

## POLLEN MORPHOLOGY AND ULTRASTRUCTURE OF CALYCERACEAE

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**Abstract:** Pollen morphology of 13 species from all six genera of Calyceraceae (*Acicarpha*, *Boopis*, *Calycera*, *Gamocarpha*, *Moschopsis*, and *Nastanthus*) and representatives of the Campanulaceae and Goodeniaceae is examined with light (LM), scanning (SEM), and transmission (TEM) electron microscopy. *Acicarpha*, *Calycera*, and *Nastanthus* pollen grains are distinguished by angulaperturate apertures, colpar ledges and surface depressions between colpi known as intercolpar concavities (IC). Pollen of *Gamocarpha* and *Moschopsis* is tricolporate rather than angulaperturate and without an IC. Some species of *Boopis* are similar to the preceding genera (e.g., *B. graminea*), while others (e.g., *B. gracilis*) are angulaperturate with ICs. Structural features derived from fractured pollen in SEM and sections in TEM show pollen walls composed of prominent columellae ca. 0.55–1.1  $\mu\text{m}$  high and  $<0.25 \mu\text{m}$  wide. The columellae terminate distally into a complex of shortened columellae ca. 1.5  $\mu\text{m}$  in length and are separated by an ill-defined irregular internal tectum layer. This structural complex is well known in several tribes of the Asteraceae and referred to as the Anthemoid type. In those grains with an IC, the structure consists of essentially short (ca. 1  $\mu\text{m}$ ), unbranched columellae, similar to those found within the Asteraceae subfamily Barnadesioideae (*Dasyphyllum* and *Schlechtendalia*). Goodeniaceae (including *Brunonia*) pollen has angulaperturate apertures, spinules (i.e., minute spines), problematic IC and some structural similarity to Calyceraceae pollen. The tendency within Calyceraceae to develop colpar ledges, ektexine bridges, and ICs may be a synapomorphy uniting the family with Goodeniaceae. If the ancestral pollen type for the Calyceraceae, Asteraceae, and Goodeniaceae clade is the *Gamocarpha* type (convex intercolpar regions; no colpar ledges and no ektexine bridges), then the appearance of these structures within each family may be a synapomorphy supporting their close phylogenetic relationship suggested by molecular analyses.

**Keywords:** Asteraceae, Barnadesioideae, Calyceraceae, Goodeniaceae, Asterales, intercolpar concavities (IC), pollen.

Calyceraceae consist of six genera with approximately 50 species. Some members of the family, especially *Acicarpha*, have capitula strikingly similar to Asteraceae, but they lack fully fused anthers and are characterized by a single pendulous ovule per achene. The first published evidence of a close relationship between Asteraceae and Calyceraceae was provided by an extensive pollen survey (Skvarla et al., 1977). One of the outcomes of this study was the suggestion that Calyceraceae were most closely related to Asteraceae based on the ultrastructural similarity of *Nastanthus* (Calyceraceae) and *Dasyphyllum* (Asteraceae, subfamily Barnadesioideae). Molecular phylogenetic analyses

(Jansen and Kim, 1996; Gustaffson et al., 1996; Lundberg and Bremer, 2003) support the monophyly of a clade that includes Goodeniaceae, Calyceraceae, and Asteraceae, and pollen data continue to provide insights into relationships among these families and other members of the Asterales (Polevova, 2006). Hansen (1992) published a survey of Calyceraceae pollen. One question Hansen raised was whether the intercolpar concavities (IC) present in some members of Barnadesioideae and Calyceraceae represent a synapomorphy.

There is strong evidence based on molecular (Jansen and Kim, 1996; Gustaffson et al., 1996; Lundberg and Bremer,

TABLE 1. Calyceraceae, Goodeniaceae and Campanulaceae pollen examined and collection data.

Taxon	Locality	Collector	Herbarium	Figures
<i>Acicarpa tribuloides</i> Juss	Argentina	Burkart & Troncoso 28056	TEX	1a-d
<i>Boopis gracilis</i> Phil.	Argentina	DeVore 1747	TEX	1e-h, 6a
<i>B. graminea</i> Phil.	Chile	DeVore 1249	TEX	1i-L
<i>Calycera calcitrapa</i> Griseb.	Argentina	Cabrera 18119	TEX	2a-d
<i>C. eryngioides</i> Remy	Chile	DeVore 1303	TEX	2e-g
<i>C. herbacea</i> Cav.	Chile	DeVore 1216	TEX	2h-k, 6b
<i>C. involucrata</i> Phil.	Argentina	DeVore 1737	TEX	2L, 3a-c
<i>C. leucanthema</i> Kuntze	Chile	DeVore 1143	TEX	3d-f
<i>C. spinulosa</i> Gill. ex Miers	Argentina	DeVore 1730	TEX	3g-j
<i>Gamocarpha alpina</i> (Poepp. ex Less.) H.V. Hansen	Chile	DeVore 1250	TEX	3k, L, 4a, b, 6c
<i>Moschopsis</i> sp.	Argentina	Moore 1966	US	4c-e, 6d
<i>Nastanthus andina</i> (Miers. H.V. Hansen)	Argentina	DeVore 1723	TEX	4f-h
<i>N. spathulatus</i> Miers	Chile	DeVore 1427	TEX	4i-L
<i>Campanula rotundiflora</i> L. (Campanulaceae)	United States	Powell 321110	TEX	5a, b
<i>Goodenia ovata</i> Smith (Goodeniaceae)	Australia	Vasek 680929-10	TEX	5c-f

2003), morphological (DeVore, 1994; DeVore and Stuessy, 1995; Pescreta et al., 1994; Carlquist and DeVore, 1998) and phytochemical (Bohm and Stuessy, 1995; Bohm et al., 1995) data that Calyceraceae are sister to Asteraceae. Pollen data have provided some of the strongest support for a close relationship between these families (Skvarla et al., 1977) and additional palynological surveys of Calyceraceae are valuable because they could elucidate morphological evolution within these families. In particular, these data are needed to reconstruct the ancestral features of Asteraceae.

In the present study, pollen morphology (Erdtman, 1960) of 13 species from all six genera of Calyceraceae and two representatives of Campanulaceae and Goodeniaceae is examined with light (LM), scanning (SEM) and transmission (TEM) microscopy. In addition to providing a palynologic overview of the Calyceraceae, which both complements and extends the original pollen morphologic data of Hansen (1992), we also compare pollen morphology of Calyceraceae with other families of Asterales.

## MATERIALS AND METHODS

Pollen was obtained from herbarium specimens of 13 species representing all six genera of Calyceraceae. Also included for comparative purposes was one species from two other families of Asterales, Goodeniaceae (*Goodenia ovata* Sm.), and Campanulaceae (*Campanula rotundiflora* L.). Table 1 shows collection information for all taxa examined as well as SEM and TEM figure citations.

All pollen samples were initially acetylated (Erdtman, 1960) to enhance viewing of the exine surfaces in (LM) and (SEM). For LM, 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).

For SEM, pollen was 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). Whole, intact

pollen grains were dehydrated 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 were prepared following the method described by Vezey et al. (1994). Sections were then sputter-coated and examined as described above.

TEM sample preparation followed the method described by Skvarla (1966, 1973). Examination and photography were performed with a JEOL 2000-FX TEM.

SEM and TEM negatives were scanned and final plates prepared using Adobe PhotoShop 7.

## RESULTS

**Pollen Descriptions.** All examined pollen samples are described below. All quantitative numbers in pollen description are the average of five random measurements.

