

ULTRASTRUCTURE OF HETEROCOLPATE POLLEN IN *CRYPTANTHA* (BORAGINACEAE)

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Pollen of eight species of *Cryptantha* was studied using scanning electron microscopy. In addition, transmission electron microscopy was used in *Cryptantha intermedia* to elucidate the ultrastructural basis for their heterocolpate pollen, in which three “true” colporate apertures alternate with three “pseudoapertures.” Both apertures and pseudoapertures are regions of the wall in which ektexine is largely absent. However, apertures have an outer band of verrucate exinous deposits, a central region of inner intine wall material, and a concentration of cytoplasmic vesicles. In addition, apertures are shorter in length and wider at the equator than are pseudoapertures. The exine wall structure in *C. intermedia* consists of a typical inner, homogeneous endexine and, in regions other than apertures and pseudoapertures, an outer, essentially imperforate, tectate-columellate ektexine. Curious triangular “polar pseudoapertures,” devoid of ektexine, occur at each pole and resemble the pseudoapertures in sculpturing and wall structure. The described heterocolpate pollen in the Boraginaceae may represent a major apomorphy for the tribes Eritricheae and Cynoglosseae and possibly other taxa. However, further comparative studies within these groups are needed to assess definitively the extent of this putative apomorphy and to identify and characterize palynological features that may be useful within these groups.

Keywords: Boraginaceae, *Cryptantha*, heterocolpate, palynology, pollen, ultrastructure.

Introduction

Cryptantha is a genus of annual, biennial, or perennial flowering plants with ca. 150 species. The genus is distinctive in that the nutlets have a ventrally located longitudinal groove. Species of the genus are found largely in the American southwest and northern Mexico, with some reported in South America (Johnston 1925; Higgins 1971). *Cryptantha* is classified as a member of the tribe Eritricheae of the Boraginaceae, a large flowering plant family that consists of annual and perennial herbs, shrubs, trees, and some lianas, containing between 100 and 154 genera and between 2000 and 2500 recognized species (Mabberley 1997).

Both Cronquist (1981) and Takhtajan (1997) treat the Boraginaceae broadly (termed here “Boraginaceae, s.l.”) as composed of five subfamilies: Boraginoideae, Cordioideae, Ehretioideae, Heliotropoideae, and Wellstedioideae. The subfamilies are distinguished from one another on the basis of differences in plant habit, gynoeceal morphology, and fruit characters. The Boraginaceae is treated even more broadly by the Angiosperm Phylogeny Group (1998), who included as part of the family not only the traditional subfamilies cited above but also the family Hydrophyllaceae. In another classification, Heywood (1985) recognized only the subfamilies Boraginoideae and Heliotropoideae, classifying the Cordioideae and Ehretioideae together in a separate family, Ehretiaceae. Alternatively, recent evidence supports the treatment of the subfamilies of the Boraginaceae, s.l., as the separate families Boraginaceae, s.s.; Cordiaceae; Ehretiaceae; and Heli-

otropaceae (Böhle and Hilger 1998; Gottschling et al. 2001; note that the Wellstedioideae was not studied by these authors). By this latter classification, the Boraginaceae is essentially equivalent to the subfamily Boraginoideae, termed here “Boraginaceae, s.s.” The Boraginoideae, or Boraginaceae, s.s., has the characteristic feature of a schizocarp, splitting into usually four nutlets. This group is often divided into five tribes—Boragineae, Eritricheae, Lithospermeae, Cynoglosseae, and Echieae—on the basis of differences in nutlet and gynobase morphology (Mabberley 1997).

The Boraginaceae, s.l., are a palynologically heterogeneous family (Erdtman 1969; Nowicke and Miller 1990; Nilsson and Praglowski 1992). From previous studies of the family, it was noted that two tribes, Cynoglosseae and Eritricheae, have a specialized pollen morphology (Sahay 1973; Palacios-Chavez and Quiroz-Garcia 1985; Quiroz-Garcia and Palacios-Chavez 1985; Ahn and Lee 1986; Diez 1994). Pollen grains of these tribes were described as being heterocolpate, consisting of three normal, “true” apertures and three intervening “pseudoapertures.”

The purpose of this study is to evaluate the ultrastructural basis of the heterocolpate pollen morphology in the Eritricheae. Pollen of *Cryptantha intermedia* (A. Gray) E. Greene, a common species widely distributed throughout the American southwest and Baja, California, was examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) in order to describe the ultrastructural morphology. Light microscopic (LM) observations of acetolyzed pollen were also made to examine the extent of the endoaperture. In addition, comparative SEM observations were made on seven other species in the genus. The extremely small size of pollen grains in *Cryptantha* necessitates the use of electron micros-

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copy to study any aspect of morphology in detail. Analysis of the ultrastructural pollen morphology of *Cryptantha* allows an assessment of taxonomic relationships and evolutionary trends within the Eritricheae. We hope to incorporate this base study into a future project of comparative pollen ultrastructure of the Eritricheae and relatives.

Material and Methods

For SEM observations, pollen was either critical-point dried from material fixed in formalin–acetic acid–alcohol (FAA) (*Cryptantha intermedia*), air dried from FAA-fixed material after transfer to 100% ethanol (*Cryptantha micromeres*), or taken directly from dried material (all other species). For critical-point drying, pollen was dehydrated to 100% ethanol, which was used as the intermediate fluid. Material was placed in a metal capsule and critical-point dried with a Tousimis critical-point dryer using pressurized carbon dioxide as the transition fluid. Once dried, all material was transferred onto a stub covered with double-stick tape, sputter-coated with gold/palladium in a Hummer-4 sputtering apparatus, and photographed on a Hitachi S500 scanning electron microscope (20 kV).

For TEM observations, fresh samples of whole anthers of *C. intermedia* were placed in 3% buffered glutaraldehyde overnight, rinsed in 0.1 M sodium cacodylate, and postfixed in 4% osmium tetroxide for 2 h. After water and then 10% ethanol rinses, the specimens were placed in 2% uranyl acetate for 20 min (enblock stain), rinsed in 10% ethanol, dehydrated to 100% ethanol, and transferred to propylene oxide. The specimens were then infiltrated with an eponlike resin (48.75 mL RF-2500 by Resin Formulators, 11.25 mL DDSA, 40 mL NMA, and 1 mL DMP-30), flat embedded, and polymerized at 60°C overnight. Sections 70–90 nm in thickness were prepared with a Dupont diamond knife using an LKB Ultratome and were mounted on uncoated 200-mesh copper grids. The sections were then poststained in 5% uranyl acetate followed by 0.05% lead citrate (5 min each followed by distilled water rinsing) and were viewed with a Zeiss EM 109 transmission electron microscope (80 kV).

Pollen of *C. intermedia* was acetolyzed (nine parts acetic anhydride : one part sulfuric acid) for 10 min in boiling water, stained with basic fuchsin, and mounted in 50% glycerol. Grains were observed and photographed with light microscopy using an oil-immersion objective lens with brightfield optics.

Measurements throughout the article are mean values (with ranges in parentheses) and are limited to available images from TEM and SEM micrographs. Voucher information is as follows: *Cryptantha clevelandii* Greene & Howe 4230 (SDSU 05417); *Cryptantha fastigiata* I. M. Johnston & R. Morgan 8212 (SD 54473); *Cryptantha glomeriflora* Greene & Peirson 11146 (SD 87724); *Cryptantha humilis* (Gray) Payson & B. Ertter 7182 (RSA 516990); *C. intermedia* (Gray) Greene & Burch 7IV95A (SDSU 13866); *Cryptantha jamesii* (Torr.) Payson & D. F. Howe 1621 (SDSU 05423); *C. micromeres* (Gray) Greene & Henry 13 (SDSU 05427); *Cryptantha celosioides* (Eastw.) Payson [*Cryptantha sheldoni*] & Steward 6740 (RSA 101951). Pollen terminology generally follows that of Punt et al. (1994).

