
N/A	0.9	N/A	lolongate—18.5 × 10.0	present	absent
N/A	0.8	N/A	lolongate—19.0 × 10.0	present	absent
N/A	1.0	N/A	lolongate—17.5 × 9.0	present	absent
N/A	0.8	N/A	lolongate—18.0 × 8.5	present	absent
N/A	1	N/A	lolongate—18.0 × 9.0	present	absent
one layer	0.9	N/A	lolongate—18.0 × 8.0	present	absent
N/A	1.1	N/A	lolongate—18.0 × 8.5	present	absent
N/A	0.3	pointed	lalongate—5.0 × 10.0	absent	0.2
N/A	0.4	pointed	lalongate—3.5 × 8.0	absent	0.15
N/A	0.4	pointed	lalongate—4.5 × 10.0	absent	0.3
N/A	0.25	pointed	lalongate—5.0 × 14.0	absent	0.2
N/A	0.3	pointed	lalongate—4.5 × 6.5	absent	absent
N/A	0.5	pointed	lalongate—4.0 × 7.0	absent	0.3
N/A	0.4	pointed	lalongate—5.5 × 11.0	absent	0.12
two layers	0.4	pointed	lalongate—4.5 × 10.0	absent	0.18
N/A	0.4	pointed	lalongate—3.8 × 7.0	absent	0.2
N/A	0.6	pointed	lalongate—5.0 × 12.0	absent	0.2
N/A	0.4	pointed	lalongate—3.8 × 9.5	absent	0.2
N/A	0.3	rounded	lalongate—4.0 × 9.0	present	0.17
N/A	0.3	rounded	lalongate—2.3 × 10.0	present	0.15
N/A	0.7	pointed	lalongate—2.0 × 9.0	present	0.2
N/A	0.25	pointed	lalongate—3.1 × 6.3	present	0.25
N/A	0.4	pointed	lalongate—4.5 × 10.0	present	0.5
one layer	0.4	rounded	lalongate—3.2 × 6.0	present	0.17
N/A	0.25	rounded	lalongate—5.0 × 11.5	present	0.25
N/A	0.3	pointed	lalongate—6.0 × 10.0	present	0.25
N/A	0.4	pointed	lalongate—5.0 × 14.0	present	0.15
N/A	0.42	pointed	lalongate—4.0 × 5.0	absent	0.4
two layers	0.45	pointed	lalongate—5.5 × 15.0	absent	0.3
N/A	0.5	pointed	lalongate—6.0 × 11.5	absent	0.15
two layers	0.4	pointed	lalongate—5.2 × 14.0	absent	0.25
two layers	0.45	rounded	lalongate—5.5 × 12.5	absent	1.2
one layer	0.8	N/A	lolongate—18.0 × 10.0	absent	absent
two layers	0.4	pointed	lalongate—3.5 × 10.5	absent	0.3
two layers	0.5	pointed	lalongate—4.0 × 8.0	absent	0.1