### Calyceraceae

*Acicarpha*—A genus of annuals to perennials distributed from the altiplano of Bolivia and Peru to southern Brazil and northern Argentina. *Acicarpha tribuloides* Juss. has also been collected in New Zealand and eastern North America where it was introduced in ballast dumps and never survived and reproduced past the year it was introduced (DeVore, 1991). *Acicarpha* is the genus within Calyceraceae that most resembles Asteraceae in capitular morphology. Like Asteraceae, members of the genus possess capitula that develop centripetally. Other distinctive features of the genus include lateral fusion of the filaments above nectaries and a prominent flared connective base (Pescreta et al., 1994).

*Acicarpha tribuloides* Juss.—Figs. 1a–d.

Grains spheroidal-rhombohedral, 13.0  $\mu\text{m}$   $\times$  12.5  $\mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions strongly concaved; intercolpar ledges present; bridge over pore pronounced; colpi 10.4  $\mu\text{m}$   $\times$  0.9  $\mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium 2.5  $\mu\text{m}$   $\times$  6.0  $\mu\text{m}$ , lalongate, lateral ends acute.

Exine 1.1  $\mu\text{m}$  at equator; foot layer-endexine 0.4  $\mu\text{m}$ ; columellae distally branched, 0.19  $\mu\text{m}$  wide, 0.55–0.90  $\mu\text{m}$  high; tectum thickness 0.15  $\mu\text{m}$ ; surface spinulate, 0.15  $\mu\text{m}$  high, 0.4  $\mu\text{m}$  wide.

*Boopis*. A genus of annuals and perennials ranging from central Chile and northern Argentina south to Tierra del Fuego. Capitula consist of cymose subunits and achenes characterized by calycine lobes of equal length, and indicated here and elsewhere (i.e., Patel, 1976; Hellwig, 2007) the genus has dimorphic pollen.

*Boopis gracilis* Phil.—Figs. 1e–h, 6a.

Grains spheroidal, 18.9  $\mu\text{m}$   $\times$  18.8  $\mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat; intercolpar ledges present; bridge over pore; colpi 14.2  $\mu\text{m}$   $\times$  1.0  $\mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium 1.8  $\mu\text{m}$   $\times$  5.2  $\mu\text{m}$ , lalongate, narrowly lenticulate.

Exine 1.2  $\mu\text{m}$  at equator; foot layer-endexine 0.48  $\mu\text{m}$ ; columellae distally branched, 0.2  $\mu\text{m}$  wide, 0.52–0.90  $\mu\text{m}$  high; tectum thickness 0.2  $\mu\text{m}$ ; surface spinulate, 0.2  $\mu\text{m}$  high, 0.4  $\mu\text{m}$  wide.

*Boopis graminea* Phil.—Figures 1i–L.

Grains subspheroidal, 22.3  $\mu\text{m}$   $\times$  18.3  $\mu\text{m}$ .

Tricolporate; intercolpar regions and ledges absent; bridge over pore absent; colpi 16.3  $\mu\text{m}$   $\times$  3.0  $\mu\text{m}$ , furrow nearly same width with rounded ends; endocolpium 2.0  $\mu\text{m}$   $\times$  8.0  $\mu\text{m}$ , lalongate, narrowly lenticulate.

Exine 1.7  $\mu\text{m}$  at equator; foot layer-endexine 0.44  $\mu\text{m}$ ; columellae distally branched, 0.3  $\mu\text{m}$  wide, 1.1  $\mu\text{m}$  high; tectum thickness 0.16  $\mu\text{m}$ ; surface spinulate, 0.23  $\mu\text{m}$  high, 0.4  $\mu\text{m}$  wide.

*Calycera*. A genus of annuals and perennials with a principal distribution

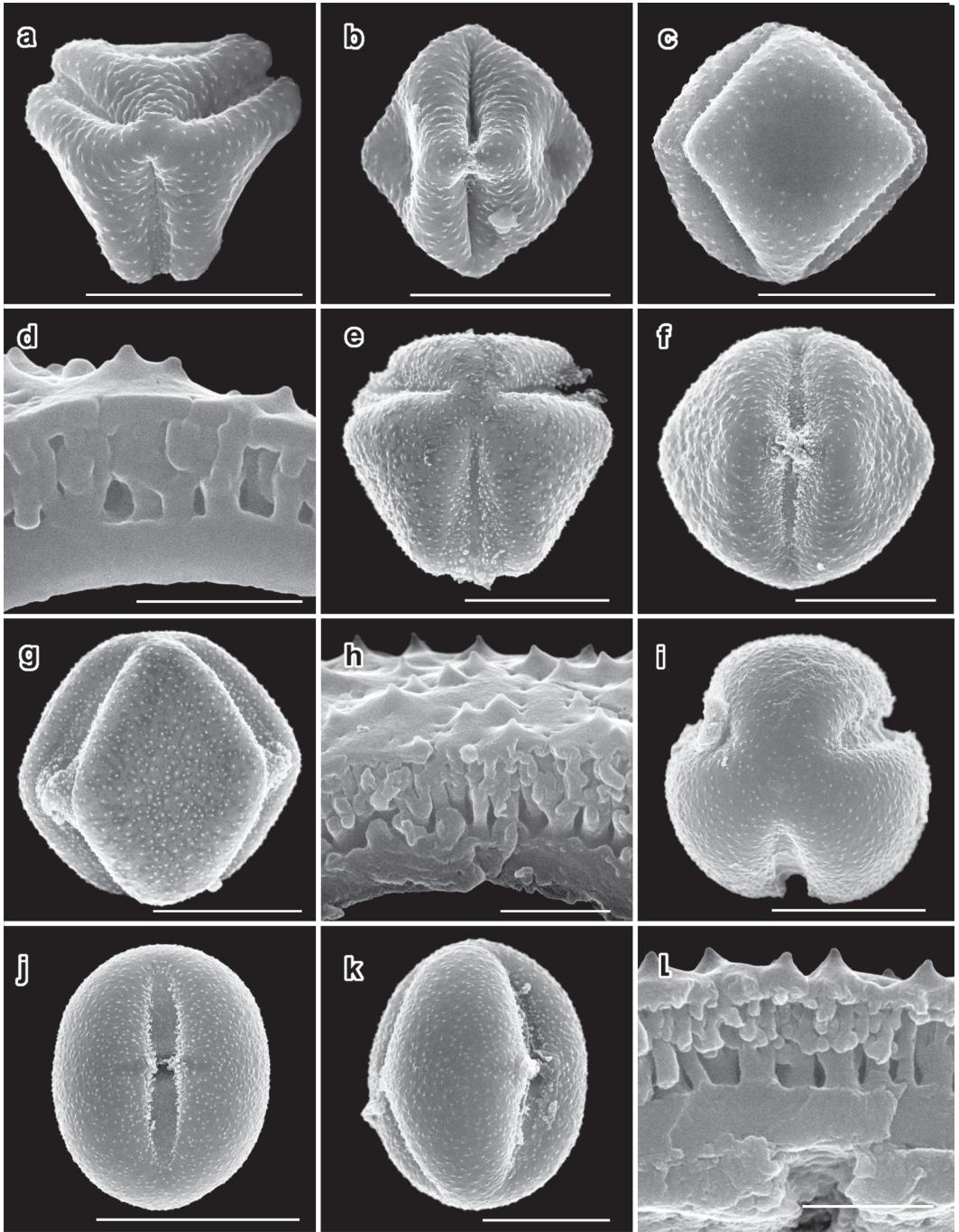


FIG. 1. a–L. SEMs of Calyceraceae pollen. Scale bars for whole pollen grains = 10  $\mu\text{m}$ ; for fractured grains scale bars = 1  $\mu\text{m}$ . a–d. *Acicarpha tribuloides*; e–h. *Boopis gracilis*; i–L. *B. graminea*.



along the Andes of Argentina and Chile. *Calycera* consists of two distinct groups of species: Argentinean taxa (with the exception of *C. herbacea* Cav.) with chromosome numbers of  $n = 21$  and anthers lacking tails; Chilean species (with the exception of *C. pulvinata* Remy) consisting of taxa with variable chromosome numbers and anthers with distinct tails (DeVore, 1994). Capitula consist of cymose subunits. The genus has been delimited from other genera within Calyceraceae based on the presence of dimorphic achenes (achenes with different calycine lobe lengths).