Results

Cryptantha intermedia

Pollen grains are shed as monads (fig. 1A, 1B), measuring 5.5 (5.2–5.8) μm along the longer, polar axis. Grains are radial in symmetry and isopolar, perprolate, and hourglass shaped, oblong in equatorial view with rounded ends and a constricted midregion (fig. 1A, 1D; fig. 2A, 2B). In polar view, grains have a circular outline (amb) (figs. 1C, 4B). The equatorial diameter measures 2.4 (2.0–2.5) μm at the constricted midregion; the diameter at the widest point of the rounded ends is 2.8 (2.7–3.0) μm . The polar : equatorial diameter (P : E) ratio is 2.3 (2.1–3.0).

Pollen grains are heterocolpate with three true apertures (also termed simply “apertures”), three alternating pseudoapertures, and two polar pseudoapertures. The apertures are narrowly rhombic in shape with slightly inwardly curved edges (fig. 1A, 1B, 1D) and extend ca. 0.6 (0.5–0.7) times the length of the pollen grain. The exine along the margin of the aperture consists of somewhat discrete baculate to verrucate deposits, giving the margin a toothed appearance (fig. 1D). A band of verrucate exine deposits occurs along the equatorial plane of the apertures (fig. 1A, 1D; fig. 4A). A narrow, transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (fig. 1A).

The three pseudoapertures alternate between and are parallel to the three apertures (fig. 1A, 1D; fig. 4C). Pseudoapertures are nearly linear in shape, slightly wider at the equator, and narrowing to a sharp point near the poles (fig. 1A, 1D). The pseudoapertures extend ca. 0.8 times that of the pollen grain length and are thus slightly longer than the apertures. The exine along the edge of the pseudoapertures also consists of baculate to verrucate deposits (fig. 1D); median deposits, like those of the apertures, are absent. The two polar pseudoapertures, located at both polar ends, are roughly triangular in shape, with the triangular apex oriented in line with the three apertures (fig. 1B, 1C; fig. 2B). Baculate to verrucate deposits are present along the margins of the polar pseudoapertures (fig. 1C), similar to those found in the apertures and pseudoapertures. A single median baculate to verrucate deposit may also occur on the surface near the center of the polar pseudoapertures (fig. 1C).

The pollen wall in the nonapertural region consists of a typical inner intine layer and outer exine layer (figs. 3A, 4A). The intine is relatively electron translucent and is only 3 (1–10) nm thick in the nonapertural region but much thicker at the apertures. The exine of the nonapertural region consists of an inner endexine layer that is 23 (9–82) nm thick and an outer ektexine layer 83 (64–104) nm thick (fig. 3A), thus having an average total exine thickness of 106 nm. The ektexine is tectate columellate, consisting of an inner foot layer (15–18 nm thick), central columellae (16–50 nm thick), and an outer, mostly imperforate tectum (18–24 nm thick) (fig. 3A); perforations in the tectum occur only rarely (fig. 3B). The endexine, foot layer, and tectum are of a similar homogeneous density; the endexine below the foot layer is slightly more electron dense (fig. 3A). Electron-dense deposits are located in the columellar interstitial layer (fig. 3A, 3B, 3D); these deposits are found in greater concentration toward the polar ends and appear absent near

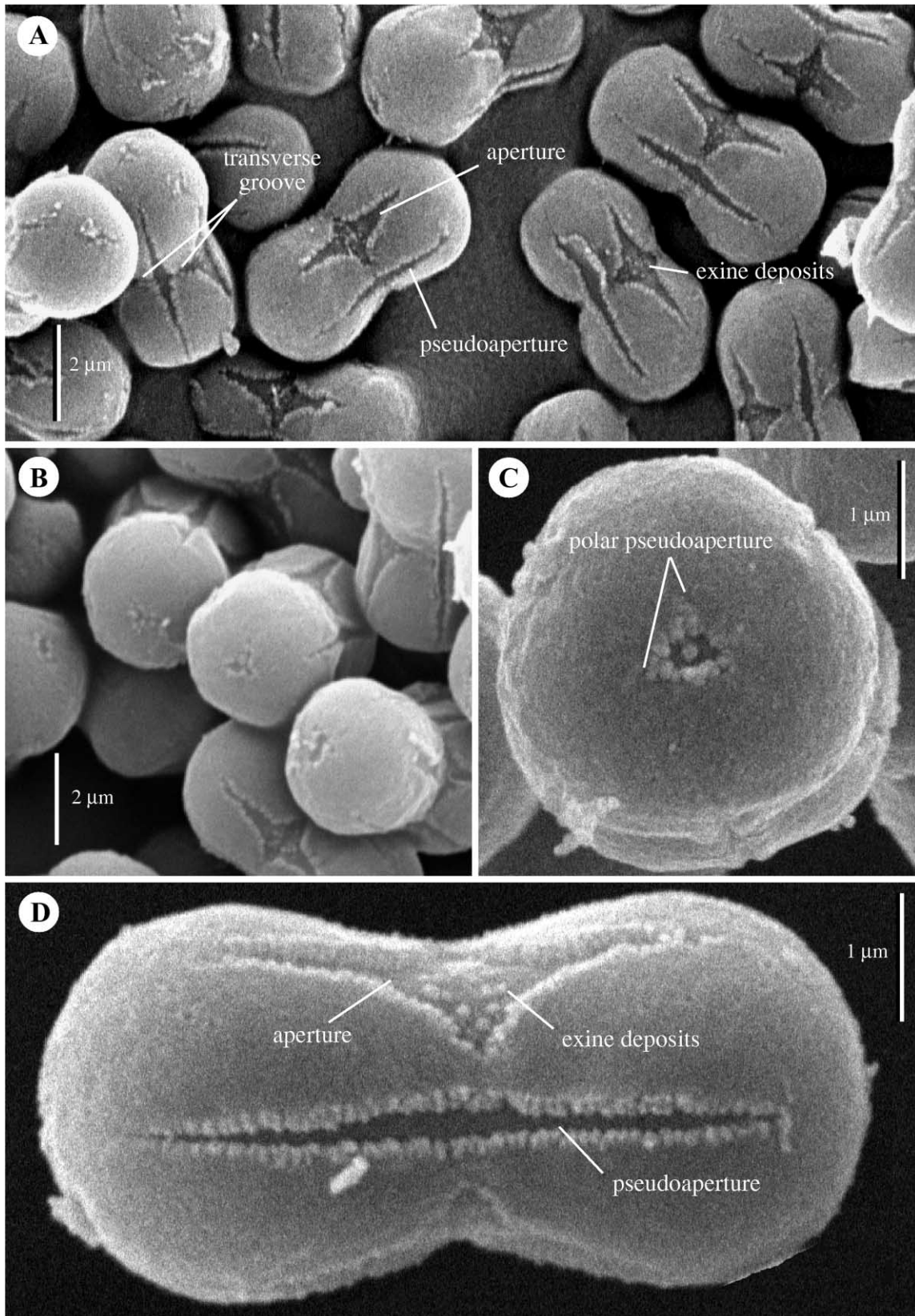


Fig. 1 *Cryptantha intermedia* pollen (SEMs). *A*, Whole grains, mostly equatorial view. Note apertures, with outer exine deposits, and pseudoapertures lacking such deposits; also note transverse groove running between apertures and pseudoapertures. *B*, Grains in polar view. *C*, Polar view, close-up, showing triangular-shaped polar pseudoaperture with central exine deposit. *D*, Single grain in equatorial view, showing aperture and pseudoaperture, the former with outer exine deposits.