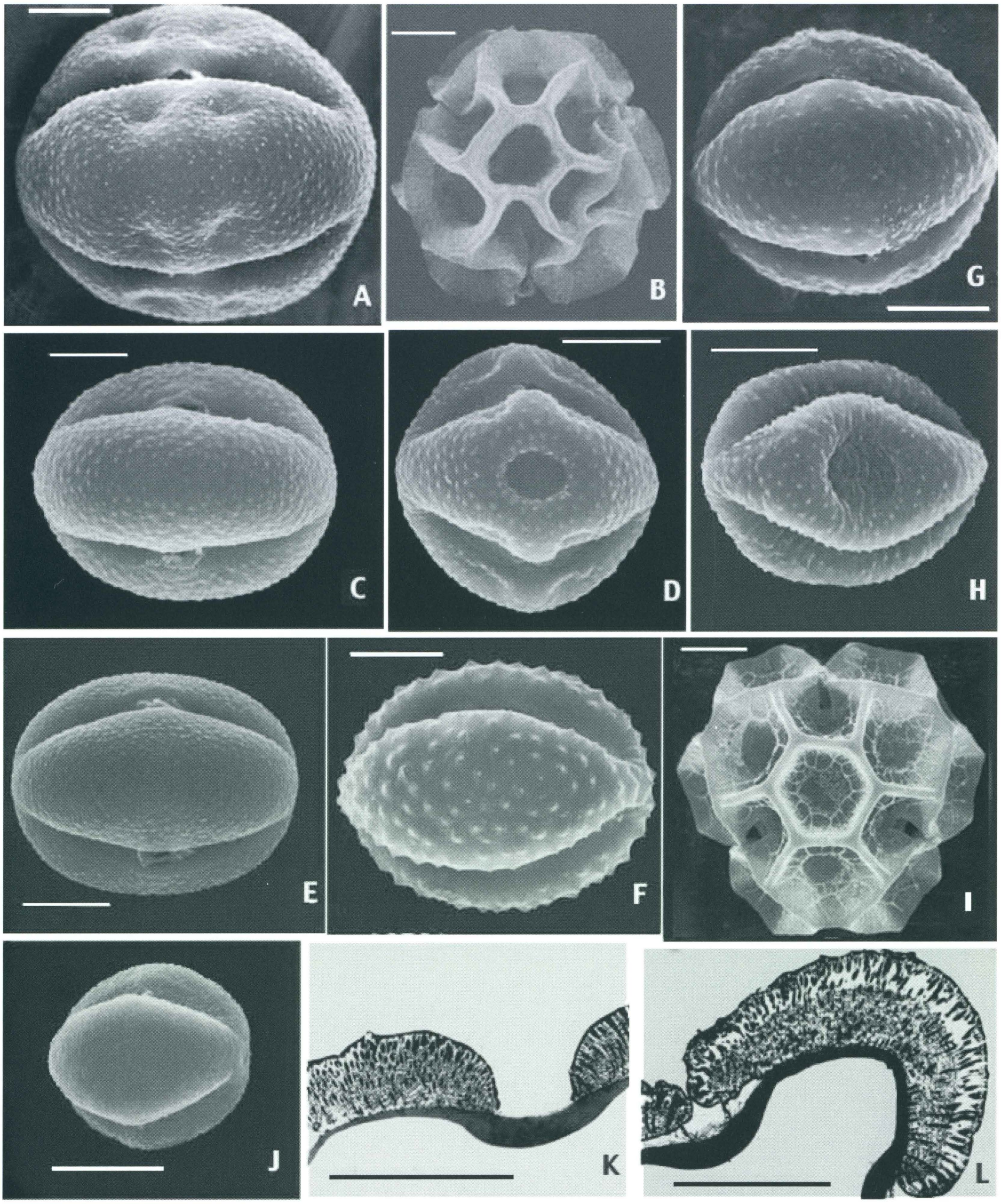

FIG. 1. A–J SEM micrographs, K–L TEM micrographs (bar length 10 um). A. *Arnaldoa weberbaueri* × 1400. B. *Huarpea andina* × 1300. C. *Doniophyton anomalum* × 1800. D. *Schlechtendalia luzulaefolia* × 2000. E. *Duseniella patagonia* × 1800. F. *Fulcaldea laurifolia* × 1800. G. *Chuquiraga rotundifolia* × 1800. H. *Dasyphyllum horridum* × 2500. I. *Barnadesia spinosa* × 1300. J. *Gamocarpha alpina* × 3000. K. *Arnaldoa weberbaueri* × 5000. L. *Chuquiraga rotundifolia* × 4000.