*Calycera calcitrapa* Griseb.—Figs. 2a–d.

Grains spheroidal-rhombohedral,  $16.0 \mu\text{m} \times 16.5 \mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat-slightly concave; intercolpar ledges present; bridge over pore; colpi  $16.3 \mu\text{m} \times 3.0 \mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium  $2.0 \mu\text{m} \times 5.5 \mu\text{m}$ , lalongate, lateral ends acute.

Exine  $1.5 \mu\text{m}$  at equator; foot layer-endexine  $0.4 \mu\text{m}$ ; columellae distally branched,  $0.18 \mu\text{m}$  wide,  $0.9 \mu\text{m}$  high; tectum thickness  $0.2 \mu\text{m}$ ; surface spinulate,  $0.15 \mu\text{m}$  high,  $0.3 \mu\text{m}$  wide.

*Calycera eryngioides* Remy—Figs. 2e–g.

Grains spheroidal-rhombohedral,  $21.6 \mu\text{m} \times 28.0 \mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions strongly concave; intercolpar ledges present and pronounced; bridge over pore; colpi  $18.0 \mu\text{m} \times 2.6 \mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium  $2.8 \mu\text{m} \times 6.0 \mu\text{m}$ , lalongate, lateral ends rounded.

Exine  $2.1 \mu\text{m}$  at equator; foot layer-endexine  $0.82 \mu\text{m}$ ; columellae distally branched,  $0.2 \mu\text{m}$  wide,  $1.1 \mu\text{m}$  high; tectum thickness  $0.2 \mu\text{m}$ ; surface spinulate,  $0.05 \mu\text{m}$  high,  $0.15 \mu\text{m}$  wide.

*Calycera herbacea* Cav.—Figs. 2h–k, 6b.

Grains spheroidal-rhombohedral,  $17.6 \mu\text{m} \times 20.8 \mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat-slightly concave; inter-

colpar ledges present; bridge over pore; colpi  $15.6 \mu\text{m} \times 3.6 \mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium  $2.2 \mu\text{m} \times 7.2 \mu\text{m}$ , lalongate, lateral ends acute.

Exine  $1.5 \mu\text{m}$  at equator; foot layer-endexine  $0.44 \mu\text{m}$ ; columellae distally branched,  $0.24 \mu\text{m}$  wide,  $0.9 \mu\text{m}$  high; tectum thickness  $0.16 \mu\text{m}$ ; surface spinulate,  $0.2 \mu\text{m}$  high,  $0.35 \mu\text{m}$  wide.

*Calycera involucrata* Phil.—Figs. 2L, 3a–c.

Grains spheroidal-rhombohedral,  $17.6 \mu\text{m} \times 19.8 \mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat-slightly concave; intercolpar ledges present; bridge over pore; colpi  $15.2 \mu\text{m} \times 3.8 \mu\text{m}$ , furrow nearly same width with rounded ends; endocolpium  $2.0 \mu\text{m} \times 6.0 \mu\text{m}$ , lalongate, lateral ends acute.

Exine  $1.4 \mu\text{m}$  at equator; foot layer-endexine  $0.5 \mu\text{m}$ ; columellae distally branched,  $0.22 \mu\text{m}$  wide,  $0.75 \mu\text{m}$  high; tectum thickness  $0.15 \mu\text{m}$ ; surface spinulate,  $0.15 \mu\text{m}$  high,  $0.35 \mu\text{m}$  wide.

*Calycera leucanthema* Kuntze—Figs. 3d–f.

Grains spheroidal-rhombohedral,  $20.0 \mu\text{m} \times 23.0 \mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions strongly concave; intercolpar ledges present and pronounced; bridge over pore pronounced; colpi  $17.7 \mu\text{m} \times 2.4 \mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium  $3.1 \mu\text{m} \times 7.5 \mu\text{m}$ , lalongate, lateral ends rounded.

Exine  $0.9 \mu\text{m}$  at equator; foot layer-endexine  $0.25 \mu\text{m}$ ; columellae distally branched,  $0.17 \mu\text{m}$  wide,  $0.5 \mu\text{m}$  high; tectum thickness  $0.15 \mu\text{m}$ ; surface spinulate,  $0.1 \mu\text{m}$  high,  $0.3 \mu\text{m}$  wide.

*Calycera spinulosa* Gilles ex Miers.—Figs. 3g–j.

Grains spheroidal-rhombohedral,  $20.0 \mu\text{m} \times 22.7 \mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat-slightly concave; intercolpar ledges present; bridge over pore; colpi