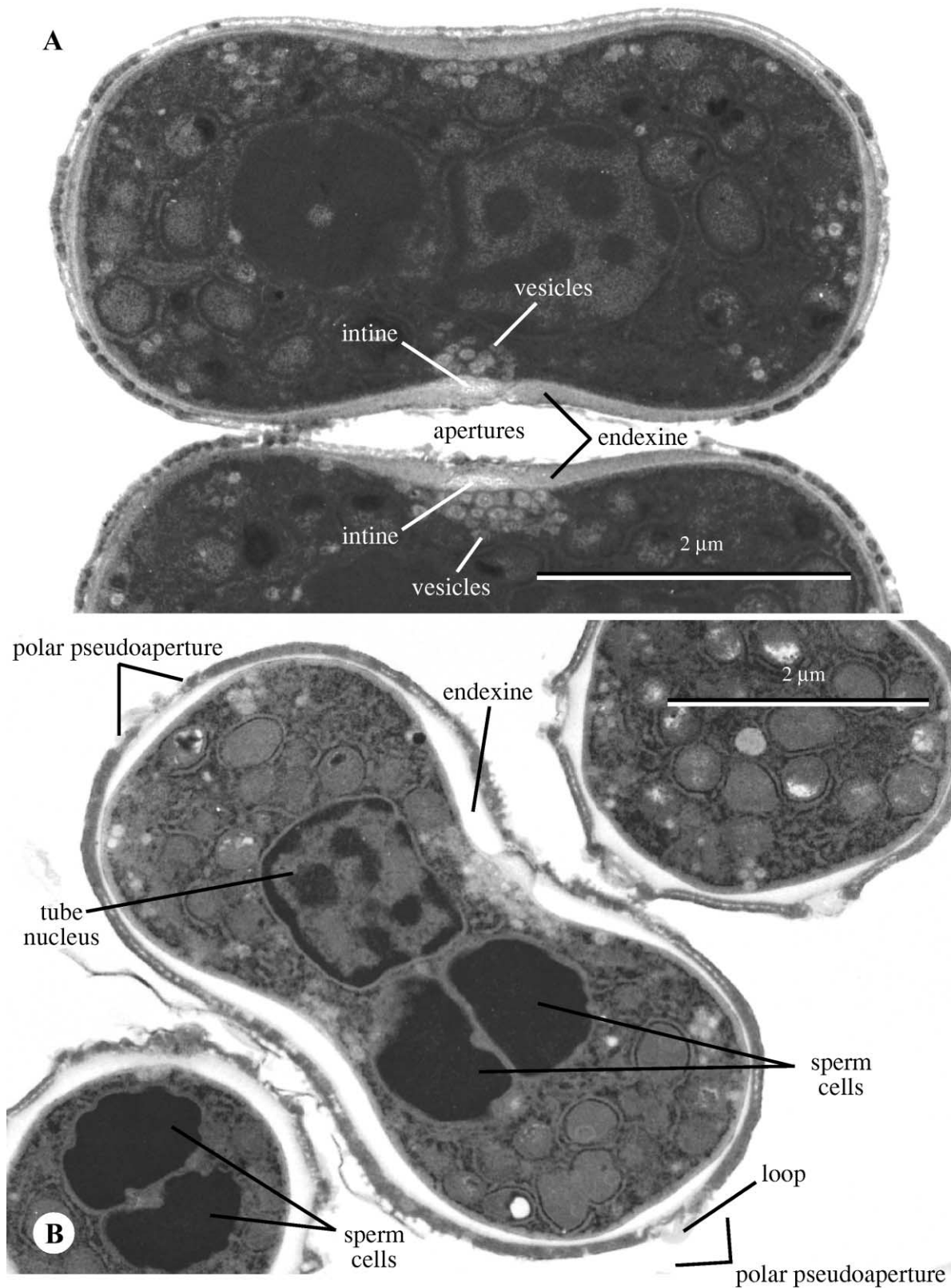


Fig. 2 *Cryptantha intermedia* pollen (TEMs). *A*, Pollen grains, longitudinal section. Note adjacent apertures, showing endexine and inner layer of intine. Also note slight thickening of endexine at equators and concentration of vesicles beneath apertural intine. *B*, Pollen grains, longitudinal and cross sections. Note tube nucleus and two sperm cells per grain. Also note polar pseudoapertures, having an interrupted exine layer, and thickened endexine (staining light in this section) at poles and equatorial region.