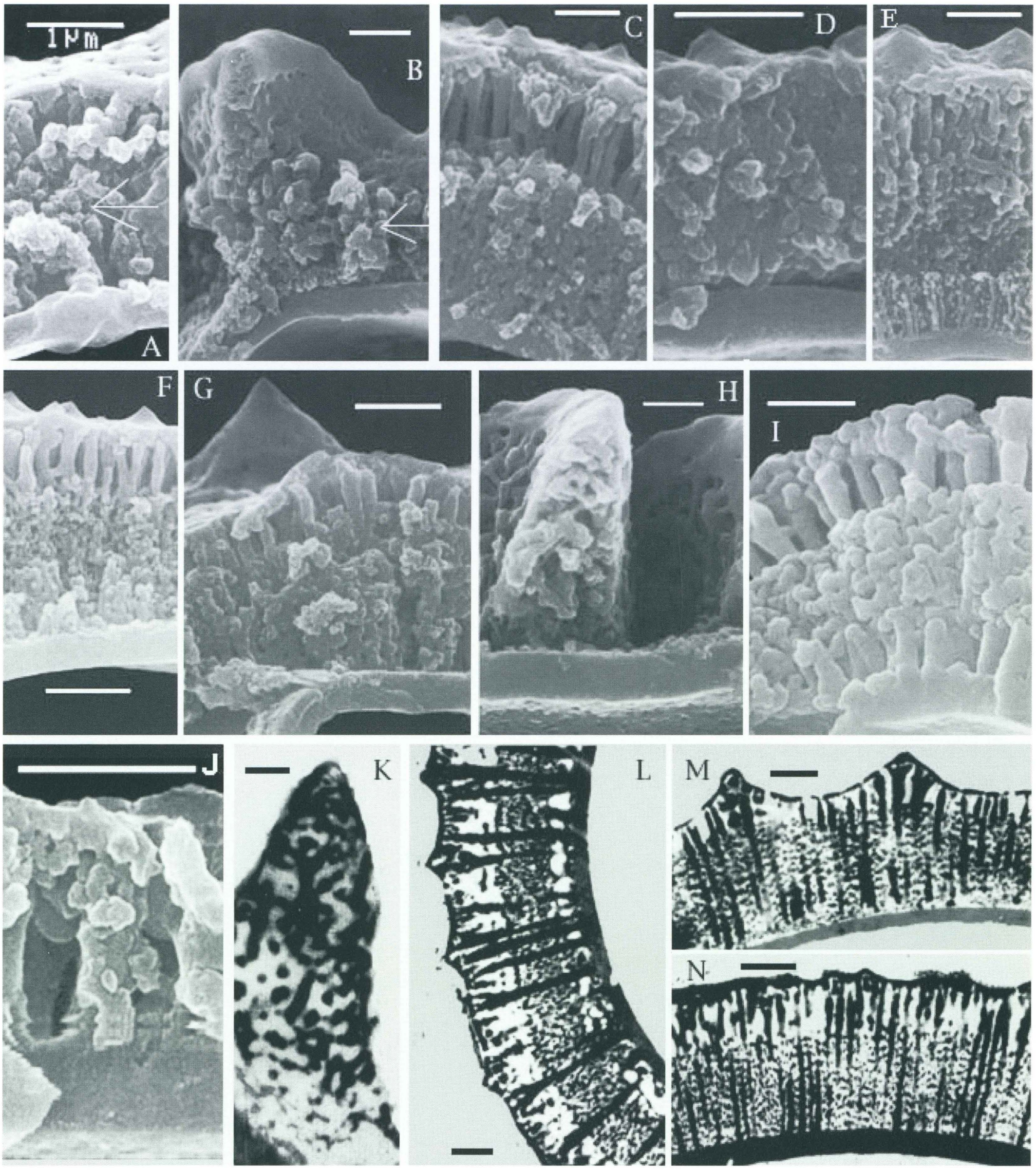

FIG. 2. A–J SEM micrographs of pollen wall (bar length 1 um). K–L TEM micrographs of pollen wall (bar length 1 um). A. *Arnaldoa weberbaueri* × 16000. B. *Barnadesia arborea* × 6000. C. *Chuquiraga rotundifolia* × 10000. D. *Dasyphyllum horridum* × 20000. E. *Doniophyton anomalum* × 10000. F. *Duseniella patagonia* × 15000. G. *Fulcaldea laurifolia* × 14000. H. *Huarpea andina* × 10000. I. *Schlechtendalia luzulaefolia* × 14000. J. *Gamocarpha alpina* × 27000. K. *Huarpea andina* × 6300. L. *Schlechtendalia luzulaefolia* × 5000. M. *Fulcaldea laurifolia* × 8000. N. *Duseniella patagonia* × 8000.