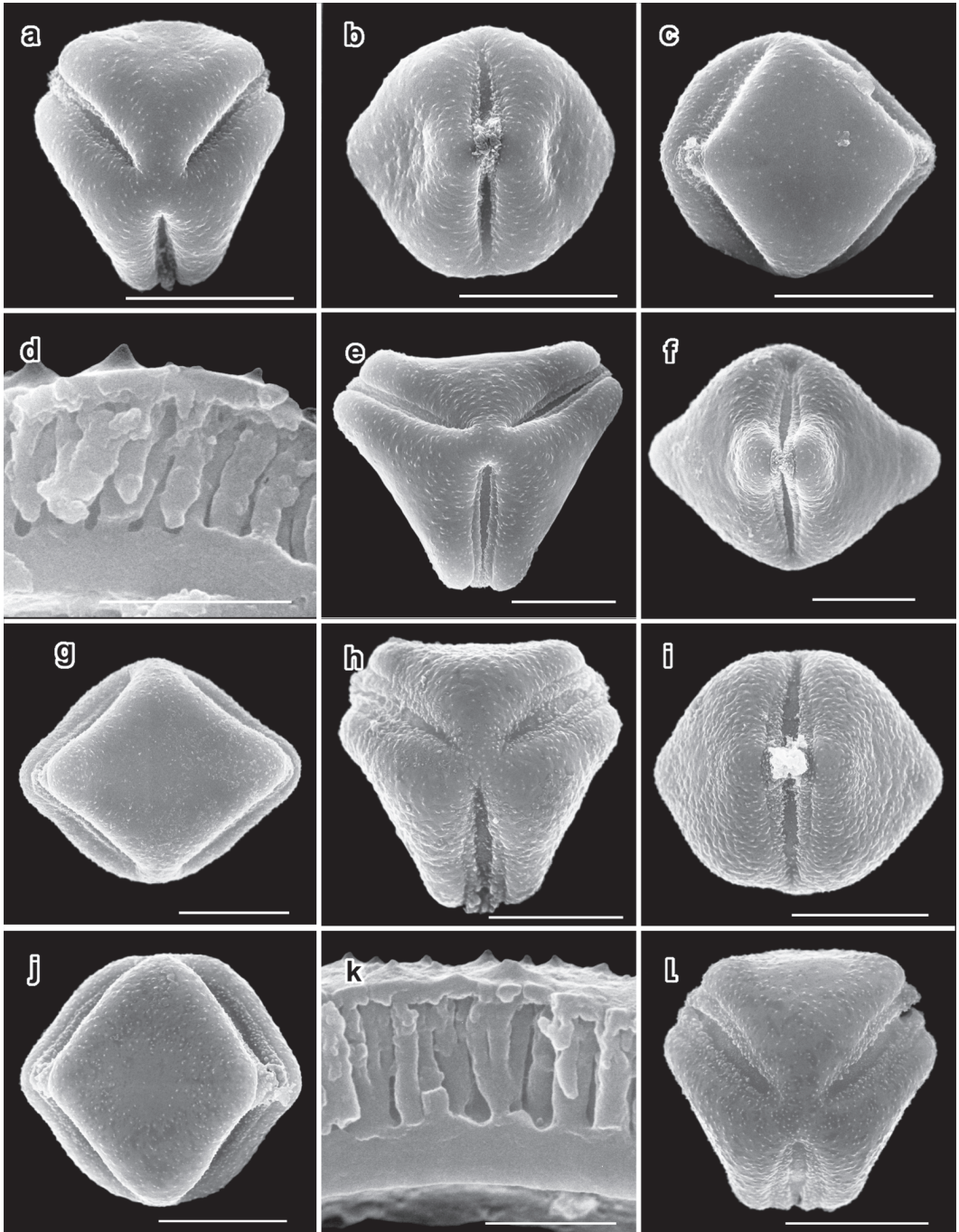


FIG. 2. a–l. SEMs of Calyceraceae pollen. Scale bars for whole pollen grains = 10  $\mu$ m; for fractured grains scale bars = 1  $\mu$ m. a–d. *Calycera calcitrapa*; e–g. *C. eryngioides*; h–k. *C. herbacea*; l. *C. involucrata*.

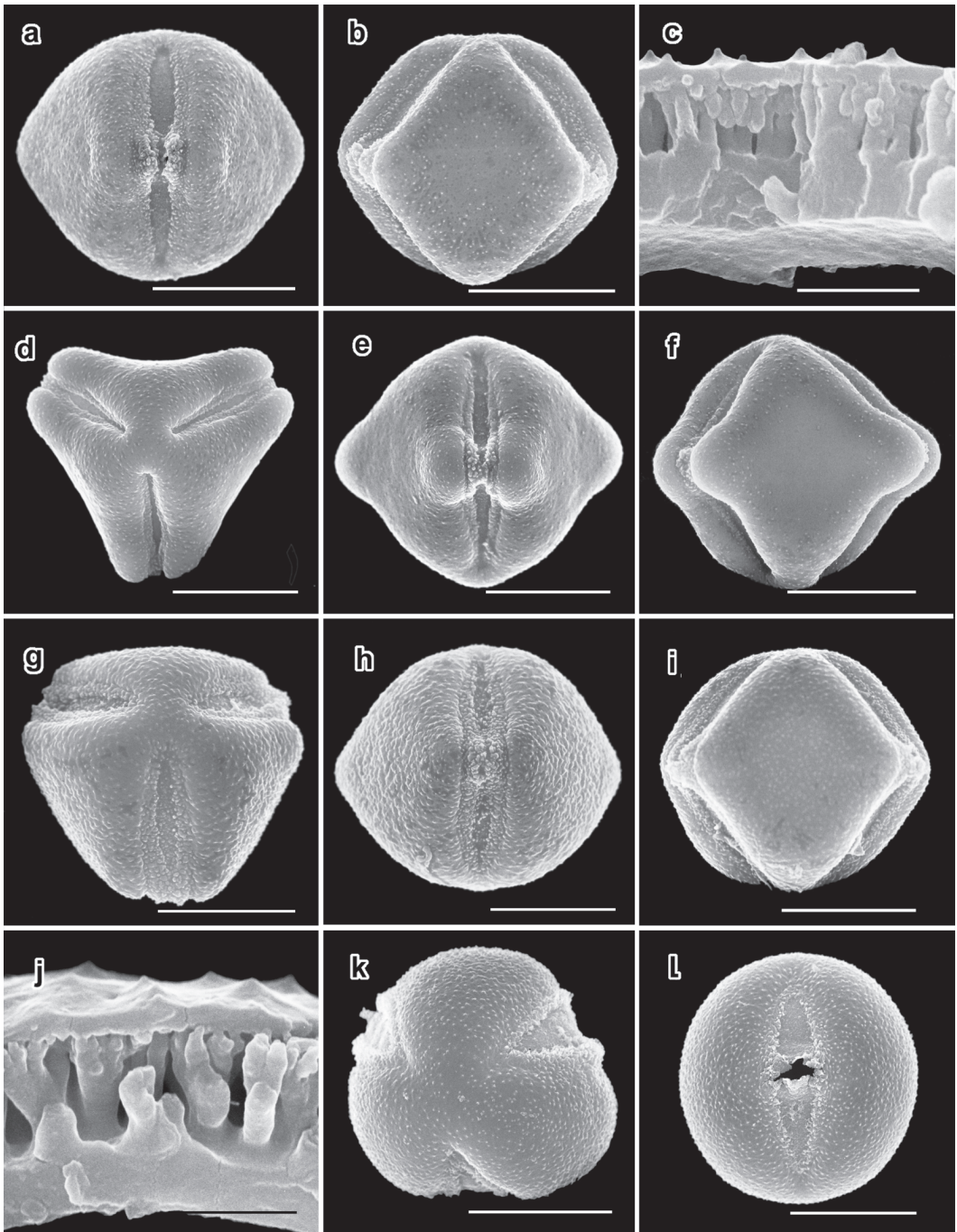


FIG. 3. a–l. SEMs of Calyceraceae pollen. Scale bars for whole pollen grains = 10  $\mu\text{m}$ ; for fractured grains scale bars = 1  $\mu\text{m}$ . a–c. *Calycera involucrata*; d–f. *C. leucanthera*; g–j. *C. spinulosa*; k, l. *Gamocarpha alpina*.



17.3  $\mu\text{m}$   $\times$  3.6  $\mu\text{m}$ , furrow nearly same width with rounded ends; endocolpium 1.8  $\mu\text{m}$   $\times$  7.2  $\mu\text{m}$ , lalongate, lateral ends acute.

Exine 1.52  $\mu\text{m}$  at equator; foot layer-endexine 0.4  $\mu\text{m}$ ; columellae distally branched, 0.2  $\mu\text{m}$  wide, 0.9  $\mu\text{m}$  high; tectum thickness 0.22  $\mu\text{m}$ ; surface spinulate, 0.18  $\mu\text{m}$  high, 0.35  $\mu\text{m}$  wide.

*Gamocarpha*. A genus of rhizomatous perennials of Andean regions of southern Argentina and Chile. Capitula are composed of heads consisting of cymes surrounded by fused pales.

*Gamocarpha alpina* (Poepp. ex Less.) H. V. Hansen—Figs. 3k, L; 4a, b; 6c.

Grains spheroidal, 20.0  $\mu\text{m}$   $\times$  18.5  $\mu\text{m}$ .

Tricolporate; intercolpar regions and ledges absent; bridge over pore absent; colpi 14.6  $\mu\text{m}$   $\times$  4.2  $\mu\text{m}$ , furrow ends pointed; endocolpium 4.0  $\mu\text{m}$   $\times$  8.0  $\mu\text{m}$ , lalongate, lateral ends acute.

Exine 1.9  $\mu\text{m}$  at equator; foot layer-endexine 0.5  $\mu\text{m}$ ; columellae distally branched, 0.3  $\mu\text{m}$  wide, 1.3  $\mu\text{m}$  high; tectum thickness 0.1  $\mu\text{m}$ ; surface spinulate, 0.1  $\mu\text{m}$  high, 0.2  $\mu\text{m}$  wide.