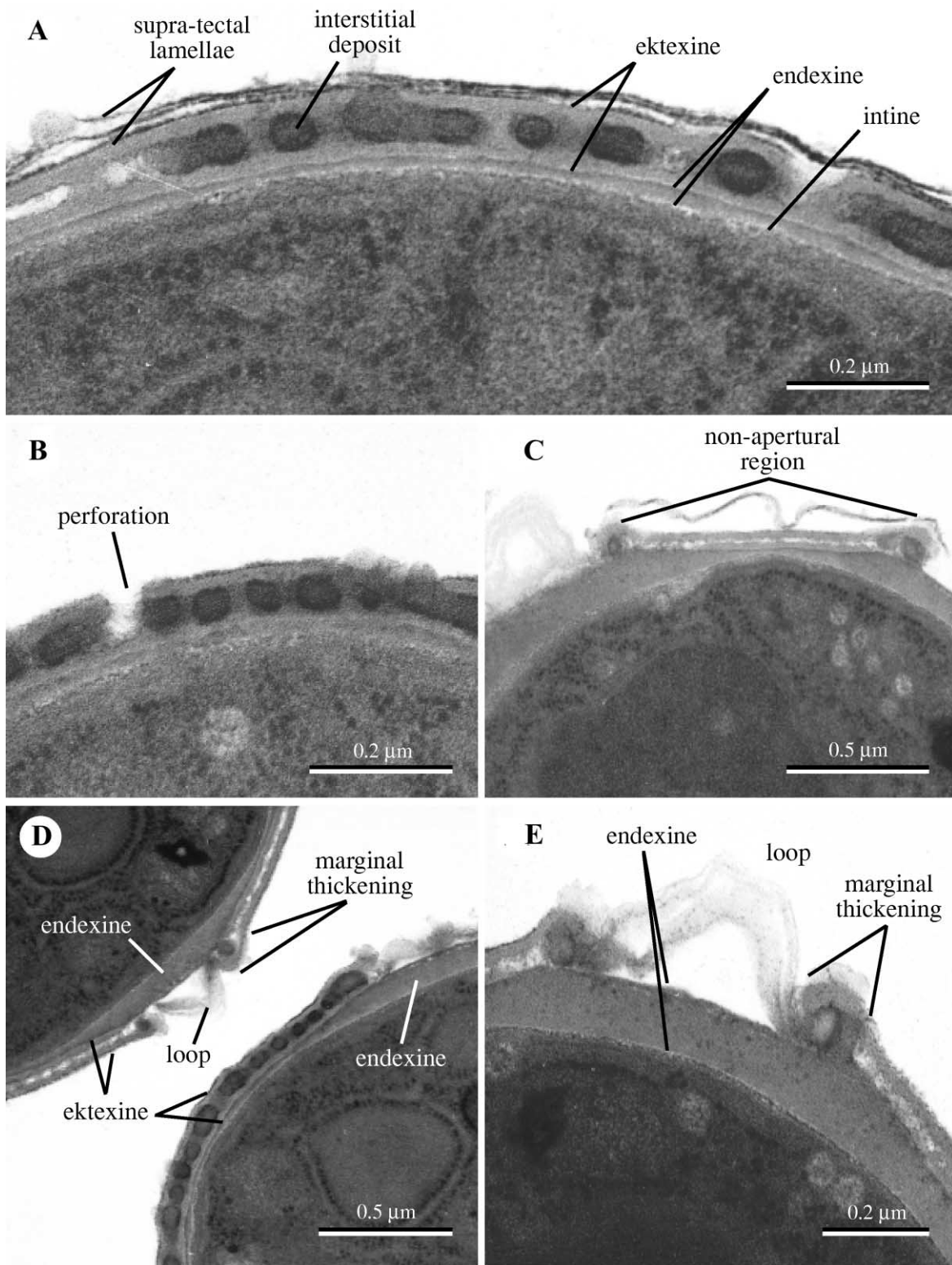


Fig. 3 *Crypantha intermedia* pollen (TEMs). **A**, Nonapertural wall region away from equatorial plane. Note scanty intine, relatively thin endexine, and tectate-columellate Ektexine, with occasional supratectal, lamellar deposits. The interstices of the Ektexine are filled with an electron-dense deposit. **B**, Nonapertural region, showing perforation in Ektexine. **C**, Pollen grain cross section near equatorial plane, showing nonapertural region with flattening of Ektexine. **D**, Adjacent pollen grains in cross section near equatorial plane, probably at pseudoapertures. Note endexine, thickened in pseudoapertural region. Also note Ektexine with interstitial deposits and thickenings at the margin of the pseudoaperture. A looplike deposit extends across the outer pseudoaperture. **E**, Close-up of probable pseudoaperture region, showing thick endexine, thickening of marginal Ektexine, and outer looplike deposit.

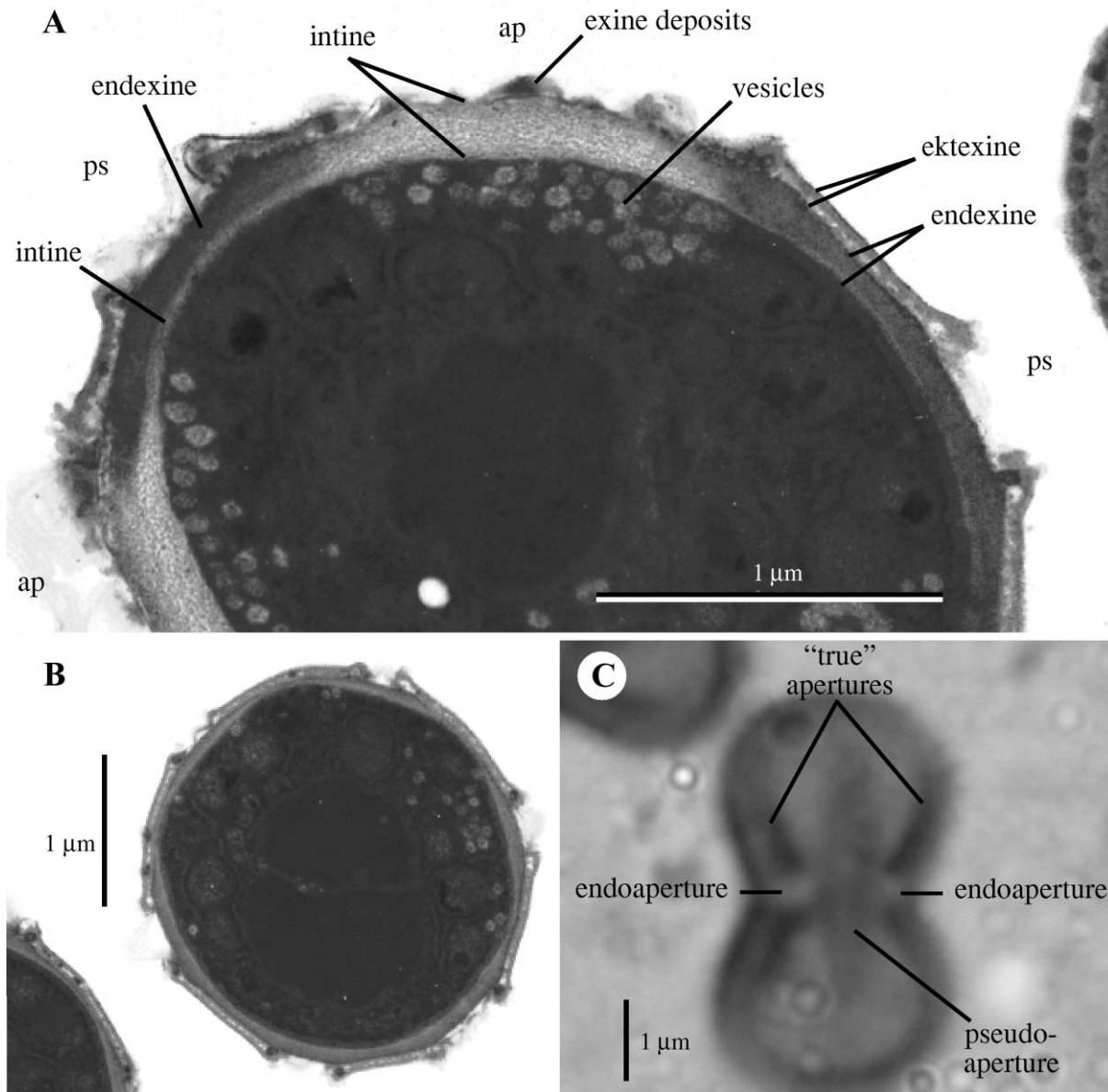


Fig. 4 *Cryptantha intermedia* pollen. A–B, TEMs. A, Pollen grain, slightly oblique cross section near equatorial plane. Apertures (*ap*) have a concentration of cytoplasmic vesicles, a thick layer of intine, and scattered, outer exine deposits. Pseudoapertures (*ps*) have a very thin intine layer and a thick endexine. Regions between apertures and pseudoapertures have a very thin intine, thick endexine, and outer ektexine. B, Pollen grain, cross section between equatorial plane and pole. Note six regions devoid of exine, corresponding to apertures or pseudoapertures. C, Pollen grain, acetolyzed, brightfield light microscopy. Note central, elongate pseudoaperture and peripheral “true” apertures, the latter with central, elliptical bright regions corresponding to endoapertures.

the equatorial region (fig. 2A). There is a fine layer of electron-dense granular material directly on the tectum; this is occasionally overlain by two additional lamellar layers of a similarly appearing substance, sometimes appearing detached (fig. 3A). These suprategical layers are not evident from SEM observations and are only occasionally observable by TEM.