TABLE 3. Pollen types in subfamily Barnadesioideae.

Arnaldoa-type—surfaces with microspines, intercolpar concavities and depressions
- A. *Arnaldoa* (Figs. 1A, 1K, 2A)

Barnadesia-type—psilolophate surfaces
- A. *Barnadesia* (Figs. 1I, 1L)
- B. *Huarpea* (Fig. 1B)

Chuquiraga-type—surfaces with microspines
- A. *Chuquiraga* (Figs. 1G, 2C)
- B. *Doniophyton* (Figs. 1C, 2E)
- C. *Duseniella* (Figs. 1E, 2F, 2N)
- D. *Fulcaldea* (Figs. 1F, 2G, 2N)

Dasyphyllum-type—surfaces with microspines and intercolpar concavities
- A. *Dasyphyllum* (Figs. 1H, 2D)
- B. *Schlechtendalia* (Figs. 1D, 2I, 2L)

Wodehouse (1928) and he termed such concavities as intercolpar (Fig. 1H). The intercolpar regions of some species of *Dasyphyllum* are strongly concave while others are only slightly concave or even somewhat flattened, or perhaps absent as noted by Urtubey and Telleria (1998) for *D. donianum, D. infundibulare, D. reticulum* and *D. velutinum. Dasyphyllum* is divided into two subgenera (Cabrera 1959), *Archidasyphyllum* (with two species) and *Dasyphyllum* (with 34 species) with two sections (sect. *Dasyphyllum* (= *Microcephala*) and sect. *Macrocephala*). The only species of subg. *Archidasyphyllum* examined, *D. excelsum*, has strongly concave intercolpar concavities. In sect. *Microcephala* (with 24 species) four examined species have strongly concave intercolpar regions and in sect. *Macrocephala* (with 10 species) pollen of the two examined species have more or less flat intercolpar regions. This suggests concavities may be useful for distinguishing the two sections. Urtubey and Telleria (1998) indicate that intercolpar depressions also characterize *Archidasphyllum*, most species of sect. *Microcephala* and only 3 of 11 species of sect. *Macrocephala.* Urtubey and Telleria (1998) examined 39 species of *Dasyphyllum* and established two major types and 3 subtypes (of Type 2) based on the strength and weakness of intercolpar depressions. Pollen of *Schlechtendalia* is a modified dasyphylloid type (Figs. 1D, 2I, 2L). Like *Dasyphyllum,* the pollen grains have a large equatorial depression in each intercolpar region. Also present in *Schlechtendalia* are two small depressions on each side of the furrows. *Schlechtendalia* does not have a cavus above the foot layer as is always noted in *Dasyphyllum.* This confirms similar observations by Skvarla et al. (1977) and Urtubey and Telleria (1998). In a comprehensive examination of *Dasyphyllum* (39 species examined) they noted an absence of a cavus only in *D. velutinum.*

Arnaldoa-type. Pollen grains of *Arnaldoa* (Figs. 1A, 1K, 2A) appear intermediate between *Chuquiraga*- and *Dasyphyllum*-types. They resemble the former in lacking distinctive intercolpar concavities and the latter in having them, although modified, occurring as four depressions surrounding each aperture (i.e., paraporal depressions) which are symmetrical to the long axis of the pollen grain (Fig. 1A). Urtubey and Telleria (1998) noted that these depressions can vary from weak to very strong. A cavus is also present in this pollen, as is present in *Dasyphyllum.*

Chuquiraga-type. The prolate-subspheroidal pollen grains of *Chuquiraga* (Fig. 1G) are the smallest of all Barnadesioideae species and the pollen is very similar to each other. *Fulcadea* pollen is somewhat different from the other chuquiragoid members in having wider germinal furows and larger microspines (Fig. 2G, Table 2).