*Moschopsis*. A genus of perennial herbs extending from the Andes of Argentina and Chile to Tierra del Fuego. The only character that delimits the genus is the absence of a clearly defined involucre.

*Moschopsis* sp.—Figs. 4c–e, 6d.

Grains spheroidal, 17.5  $\mu\text{m}$   $\times$  17.3  $\mu\text{m}$ .

Tricolporate; intercolpar regions and ledges absent; bridge over pore absent; colpi 14.2  $\mu\text{m}$   $\times$  2.2  $\mu\text{m}$ , furrow nearly same width with rounded ends; endocolpium 2.9  $\mu\text{m}$   $\times$  7.2  $\mu\text{m}$ , lalongate, broadly lenticulate.

Exine 1.6  $\mu\text{m}$  at equator; foot layer-endexine 0.4  $\mu\text{m}$ ; columellae distally branched, 0.26  $\mu\text{m}$  wide, 1.0  $\mu\text{m}$  high; tectum thickness 0.2  $\mu\text{m}$ ; surface spinulate, 0.1  $\mu\text{m}$  high, 0.2  $\mu\text{m}$  wide.

*Nastanthus*. This unusual genus, once described as appearing like a cauliflower (Miers, 1860), extends from the northern to southern Andean ranges of Chile and

Argentina. The generic delimitation between *Nastanthus* and *Boopis* has been unclear. The only noted difference between the two genera has been the size of the receptacle.

*Nastanthus andina* (Miers) H. V. Hansen—Figs. 4f–h.

Grains spheroidal-rhombohedral, 23.5  $\mu\text{m}$   $\times$  25.5  $\mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat to slightly concave; intercolpar ledges present; bridge over pore; colpi 20.2  $\mu\text{m}$   $\times$  4.1  $\mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium 3.1  $\mu\text{m}$   $\times$  7.0  $\mu\text{m}$ , lalongate, lateral ends acute.

Exine 1.85  $\mu\text{m}$  at equator; foot layer-endexine 0.41  $\mu\text{m}$ ; columellae distally branched, 0.25  $\mu\text{m}$  wide, 1.25  $\mu\text{m}$  high; tectum thickness 0.19  $\mu\text{m}$ ; surface spinulate, 0.18  $\mu\text{m}$  high, 0.32  $\mu\text{m}$  wide.

*Nastanthus spathulatus* Miers.—Figs. 4i–L.

Grains spheroidal-rhombohedral, 20.0  $\mu\text{m}$   $\times$  19.0  $\mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions flat slightly concave; intercolpar ledges present; bridge over pore; colpi 15.5  $\mu\text{m}$   $\times$  5.2  $\mu\text{m}$ , furrow nearly same width with pointed ends; endocolpium 3.0  $\mu\text{m}$   $\times$  7.5  $\mu\text{m}$ , lalongate, lateral ends acute.

Exine 1.2  $\mu\text{m}$  at equator; foot layer-endexine 0.5  $\mu\text{m}$ ; columellae distally branched, 0.22  $\mu\text{m}$  wide, 0.6  $\mu\text{m}$  high; tectum thickness 0.1  $\mu\text{m}$ ; surface spinulate, 0.1  $\mu\text{m}$  high, 0.2  $\mu\text{m}$  wide.

#### Campanulaceae

*Campanula rotundiflora* L.—Figs. 5a–b.

Grains spheroidal, 32.2  $\mu\text{m}$   $\times$  30.6  $\mu\text{m}$ .

Porate with at least 4 rounded pores; exine 0.67  $\mu\text{m}$ ; highly reduced columellae; surface entirely spinate, spines 2.2  $\mu\text{m}$  high and 0.9  $\mu\text{m}$  wide.

#### Goodeniaceae

*Goodenia ovata* Sm.—Figs. 5c–f.

Grains spheroidal, 23.4  $\mu\text{m}$   $\times$  24.7  $\mu\text{m}$ .

Tricolporate and angulaperturate; intercolpar regions convex; intercolpar ledges absent; bridge over pore absent; colpi 16.8  $\mu\text{m}$   $\times$  2.6  $\mu\text{m}$ , furrow ends rounded;



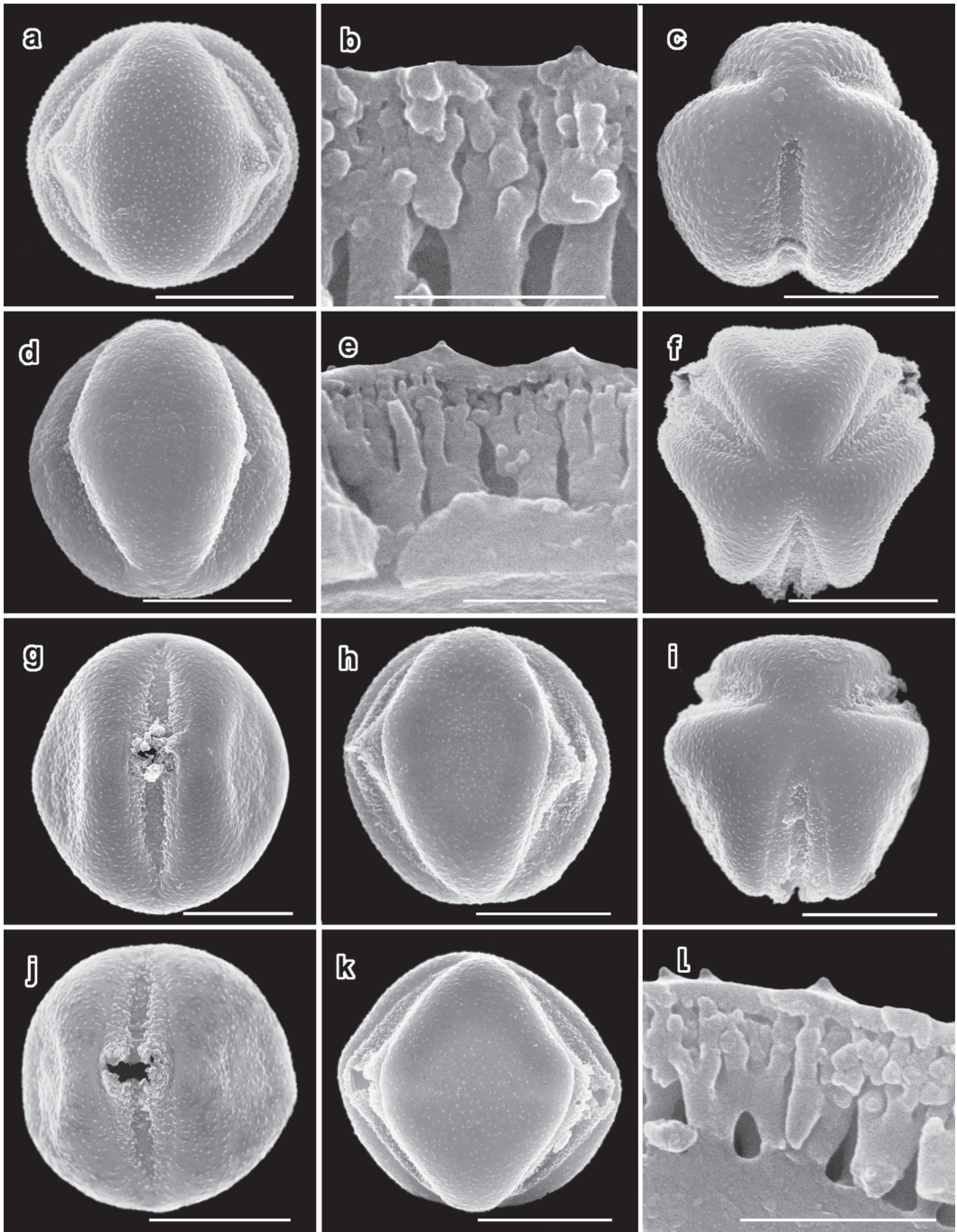


FIG. 4. a–L. SEMs of Calyceraceae pollen. Scale bars for whole pollen grains = 10  $\mu\text{m}$ ; for fractured grains scale bars = 1  $\mu\text{m}$ . a, b. *Gamocarpha alpina*; c–e. *Moschopsis* sp.; f–h. *Nastanthus andina*; i–L. *N. spathulatus*.