The apertural and pseudoapertural regions are characterized by a general absence of ektexine (figs. 3E, 4A). At the margins of both the apertures and pseudoapertures, the tectum of the ektexine is raised and arched at the borders, with an interstitial

deposit often located beneath the raised marginal thickening of this ektexine (fig. 3D, 3E). These marginal thickenings appear to correspond with the marginal baculate to verrucate structures observed with SEM (fig. 1D). The ektexine layer is typically flattened near the equator (fig. 3C), becoming more rounded at the poles. The apertures, pseudoapertures, and polar pseudoapertures are often associated with an outer, translucent, faintly lined or layered deposit that has a looplike appearance; these deposits appear to attach at the base of each marginal thickening at either side of the apertural border and

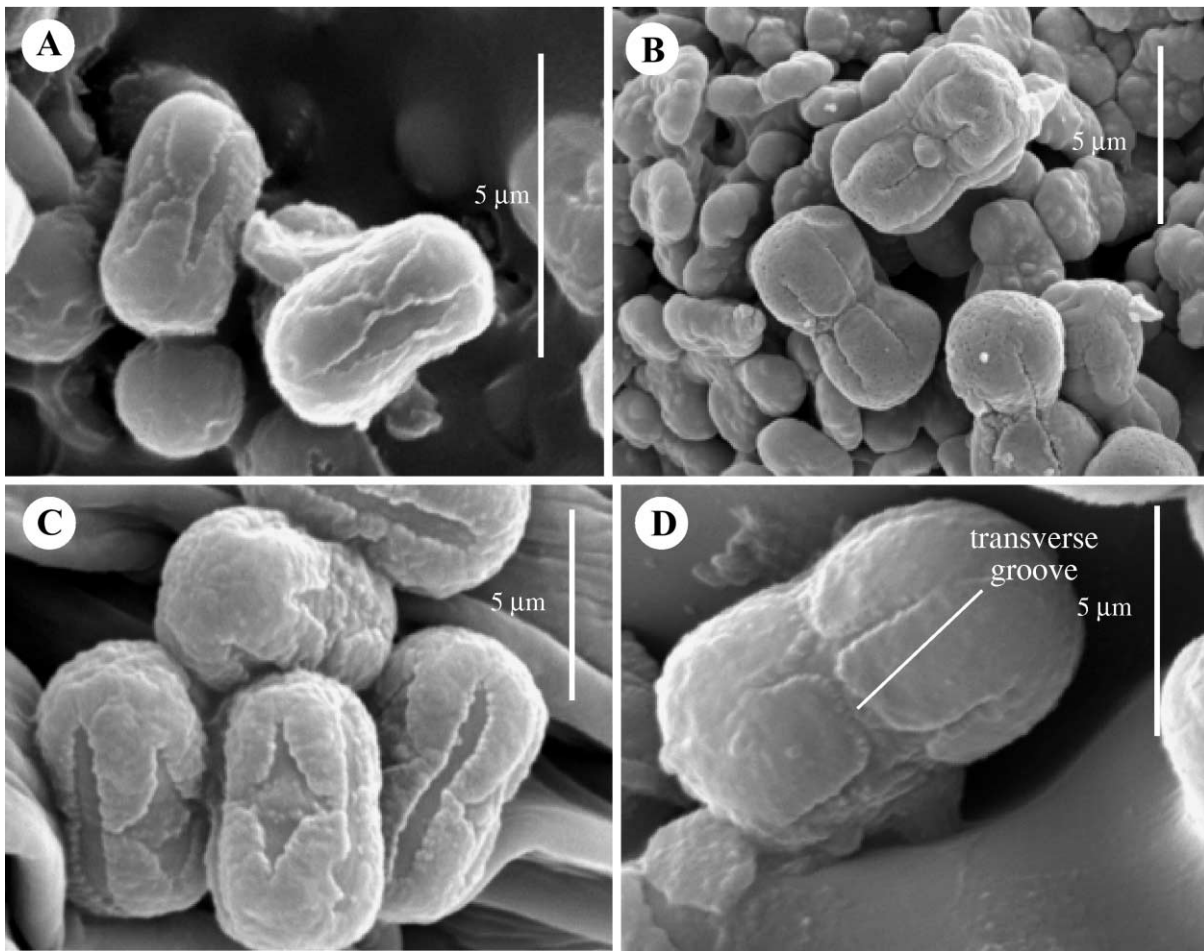


Fig. 5 *Cryptantha* spp. pollen (SEMs). A, *Cryptantha clelandii*. B, *Cryptantha fastigiata*. C, *Cryptantha glomeriflora*. D, *Cryptantha humilis*, with prominent transverse groove.

arch across (fig. 2B; fig. 3D, 3E). These loops are not preserved from SEM preparations and are only partly preserved by TEM.

In the equatorial plane, the intine beneath the true apertures is greatly thickened and fibrillar (fig. 4A), with an aggregation of numerous electron-translucent vesicles in the adjacent cytoplasm (figs. 2A, 4A). These vesicles are much less concentrated in the regions of the pseudoapertures and polar apertures (figs. 2A, 4A). The endexine is slightly thicker at the poles and is considerably thicker in the equatorial region, except where the intine is thickened (fig. 2A). In addition, away from the equatorial region, the endexine is thicker at the apertures and pseudoapertures, in contrast to the intervening nonapertural regions (figs. 3C, 4B). A comparison of longitudinal (fig. 2A) and transverse sections near the equator (fig. 4A) shows that the apertures consist of a thick layer of endexine, except at the immediate equator, where it consists almost entirely of fibrillar intine, forming a central, ellipsoid band. This band of intine measures ca. 1.2 μm in length (parallel to the equatorial plane; fig. 4A) and 0.5 μm in width (parallel to the polar axis; fig. 2A).

Pollen grains are trinucleate, containing two adjacent sperm cells (the nuclei enveloped by a plasmalemma) and one free

tube nucleus (fig. 2B). The tube nucleus and sperm cells are positioned semimedially on either side of the equatorial constriction (fig. 2B).

The light microscopic observations of acetolyzed pollen (fig. 4C) show the presence of three slightly shorter apertures, each with a central, transversely elliptic bright region that corresponds to the endoaperture (where the intine was digested by the acetolysis mixture). Three slightly longer pseudoapertures, lacking an endoaperture, alternate with the true apertures (fig. 4C). Note that the extremely small pollen grain size of *Cryptantha intermedia* taxed the limit of the light microscope.

Cryptantha clelandii (Fig. 5A)

Pollen grains are shed as monads, measuring 4.1 μm along the longer, polar axis. Grains are radial in symmetry and isopolar, prolate, and oblong in equatorial view with rounded ends and a slightly constricted midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 2.2 μm at the constricted midregion; the diameter at the widest point of the rounded ends is 2.6 μm . The polar : equatorial diameter (P : E) ratio is 2.2.

Pollen grains appear to have three true apertures (tricolporate) and three alternating pseudoapertures; polar apertures were not observed. The true apertures are narrowly rhombic with slightly inwardly curved edges and extend ca. 0.6 times the length of the pollen grain. A suggestion of exine deposits occurs at the true apertures along the equator. Pseudoapertures are nearly linear in shape, slightly wider at the equator and narrowing to a sharp point near the poles, extending ca. 0.7 times that of the pollen grain length.

Cryptantha fastigiata (Fig. 5B)

Pollen grains are shed as monads, measuring 6.2 (6.0–6.3) μm along the longer, polar axis. Grains are radial in symmetry and isopolar, perprolate, and hourglass shaped, oblong in equatorial view with rounded ends and a slightly constricted midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 2.5 μm at the constricted midregion; the diameter at the widest point of the rounded ends is 3.4 (3.3–3.5) μm . The polar : equatorial diameter (P : E) ratio is 2.4.