In summary, these four pollen types, based on sculptural features of the pollen wall, essentially agree with the types established by Urtubey and Telleria (1998) who used presence or absence of intercolpar depressions and lophate pollen in *Barnadesia* to recognize three pollen types. Their "Type I" corresponds to our *Barnadesia*-type, "Type II" to our *Dasyphyllum*- and *Arnal-*

doa-types, and "Type III" to our *Chuquiraga*-type.

EXINE STRUCTURE. Urtubey and Telleria (1998) suggested that exine layering was an important phylogenetic character for cladistic analyses of the Barnadesioideae. Using LM they recognized one-, two- and three-layered exines based on observations of bleached pollen grains. Our study, which includes freeze fractured SEM and limited TEM, provides an excellent opportunity to extend such observations. Micrographs from fractured (SEM) and sectioned pollen (TEM) indicate that exine structure is not clearly defined in the Barnadesioideae. Indeed, these micrographs reveal somewhat diffuse structural patterns. We recognize three structural patterns.

Single-layer exines—Consist of thick columellae extending through the exine. They are present in lophate grains (i.e., *Barnadesia*-type, Fig. 2K). As indicated elsewhere (Skvarla et al. 1977), they are also present in intercolpar portions of *Dasyphyllum* and *Schlechtendalia* exines (while other portions are more complex). A single layer may also be present in *Arnaldoa* but freeze-fracture SEM is inconclusive. Similar doubt of *Arnaldoa* exine structure was expressed by Urtubey and Telleria (1998).

Double-layer exines—Consist of a basal layer composed of thick columellae and an upper layer of highly branched or divided columellae. Exine columellae are rod-like (as is the case with all Barnadesioideae taxa) with the rods close to the pollen surface palisade-like and the rods close to the exine base partly broken up to form minute globular bodies (Fig. 1L). Double-layer exines occur in *Chuquiraga, Duseniella* (Fig. 2N), *Fulcaldea* (Fig. 2G) and *Doniophyton* (see Fig. 23E of Skvarla et al. 1977).

Triple-layer exines—*Schlechtendalia* (Fig. 2L) and *Dasyphyllum* (Fig. 2D), in addition to possessing single-layers (see above) these taxa also have complex layering. The basal layer is about 0.5 μm wide and has a highly reduced reticulate layer (some portions of the basal layer are totally devoid of reticulate exine). The medial layer consists of both fine and thickened columellae and the upper layer consists only of short and thick columellae. Similar morphology was observed by TEM in these taxa elsewhere (see Figs. 23B, C of Skvarla et al. 1977). Triple-layered exines were observed by Urtubey and Telleria only in *Schlechtendalia.*

The three types of exine layers discussed above are in close agreement with those proposed by Urtubey and Telleria (1998) and any disparities have already been noted. Additionally, these data allow general comparisons with pollen of Calyceraceae (to be discussed later).

There may be a biomechanical factor that favors a single-layered exine in lophate pollen and in the intercolpar concavity regions of grains such as *Schlechtendalia* and *Dasyphyllum,* which otherwise, are triple-layered. One may assume that triple-layered exines would be more widely distributed within the family if they better fit the functional role of lophate pollen and/or grains with intercolpar concavities. The ultrastructure of *Fulcaldea* provides a possible clue to the evolution of pollen within the subfamily. *Fulcaldea* shows a nearly single-layer exine structure. This suggests that the reticulate layer, which is distinct from the columellae, became more developed in derived members of the Barnadesioideae.