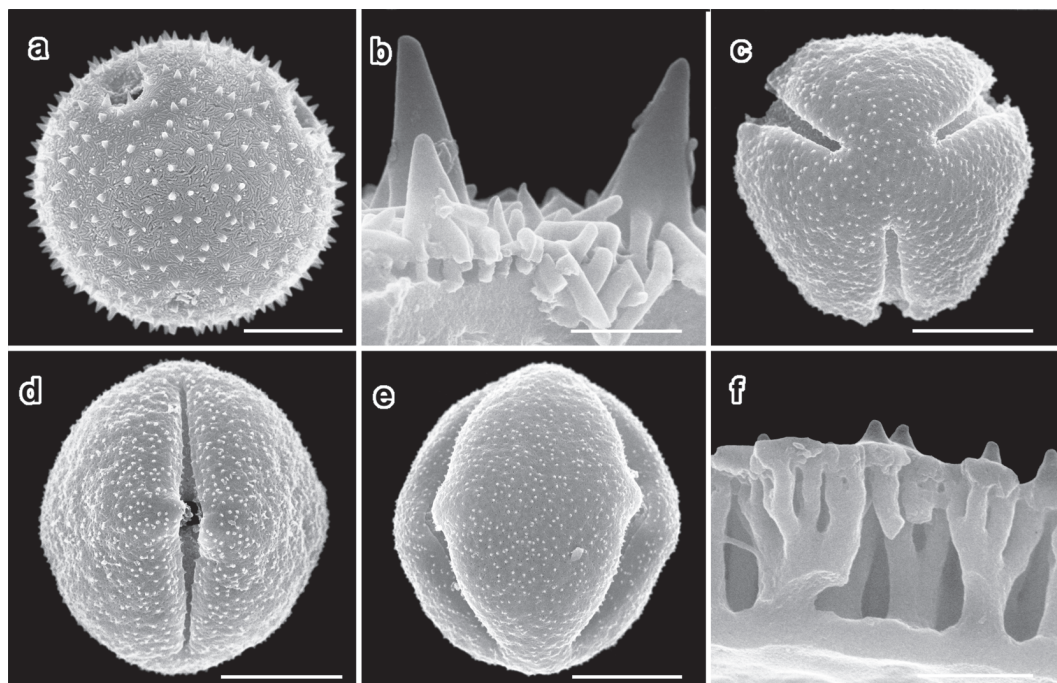


FIG. 5. a–f. SEMs of Campanulaceae (a, b) and Goodeniaceae (c–f) pollen. Scale bars for whole pollen grains = 10  $\mu\text{m}$ ; for fractured grains scale bars = 1  $\mu\text{m}$ . a, b. *Campanula rotundiflora*; c–f. *Goodenia ovata*.

endocolpium 2.8  $\mu\text{m}$   $\times$  9.6  $\mu\text{m}$ , lalongate, narrowly lenticulate.

Exine 1.8  $\mu\text{m}$  at equator; foot layer-endexine 0.35  $\mu\text{m}$ ; columellae distally branched, 0.35  $\mu\text{m}$  wide, 1.2  $\mu\text{m}$  high; tectum thickness 0.25  $\mu\text{m}$ ; surface spinulate, 0.3  $\mu\text{m}$  high, 0.45  $\mu\text{m}$  wide.

#### DISCUSSION

MAJOR FEATURES OF POLLEN MORPHOLOGY OF CALYCERACEAE. Pollen Types: Pollen of three surveyed taxa (*Gamocarpha*, *Moschopsis* and *Boopis graminea*) is very similar in several aspects: nearly rounded shape, no pouch (ektexine bridge), no intercolpar concavities and no intercolpar ledges. We can designate these three taxa as possessing *Gamocarpha* type pollen, equivalent to *Moschopsis* type of Avetisian (1980). The *Gamocarpha* type may represent the ancestral pollen type of the family based on

trends observed in Barnadesioideae and Goodeniaceae and interpreted in the context of phylogenetic studies (Gustafsson et al., 1996; Urtubey and Telleria, 1998; Zhao et al., 2000; Lundberg and Bremer, 2003; Hellwig, 2007).

A second pollen type is represented by four taxa examined during our survey. These taxa, *Acicarpha tribuloides* and three species of Chilean *Calycera*, herein called *Acicarpha* type (equivalent to Avetisian's (1980) *Calycera* type), share several derived characters: pronounced pouches (ektexine bridges), intercolpar ledges, and pronounced intercolpar concavities. The degree of development of these features is not as great in other species, including Argentinean species of *Calycera*. These taxa have slightly flat intercalary areas, more or less ektexine bridges and some weakly developed intercolpar ledges. Because some species of *Calycera* fall into this category, we elect to refer to the