Pollen grains appear to have three true apertures (tricolporate) and three alternating pseudoapertures; polar apertures were not observed. The true apertures are narrowly linear but abruptly widened at the equator and extend ca. 0.6 times the length of the pollen grain. Scanty baculate to verrucate deposits occur at the true apertures along the equator. Pseudoapertures are very narrowly linear in shape, extending ca. 0.7 times that of the pollen grain length. A very narrow transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (fig. 5B).

Cryptantha glomeriflora (Fig. 5C)

Pollen grains are shed as monads, measuring 7.7 (7.6–7.8) μm along the longer, polar axis. Grains are radial in symmetry and isopolar, prolate, and oblong in equatorial view with rounded ends and a slightly constricted midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 4.3 μm at the constricted midregion; the diameter at the widest point of the rounded ends is 4.6 (4.6–4.7) μm . The polar : equatorial diameter (P : E) ratio is 1.7.

Pollen grains appear to have three true apertures (tricolporate) and three alternating pseudoapertures; polar apertures were not observed. The true apertures are narrowly rhombic in shape and extend ca. 0.5 times the length of the pollen grain. Scanty verrucate deposits occur transversely at the center of the true apertures. Pseudoapertures are very narrowly linear in shape, extending ca. 0.7 times that of the pollen grain length. An obscure, transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (fig. 5C).

Cryptantha humilis (Fig. 5D)

Pollen grains are shed as monads, measuring 9.7 μm along the longer, polar axis. Grains are radial in symmetry and isopolar. Pollen grains are prolate and hourglass shaped, oblong in equatorial view with rounded ends and a slightly constricted midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 5.0 μm at the constricted midregion; the diameter at the widest point of the rounded

ends is 5.6 μm . The polar : equatorial diameter (P : E) ratio is 1.8.

Pollen grains appear to have three true apertures and three alternating pseudoapertures, but the two could not be readily distinguished; polar apertures were not observed. The apertures and pseudoapertures extend ca. 0.5–0.6 times that of the pollen grain length. A narrow transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (fig. 5D).

Cryptantha jamesii (Fig. 6A, 6B)

Pollen grains are shed as monads, measuring 6.9 (6.0–8.7) μm along the longer, polar axis. Grains are radial in symmetry and isopolar, prolate, and hourglass shaped, oblong in equatorial view with rounded ends and a slightly constricted midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 4.0 (3.3–4.6) μm at the constricted midregion; the diameter at the widest point of the rounded ends is 4.4 (3.8–5.1) μm . The polar : equatorial diameter (P : E) ratio is 1.9.

Pollen grains appear to have three true apertures (tricolporate) and three alternating pseudoapertures; polar apertures were not observed. The true apertures are narrowly rhombic with slightly inwardly curved edges and extend ca. 0.6 times the length of the pollen grain. Scanty verrucate deposits occur along the equator of the true apertures. Pseudoapertures are nearly linear in shape, slightly wider at the equator, and narrowing to a sharp point near the poles, extending ca. 0.7 times that of the pollen grain length. A very narrow transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (fig. 6A, 6B).

Cryptantha micromeres (Fig. 6C)

Pollen grains are shed as monads, measuring 6.7 (6.6–6.9) μm along the longer, polar axis. Grains are radial in symmetry and isopolar, prolate, and oblong in shape with rounded ends; there is no constriction in the midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 4.6 (4.5–4.7) μm . The polar : equatorial diameter (P : E) ratio is 1.8.

Pollen grains appear to have three true apertures and three alternating pseudoapertures, although this was unclear, and a reduced number (two of each) may be present; polar apertures were not observed. The true apertures are narrowly elliptic and extend ca. 0.5 times the length of the pollen grain. Numerous verrucate deposits occur at the true apertures, with a concentration at the equator. Pseudoapertures are narrowly oblong in shape, extending ca. 0.8 times that of the pollen grain length. No equatorial groove connecting aperture to pseudoaperture is present (fig. 6C).

Cryptantha celosioides (Fig. 6D)

Pollen grains are shed as monads, measuring 6.2 (6.0–6.4) μm along the longer, polar axis. Grains are radial in symmetry and isopolar, prolate, and hourglass shaped, oblong in equatorial view with rounded ends and a slightly constricted midregion. In polar view, grains have a circular outline (amb). The equatorial diameter measures 3.5 (3.5–3.6) μm at the con-

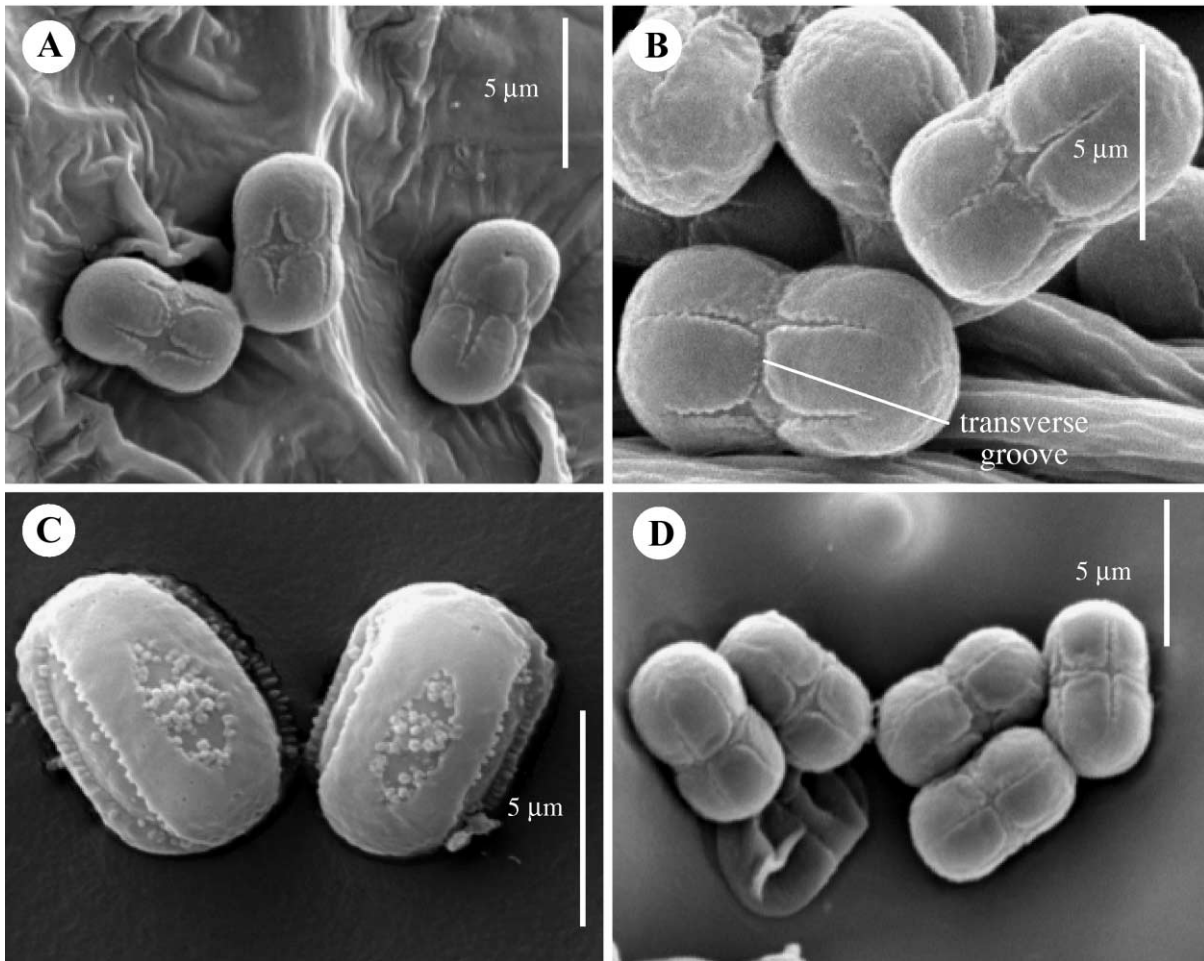


Fig. 6 *Cryptantha* spp. pollen, SEMs. A, B, *Cryptantha jamesii*, with prominent transverse groove. C, *Cryptantha micromeres*, lacking an equatorial constriction. D, *Cryptantha celosoides*.

stricted midregion; the diameter at the widest point of the rounded ends is 3.7 (3.6–3.8) μm . The polar : equatorial diameter (P : E) ratio is 1.7.