Urtubey and Telleria (1998) also suggested that the five exine sculpture patterns (i.e., granulate sparsely microechinate, microechinate, scabarate microechinate, smooth, and spinulate) in the Barnadesioideae were of phylogenetic significance. We closely examined the ultrastructure of these sculpture patterns to better ascertain their phylogenetic utility. All sculpture patterns, except for smooth exine, are derived from the conjunction of columellae (Fig. 2L–N). The columellae extend up into the apex of the spinulate tip. The various sculpture patterns differ in the relative length of the central columellae and in the degree of concavity among adjacent spinules. Among the five patterns recognized by Urtubey and Telleria (1998), the scabarate microechinate and granulate sparsely microechinate are not different based on our high magnification freeze-fracture SEM and TEM data. The surface of the scabarate microechinate pattern is flattened and Urtubey and Telleria's (1998) example of this pattern is *Doniophyton.* All of our micrographs of *Doniophyton* show the triangle spinule sculpture pattern (Fig. 1C, 2E). The granulate sparsely microechinate pattern has continuous nodules and Urtubey and Telleria (1998) use *Schlechtendalia* as their example of this exine type. Our data (Figs. 1D, 2L) clearly demonstrate that *Schlechtendalia* has the triangle spinule sculpture pattern. The only difference between the microechinate and spinulate patterns is the size of the spinules.

Exine structure of the Calyceraceae—Pollen ultrastructural studies of *Nastanthus* (Skvarla et al. 1977) and *Gamocarpha* (Fig. 2J) indicate that the Calyceraceae have a double exine layer. The basal exine layer consists of thick columellae and the upper exine consists of highly branched or divided columellae (Fig. 2J). This is not homologous with the double exines found within the Barnadesioideae for three reasons: 1) thick columellae extend through the entire exine in Barnadesioideae (Fig. 2L); 2) a reticulate, spongy network extends throughout the exine (Fig. 2M) or is only found in the basal layer in Barnadesidoideae (Fig. 2L); and 3) the reticulate layer and thick columellae are often found together.

PHYLOGENETIC IMPLICATIONS IN THE BARNADESIODEAE. It is extremely difficult to determine homologies, if any, between the subfamily Barnadesioideae and other members of the Asteraceae. This is particularly true for palynological data because the pollen ultrastructure for the subfamily is so distinctive. In particular, the pollen exine columellae layers of all genera are rodlike and more or less divided into fine divisions that appear globular in section (Fig. 2A–I). This is the only palynological characteristic that supports the monophyly of the Barnadesioideae.

Cabrera (1959, 1977) suggested that *Dasyphyllum* is basal and that it gave rise to *Chuquiraga, Barnadesia, Schlechtendalia,* and *Fulcaldea. Doniophyton* is believed to have originated from an ancestral line including *Chuquiraga,* while *Huarpea* is derived from *Barnadesia.*

Bremer (1994) provided the first phylogenetic analysis of the Barnadesioideae based on 22 morphological characters (Fig. 3). His cladogram identified two major clades, one including *Schlechtendalia, Doniophyton* and *Duseniella,* and the second including the remaining six genera. Mapping pollen morphology on Bremer's tree (Fig. 3) indicates that many of these characters change multiple times. For example, the intercolpar areas change from convex (*Chuquiraga*) to concave (*Dasyphyllum*), then to convex (*Fulcaldea*), then to concave (*Barnadesia*) again. This character is lost twice and gained twice on the Bremer phylogeny, which seems very unlikely.

Stuessy et al. (1996) proposed an alternative phylogeny based on 19 morphological characters (Fig. 4). Their cladogram suggested *Schlechtendalia* to be the basal genus. Pollen characters also change very frequently in this phylogeny. For example, the intercolpar concavities change from many (*Schlechtendalia*) to none (*Fulcaldea*) to many (*Huarpea*).