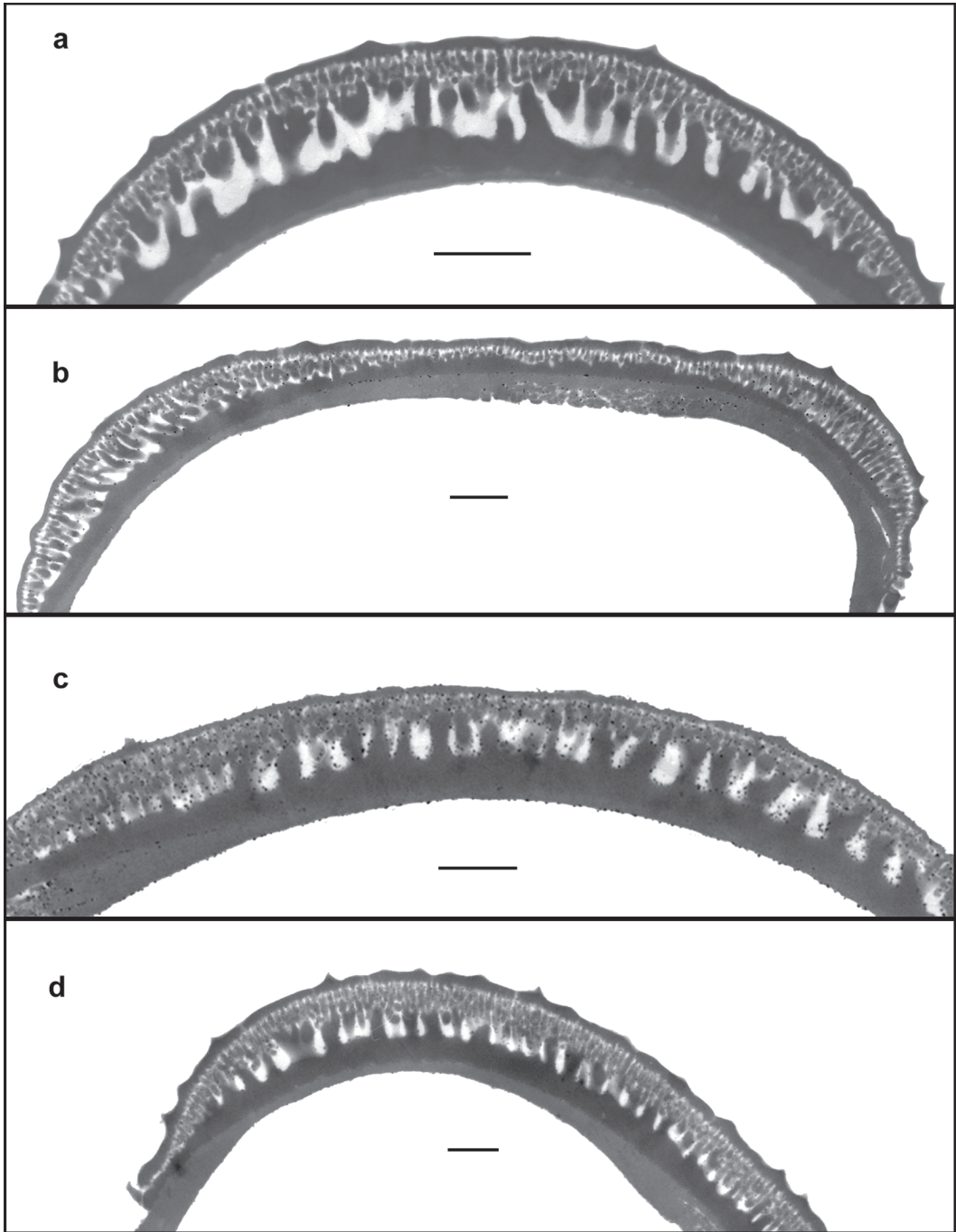


FIG. 6. a–d. TEMs of Calyceraceae pollen. Scale bars = 1  $\mu\text{m}$ . **a.** *Boopis gracilis*; **b.** *Calycera herbacea*; **c.** *Gamocarpha alpina*; **d.** *Moschopsis* sp.



pollen type with strongly developed, derived characters, as the *Acicarpha* type.

Intercolpar concavities: Skvarla et al. (1977) first noted that Asteraceae and Calyceraceae possess intercolpar concavities, and this is one of the major reasons that they suggested a close relationship between the two families. Intercolpar concavities were first described by Wodehouse (1928) in six species of *Chuquiraga*. His original description of these species was that they "... are unique in the possession of three distinct concavities appearing as the impressions one could make with the thumb in a ball of soft dough. Since these impressions are between furrows, I shall call them intercolpar concavities, and their position on the equator suggests the further designation of equatorial concavities. They have not been found elsewhere in the grains of either the Mutisieae or any other tribes of the Compositae except *Schlectendalia*." These concavities vary from simple concavities of Wodehouse to prominent depressions of Urtubey and Telleria (1998). Contemporary studies have shown that intercolpar concavities characterize *Dasyphyllum*, *Schlectendalia*, and some species of *Arnaldoa* (Skvarla et al., 1977; Urtubey and Telleria, 1998; Zhao et al., 2000; Telleria and Katinas, 2005). The terminology of Wodehouse (1928) has never found its way into any pollen glossaries or dictionaries, including the glossary in Wodehouse's 1935 book, which explained most palynological terms at that time. This may be the reason why few pollen studies of Calyceraceae did not specifically recognize these features (Erdtman, 1952; Auer et al., 1955), although they appeared in some LMs (Markgraf and D'Antoni, 1978; Wingenroth and Heusser, 1984). Perhaps unaware of Wodehouse's early description, Heusser (1971) described them for *Calycera eryngioides* as being "pinched in the middle to appear in the form of a bow." Heusser (1971) also presented clear photos of intercolpar concavities in *Boopis leucanthema* Poepp. in Less. (= *Calycera leucanthema* (Poepp. in Less.) Kuntze) and *B. pusilla* Phil. In contrast,

his descriptions of *Moschopsis monocephala* Reiche, *Gamocarpha poeppigii* DC. and *Nastanthus scapigera* Miers did not indicate their presence. The most definitive work on these structures is credited to Hansen (1992). Examining unacetolyzed pollen with SEM he recorded intercolpar concavities in four species of *Acicarpha*, three of seven species of *Boopis*, five of eight species of *Calycera*, and two of three species of *Nastanthus*. *Gamocarpha* was the only taxon uniformly lacking them. Most recently, intercolpar concavities were recognized as mesocolpal depressions in SEMs of *Boopis anthemoides* Juss. and *Moschopsis rosulata* (N. E. Br.) Dusén, several members of Goodeniaceae (*Goodenia scapigera* R. Br., *G. ovata* Sm., *Pentaptilon*, *Scaevola*, *Selliera*, *Velleia*, *Verreauxia*) (Gustafsson et al., 1997), and in *Dasyphyllum* (Urtubey and Telleria, 1998; Zhao et al., 2000).

Ektexine bridges: An ektexine bridge is an extension of the exine that projects over the pore. This feature has been observed in several groups of Asteridae including Lythraceae (Graham et al., 1985) and Boraginaceae (Nowicke and Skvarla 1974). This feature, which is a later stage of a common trend found within Asteridae, is likely derived within Calyceraceae, and there is a trend from slight (*Gamocarpha*) to well developed ektexine bridges within the Chilean species of *Calycera* and *Acicarpha*.

Colpar ledges: Colpar ledges are ridges of exine that line the inner margin of the colpi. This feature is found in both Calyceraceae and Goodeniaceae. The function of colpar ledges may be related to harmomegathy. Wodehouse (1935) showed that colpi function in accommodating volume changes as pollen passes out of the humid anther to the dry air. Bolick (1978) hypothesized that the evenly spaced colpi enable round pollen grains to approach a triangular perimeter following water loss. This triangular cross section would be the most stable cross section and would prevent further collapse of pollen. Prominent colpar ledges might prevent



additional water loss by sealing off the colpi before shrinkage reduces the diameter of the pollen grain to the extent that the colpar ledges are in contact.

GENERAL POLLEN CHARACTERISTICS OF GOODENIACEAE, CAMPANULACEAE AND SIMILARITIES WITH CALYCERACEAE: Goodeniaceae consist of about 14 genera and 300 species and exhibit a wide range of pollen variation (Patel, 1976; Skvarla et al., 1977; Gustafsson et al., 1997; Polevova, 2006). Duigan (1961) surveyed 39 species from six genera (*Brunonia*, *Dampiera*, *Goodenia*, *Scaevola*, *Selliera*, and *Velleia*) within the family using light microscopy with Erdtman (1952) and Avetisian (1973) providing additional but more limited LM observations. Skvarla et al. (1977) selected nine species from six genera (*Anthotium*, *Brunonia*, *Dampiera*, *Goodenia*, *Scaevola*, and *Velleia*) for observation using SEM and TEM. Our summary of the overall pollen morphology for use in comparison with Calyceraceae is based on these two studies and the new data generated in this paper.