Pollen grains appear to have three true apertures (tricolporate) and three alternating pseudoapertures; polar apertures were not observed. The true apertures are narrowly linear but wider and rhombic at the equator and extend ca. 0.5 times the length of the pollen grain. Scanty verrucate deposits occur transversely along the center of the apertures. Pseudoapertures are narrowly linear in shape, narrowing to a sharp point near the poles, extending ca. 0.7 times that of the pollen grain length. A very narrow transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (fig. 6D).

Discussion

Pollen grains of *Cryptantha* species are extremely small, in this study ranging in length (polar diameter) from 4.1 μm in *Cryptantha clevelandii* to 9.7 μm in *Cryptantha humilis*. These sizes fall within the “very small” (<10 μm) size category of Erdtman (1966), approaching the size of some bacteria. In fact,

Cryptantha species are among the smallest of the tribe Eritricheae (table 1; fig. 7). In addition, the exine thickness of *Cryptantha* species (0.11–0.5 μm) is among the smallest of any other investigated members of the Boraginaceae, and that of *C. clevelandii* is the smallest measured (table 1; fig. 9).

Cryptantha pollen is elongate, having a polar : equatorial ratio that is relatively high (1.7–2.4) compared with other investigated members of the family (table 1; fig. 8). The shape of *Cryptantha* species ranges from prolate to perprolate; seven of the eight investigated species are characteristically hourglass shaped, being oblong with rounded ends and a constricted equatorial region. The one exception to this shape is *Cryptantha micromeres*, which is oblong but lacks any evidence of an equatorial constriction. The other seven species vary in the extent of the equatorial constriction. This variation may be influenced by pollen grain hydration, with the more hydrated grains having a wider equatorial diameter and being less constricted.

Aperture morphology in *Cryptantha* pollen is generally uniform. All investigated *Cryptantha* species have what has been termed heterocolpate pollen grains, consisting of three true apertures alternating with three pseudoapertures. (Apertures

Table 1

List of Palynological Characters for Investigated Members of the Boraginaceae

Taxon	Source	Pollen shape	Medial constriction	Aperture type	Aperture no.		Polar length (mean)	P : E ratio (mean)	Exine (mean)
					True	Pseudo			
Boraginoideae:									
Boragineae:									
<i>Anchusa arvensis</i> -type	2	Subprolate to prolate	(-)	Isocolpate	3	0	55.5	1.31	1.20
<i>Anchusa aucheri</i> -type	2	Prolate	-	Isocolpate	3	0	50.0	1.43	0.60
<i>Anchusa hispida</i> -type	2	Spheroidal	-	Isocolpate	9	0	23.5	1.07	0.40
<i>Anchusella variegata</i> -type	2	Prolate	-	Isocolpate	4	0	48.5	1.44	1.10
<i>Borago officinales</i> -type	2	Spheroidal to subprolate	-	Isocolpate	9	0	30.5	1.19	3.00
<i>Brunnera orientalis</i> -type	2	Subprolate	(-)	Isocolpate	4	0	15.5	1.28	0.23
<i>Elizaldia calycina</i> -type	2	Spheroidal, rarely subprolate	-	Isocolpate	13	0	47.5	1.13	0.95
<i>Lithodora prostrata</i> -type	2	Subprolate to prolate	(-)	Isocolpate	7	0	21.0	1.34	0.28
<i>Nonea lutea</i> -type	2	Prolate	-	Isocolpate	5	0	46.5	1.37	1.00
<i>Nonea obtusifolia</i> -type	2	Prolate	+	Isocolpate	3	0	22.5	1.46	0.28
<i>Nonea vesicaria</i> -type	2	Spheroidal to subprolate	-	Isocolpate	8	0	34.0	1.17	0.85
<i>Pentaglottis sempervirens</i> -type	2	Subprolate to prolate	(-)	Isocolpate	4-5	0	18.5	1.35	0.23
<i>Pulmonaria obscura</i> -type	2	Spheroidal to subprolate-prolate	-	Isocolpate	4-5	0	41.0	1.25	0.85
<i>Symphytum bulbosum</i> -type	2	Subprolate to prolate	-	Isocolpate	9	0	34.5	1.33	0.95
<i>Symphytum officinale</i>	1	Prolate or subprolate	-	Isocolpate	9	0	22.4	1.31	...
<i>Trachystemon orientalis</i> -type	2	Subprolate to prolate	-	Isocolpate	6	0	26.0	1.35	0.85
Cynoglosseae:									
<i>Cynoglossum arundanum</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	21.5	1.41	1
<i>Cynoglossum asperinum</i>	1	Prolate	+	Heterocolpate	3	3	8.8	1.94	...
<i>Cynoglossum cheirifolium</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	19.0	1.42	1
<i>Cynoglossum clandestinum</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	13.4	1.37	0.5
<i>Cynoglossum creticum</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	12.6	1.59	0.5
<i>Cynoglossum dioscoridis</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	12.2	1.43	0.5
<i>Cynoglossum nebrodense</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	9.5	1.44	0.5
<i>Cynoglossum officinale</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	12.9	1.42	0.5
<i>Gyrocarion oppositifolium</i>	3	Prolate	+	Heterocolpate	3	3	9.0	1.80	0.5
<i>Omphalodes brassicifolia</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	8.3	1.88	0.5
<i>Omphalodes commutata</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	9.7	1.86	0.5
<i>Omphalodes krameri</i>	1	Prolate	+	Heterocolpate	3	3	11.2	1.95	...
<i>Omphalodes linifolia</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	8.3	1.83	0.5
<i>Omphalodes littoralis</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	9.9	1.84	0.5
<i>Omphalodes nitida</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	9.7	1.79	0.5
<i>Solenanthus reverchonii</i>	3	Subprolate to prolate	-	Heterocolpate	3	3	12.1	1.27	0.5
Eritrichieae:									
<i>Amsinckia hispida</i>	1	Prolate or subprolate	-	Heterocolpate	3	3	31.8	1.33	...
<i>Amsinckia lycopsoides</i>	3	Subprolate or prolate	-	Heterocolpate	3	3	30.8	1.39	1.75
<i>Asperugo procumbens</i>	3	Prolate	+	Heterocolpate	3	3	10.1	1.76	0.5
<i>Bothriospermum tenellum</i>	1	Prolate	+	Heterocolpate	3	3	8.8	1.72	...
<i>Brachybotrys paridiformis</i>	1	Prolate	+	Heterocolpate	3	3	12.3	1.50	...
<i>Cryptantha albida</i>	5	Perprolate	+	Heterocolpate	3	3	10.0	2.20	0.5
<i>Cryptantha celosioides</i>	6	Prolate	+	Heterocolpate	3	3	6.2	1.7	...
<i>Cryptantha clevelandii</i>	6	Perprolate	+	Heterocolpate	3	3	4.1	2.2	...
<i>Cryptantha fastigiata</i>	6	Perprolate	+	Heterocolpate	3	3	6.2	2.4	...
<i>Cryptantha glomeriflora</i>	6	Prolate	+	Heterocolpate	3	3	7.7	1.7	...
<i>Cryptantha humilis</i>	6	Prolate	+	Heterocolpate	3	3	9.7	1.8	...
<i>Cryptantha intermedia</i>	6	Perprolate	+	Heterocolpate	3	3	5.5	2.3	0.11
<i>Cryptantha jamesii</i>	6	Prolate	+	Heterocolpate	3	3	6.9	1.9	...
<i>Cryptantha micromeres</i>	6	Prolate	-	Heterocolpate	3	3	6.7	1.8	...
<i>Hackelia deflexa</i>	1	Prolate	+	Heterocolpate	3	3	14.5	1.77	...
<i>Lappula barbata</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	10.6	2.74	0.5
<i>Lappula heteracantha</i>	1	Prolate	+	Heterocolpate	3	3	14.5	1.77	...
<i>Lappula patula</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	11.2	2.53	0.5
<i>Lappula squarrosa</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	13.7	2.60	0.5
<i>Mertensia asiatica</i>	1	Prolate	+	Heterocolpate	3	3	11.5	1.68	...
<i>Myosotis alpestris</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	6.6	1.66	0.5
<i>Myosotis arvensis</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	7.0	1.74	0.5
<i>Myosotis debilis</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	8.5	1.75	0.5