PHYLOGENETIC DISTRIBUTION OF INTERCOLPAR CONCAVITIES. Skvarla et al. (1977) first noted that the Asteraceae and Calyceraceae have intercolpar concavities, and this is one of the major reasons why they suggested a close relationship between these families. Hansen (1992) suggested that the intercolpar concavities of the Calyceraceae and Barnadesioideae may represent a synapomorphy. Our pollen investigations suggest that in both the Barnade-

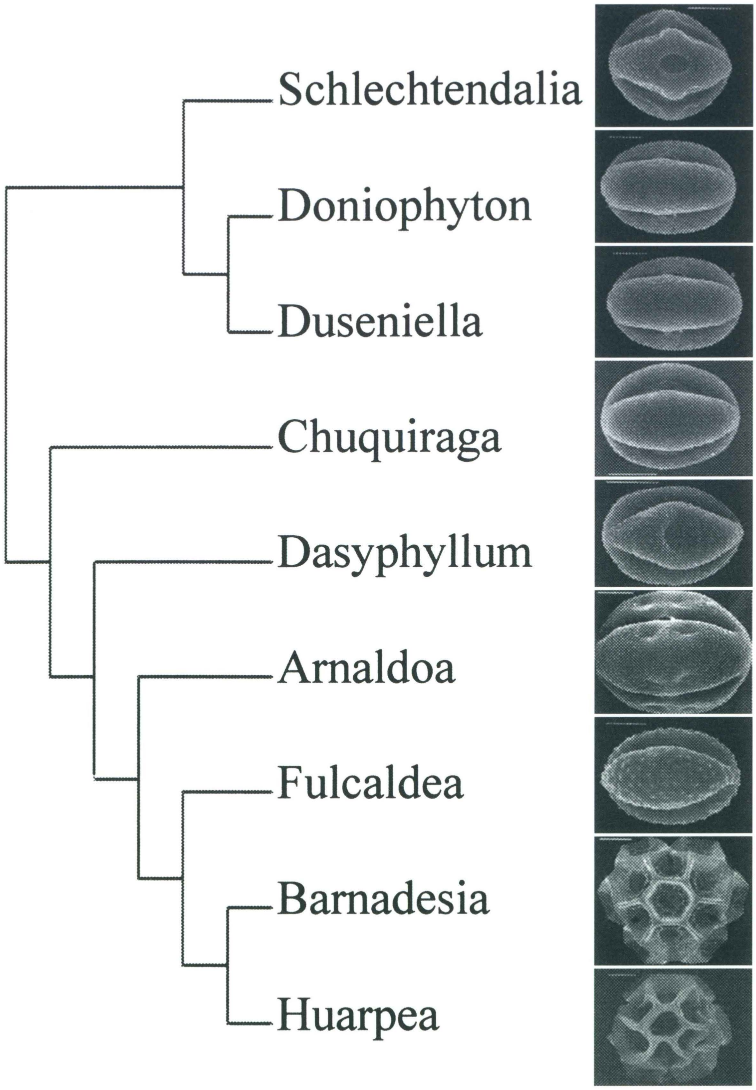

FIG. 3. Modified Bremer's (1994) phylogeny of Barnadesioideae.

sioideae and Calyceraceae the intercolpar concavities are derived from convex pollen. The only members of the Barnadesiodeae with intercolpar concavities are *Dasyphyllum* and *Schlechtendalia*. *Dasyphyllum* does not occur in a basal position in phylogenies produced by either Bremer (1994, Fig. 3) or Stuessy et al. (1996, Fig. 4) but *Schlechtendalia* is basal in the Stuessy et al. cladogram. Thus, it is likely that intercolpar concavities have evolved independently within the Barnadesioideae and Calyceraceae.