Most pollen of Goodeniaceae is tricolporate, however grains with two, four, or more colpi, as well as syncolpate grains, are found within the family (Duigan, 1961). When grains have more than three colpi, Duigan (1961) noted that these pollen grains were present within species with polyploid races that also produce larger grains. One genus, *Leschenaultia*, has tetrad pollen grains (Skvarla et al., 1977). Based on the overall pollen morphology, Duigan (1961) recognized three pollen types: 1) *Brunonia*; 2) *Dampiera*; and 3) *Goodenia*. *Brunonia*-type pollen is distinct in having colpar ledges (Duigan, 1961). These structures are layers of exine that extend along the edges of the colpi. Endocolpia are sublanceolate alongate. In polar view, *Brunonia*-type pollen appears triangular in shape, while in equatorial view, the grains are prolate-spheroidal in shape. The surface is microspinulose. *Dampiera*-type grains are characterized by non-elongated colpi with irregular outlines. In equatorial view,

the grains appear circular or elliptical, while in polar view the grains are oblate to subprolate in shape with straight, slightly convex, circular, or lobed intercolpar regions. The surface is rugulate. In contrast to the *Dampiera*-type grains, *Goodenia*-type pollen has alongate endocolpia with acute or rounded lateral ends. *Goodenia*-type grains, like those of the *Dampiera*-type, are triangular when viewed at the polar position and exhibit straight-sided colpi. Equatorial views show the grains to be circular-elliptical in shape. Unlike the other pollen types, some species with *Goodenia*-type pollen have colpi margins modified to form bridges over the endoaperture (e.g., *Scaevola ramosissima* (Sm.) K. Krause).

Observations from SEM and TEM (Patel, 1976; Skvarla et al., 1977; Polevova, 2006) were used to determine pollen surface and ultrastructural characteristics of Goodeniaceae pollen grains. Most genera within the family have spinulate surfaces, with two genera (*Dampiera* and *Anthotium*) being characterized by the presence of striae and rugulae. TEM micrographs document a relatively thin endoexine and a thick footwall with large, distally bifurcating columellae (see *Goodenia*, Fig. 5f). This bifurcating pattern can be simple (e.g., *Dampiera*) or of a higher order (e.g., *Brunonia*). In *Scaevola*, the higher order columellae are fused. More recent LM/SEM/TEM studies in the Goodeniaceae (Gustafsson et al., 1997) supported the three groups based on pollen morphology originally established by Duigan (1961) but with an expanded list of taxa. Their *Scaevola-Goodenia* group (= *Brunonia* and *Goodenia* groups of Duigan, 1961), with tricolporate, microspinulose grains having distally branched columellae was indicated by Gustafsson et al. (1997) as showing similarity to Asteraceae and Calyceraceae. Finally, Carolin's review (2007) of Goodeniaceae further supports the pollen data of Duigan (1961) and Gustafsson (1997).

Based on the available pollen data (Duigan, 1961; Skvarla et al., 1977; Gustafsson et al., 1997), as well as phylogenetic analyses based on morphology of Goodeniaceae (Carolin, 1978) and a molecular phylogeny of Asterales that included 12 species of Goodeniaceae (Gustafsson et al., 1996), one can begin to elucidate some trends in pollen morphology of Goodeniaceae. The Goodeniaceae contains two large genera with more than eighty species (*Goodenia* and *Scaevola*) that have not been fully surveyed for pollen variation. Therefore, the extent of pollen variation within these genera is unknown. In Gustafsson et al. (1996), *Goodenia ovata* groups with *Selliera radicans*, and *G. scapigera* forms a clade with *Verreuxia reinwardtii*.

Trends exist within Goodeniaceae that are shared with Calyceraceae. The most evident of these is the presence of colpar ledges in *Brunonia*. Within Calyceraceae, colpar ledges are found in *Acicarpha*, *Calycera*, and *Nastanthus*. Ektexine bridges also have evolved within both Calyceraceae and Goodeniaceae, as well as within other families (e.g., Primulaceae, Nowicke and Skvarla, 1977; Fabaceae, Endo and Ohashi, 1996). Within Calyceraceae, a trend is seen from slight (*Gamocarpha*) to well developed ektexine bridges within the Chilean species of *Calycera* and *Acicarpha*. We hypothesize that such an extension over the pore may function in diminishing water loss. According to Ficks law, evaporation rate depends on different resistances to diffusion (Salisbury and Ross, 1985). Because water molecules diffusing from a pore can travel in any direction, the boundary layer is very thin and the concentration gradient is very steep. The presence of an ektexine bridge may be instrumental in deterring water loss from the pore.

Nowicke and Skvarla (1979) proposed that congruity in wall architecture is the manifestation of long-standing structural similarity dictated by common ancestry. Based on this premise, we suggest particular pollen features (e.g., colpar ledges in both

Calyceraceae and Goodeniaceae), might follow a pathway dictated by common ancestry. In other words, taxa sharing a common ancestry would obtain features via similar, if not nearly identical, series of structural modifications of one particular shared pollen type. Utilizing a trend in pollen morphology as a phylogenetic character proved insightful in previous studies (DeVore et al., 2000) and supports the hypothesis of Nowicke and Skvarla (1979). The question still remains regarding whether intercolpar concavities in Calyceraceae and Asteraceae are a parallelism or a synapomorphy for these families.

A recent survey of pollen morphology and ultrastructure of families closely allied with the Asteraceae, Calyceraceae, and Goodeniaceae clade (Polevova, 2006) indicates that the sister group to this clade, Menyanthaceae and Campanulaceae, share pollen features that are unlike those present within Asteraceae, Calyceraceae, and Goodeniaceae. An examination of Figures 5a and b clearly show that the pollen of Campanulaceae and Menyanthaceae (not figured in the present paper) are distinctive from that of Calyceraceae by having large, unbranched colmellae in the upper exine covered by a thick tectum. Clearly the Campanulaceae-Menyanthaceae pollen type is not comparable to any type present within Calyceraceae or within its closest related families Asteraceae and Goodeniaceae.

Clarifying the trends in pollen morphology for Calyceraceae is valuable because the family contains two distinct pollen types, the less derived *Gamocarpha*-type and the highly derived *Acicarpha* type, as well as intermediate forms between these two types. In essence, the entire pathway of modifications of the pollen ground plan for the Goodeniaceae, Calyceraceae, and Asteraceae clade appears to be present within Calyceraceae (Table 2). Trends in pollen morphology within Calyceraceae also are useful in helping to provide a search image for paleopalynologists seeking to document the first appearance of Asteraceae in the fossil

TABLE 2. Summary of pollen features and trends of Goodeniaceae, Calyceraceae and Barnadesioideae. Data from Duigan (1961), Patel (1976), Skvarla et al. (1977), Avetisian (1980), Hansen (1992), Gustafsson et al. (1997), Urtubey and Telleria (1998), Zhao et al. (2000), and current study.

	Goodeniaceae	Calyceraceae	Barnadesioideae
Exine	Thickened columellae bifurcating distally, do not extend through entire exine, thick footwall reticulate, spongy network absent	Thickened columellae bifurcating distally, do not extend through entire exine, thick footwall reticulate, spongy network absent	Thickened columellae bifurcating distally, some extend through entire exine, thin footwall; reticulate, spongy network present
Colpar ledges absent to present	Present only in <i>Brunonia</i>	Present in <i>Acicarpha</i> , <i>Calycera</i> , <i>Nastanthus</i> , and some members of <i>Boopis</i>	Absent
Ektexine bridges absent to slight to well developed	Present within family	Present in <i>Acicarpha</i> , some members of <i>Boopis</i> , <i>Calycera</i> , and <i>Nastanthus</i>	Absent
Intercolpar concavities convex to flattened (mesocolpar depressions) to present	Present in several members of Goodeniaceae	Present in <i>Acicarpha</i> and some members of <i>Boopis</i> , <i>Calycera</i> , and <i>Nastanthus</i>	Present in <i>Dasyphyllum</i> and <i>Schlectendalia</i> , paraporal depressions in <i>Arnaldoa</i> and <i>Schlectendalia</i>

record using light microscopy. One would predict that the ancestral lineage potentially possessed grains similar to what we describe as the *Gamocarpha* type (nearly rounded shape, no ektexine bridge, no intercolpar concavities and no intercolpar ledges).

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