CONCLUSIONS

Phylogenetic relationships within the Barnadesioideae are still unresolved. Palynological characters, when placed within the context of Bremer's (1994, Fig. 3) and Stuessy et al.'s. (1996, Fig. 4) phylogenies, would have had to undergone some difficult character state transformations. However, of the two morphological phylogenies, pollen morphology is more concordant with Stuessy et al.'s hypothesis of

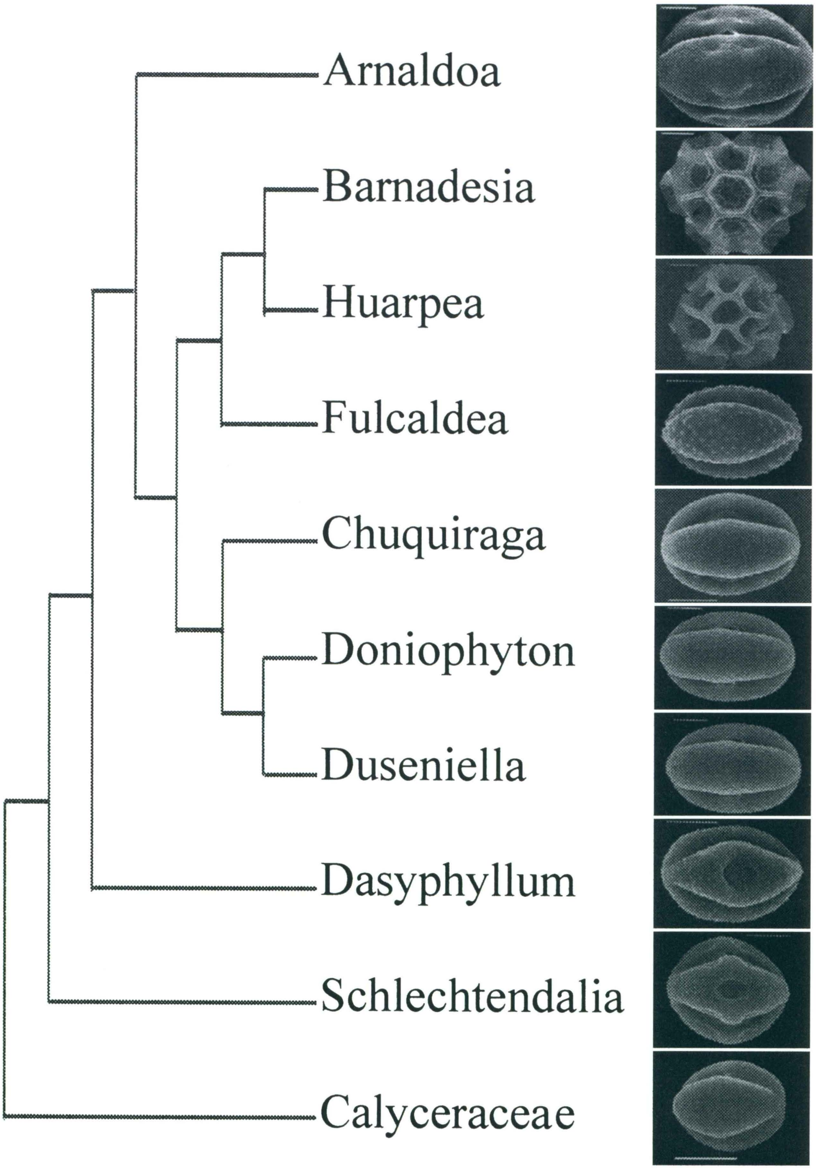

FIG. 4. Modified Stuessy et al. (1996) phylogeny of Barnadesioideae.

relationships (Fig. 4). Palynological data illustrate that further analyses of the Barnadesioideae are needed and incorporating pollen characters can enhance future comparisons.

The present study indicates that based on pollen characters alone, there are three lineages within subfamily Barnadesioideae, each with a distinctive pollen type. Members of the subfamily exhibiting *Chuquiraga*-type pollen are most likely basal within Barnadesioideae. In contrast, taxa with *Barnadesia*-type pollen probably represent the most derived lineage within the subfamily. Most importantly, those taxa within the family with intercolpar concavities are apparently not basal. This suggests that intercolpar concavities are derived independently within both the Calyceraceae and Barnadesioideae and should not be viewed as a synapomorphy uniting the Asteraceae and Calyceraceae.

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