Table 1
(Continued)

Taxon	Source	Pollen shape	Medial constriction	Aperture type	Aperture no.		Polar length (mean)	P : E ratio (mean)	Exine (mean)
					True	Pseudo			
<i>Myosotis decumbens</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	8.5	1.69	0.5
<i>Myosotis discolor</i>	3	Subprolate to perprolate	–	Heterocolpate	4–6	4–6	17.5	1.23	0.75
<i>Myosotis lamottiana</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	7.4	1.72	0.5
<i>Myosotis laxa</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	7.9	1.54	0.5
<i>Myosotis minutiflora</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	9.9	1.50	0.5
<i>Myosotis personii</i>	3	Subprolate to perprolate	–	Heterocolpate	4–6	4–6	16.6	1.32	0.5
<i>Myosotis ramosissima</i>	3	Subprolate to perprolate	–	Heterocolpate	3	3	12.5	1.65	0.5
<i>Myosotis secunda</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	7.4	1.73	0.5
<i>Myosotis stricta</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	9.9	1.68	0.5
<i>Myosotis sylvatica</i>	1	Prolate	+	Heterocolpate	3	3	7.6	1.72	...
<i>Myosotis welwitschii</i>	3	Subprolate to perprolate	+	Heterocolpate	3	3	6.9	1.70	0.5
<i>Rochelia disperma</i>	3	Prolate to perprolate	+	Heterocolpate	3	3	13.1	2.04	0.5
<i>Trigonotis coreana</i>	1	Prolate	+	Heterocolpate	3	3	9.5	1.78	...
<i>Trigonotis icumae</i>	1	Prolate	+	Heterocolpate	3	3	10.0	1.71	...
<i>Trigonotis peduncularis</i>	1	Prolate	+	Heterocolpate	3	3	10.4	1.70	...
Lithospermeae:									
<i>Lithospermum arvense</i>	1	Rectangular prolate	–	Isocolpate	6	0	12.4	1.44	...
<i>Lithospermum erythrorhizon</i>	1	Rectangular prolate	+	Isocolpate	4	0	10.7	1.69	...
<i>Lithospermum zollingeri</i>	1	Rectangular prolate	+	Isocolpate	4	0	11.8	1.52	...
Ehretioideae:									
<i>Ehretia ovalifolia</i>	1	Mostly prolate-spheroidal	–	Isocolpate	3	0	17.6	1.07	...
Heliotropoideae:									
<i>Messerschmidia sibirica</i>	1	Mostly prolate-spheroidal	–	Heterocolpate	3	3	28.6	1.12	1.31
<i>Tournefortia-I</i>	4	Subprolate	–	Heterocolpate	3	3	26.5	1.31	1.31
<i>Tournefortia-II</i>	4	Subprolate	–	Hetero- or isocolpate	3	3 or 0	26.3	1.31	1.34
<i>Tournefortia-III</i>	4	Spheroidal	–	Isocolpate	3	0	17.6	1.00	1.00
<i>Tournefortia-IV</i>	4	Spheroidal to suboblate	–	Isocolpate	3–4	0	21.5	0.91	1.43

Sources. 1 = Ahn and Lee 1986; 2 = Bigazzi and Selvi 1998; 3 = Díez and Valdés 1991; 4 = Nowicke and Skvarla 1974; 5 = Quiroz-García and Palacios-Chávez 1985; 6 = this study.

Note. P : E = polar : equatorial diameter. For references 2 (Bigazzi and Selvi 1998) and 4 (Nowicke and Skvarla 1974), taxa were grouped based on similarities in pollen morphology; for these groups, measurements are averages. Symbols for medial constriction: “+” = present; “–” = absent; “(–)” = having a barely perceptible groove, not appreciably constricted.

and pseudoapertures could not be distinguished from one another in *C. humilis*, probably because of the poor condition of the sample.) The apertures and pseudoapertures vary in shape and size. Apertures are typically wider, usually rhombic or narrowly rhombic, rarely narrowly linear with a widened region at the equator. *Cryptantha micromeres* is an exception in that it has narrowly elliptic apertures. Pseudoapertures are narrower and longer than apertures, the former being typically linear to narrowly linear in shape and less broad at the equatorial region. The ratio of aperture : pollen length in *Cryptantha* averages 0.56, whereas the pseudoaperture : pollen length ratio averages 0.73. In addition, apertures can generally be distinguished from pseudoapertures in having verrucate surface exine deposits, typically in a somewhat transverse band, parallel to the equatorial plane. Such verrucate exine deposits are almost never present on the pseudoapertures.

A transverse groove, running along the equatorial perimeter between apertures and pseudoapertures, is present in *Cryptantha fastigiata*, *C. humilis*, *Cryptantha intermedia*, *Cryptantha jamesii*, and *Cryptantha celosiooides* (e.g., figs. 5D, 6B). Such a groove is absent in *C. micromeres* and was difficult to discern in *C. clevelandii* and *Cryptantha glomeriflora*. This groove is probably a region in which the ectexine is absent,

as occurs in the aperture and pseudoapertures of *C. intermedia*. The presence or absence of this transverse groove may be a significant systematic character, warranting further investigation.

Lamellar deposits were commonly observed just outside the tectum (fig. 3A), and electron-dense interstitial deposits were common, particularly near the poles (figs. 2A, 3A). The function of these deposits is not known, although the interstitial deposits may function in incompatibility reactions. Arched deposits, which appear as loops in section, often extend from one border to the next (fig. 3E); these looplike deposits could have some function in, or relation to, pollen wall or aperture development.

The current study provides a distinction in wall ultrastructure between the true apertures and pseudoapertures of *Cryptantha* and presumably of other members of the tribes Eritricheae and Cynoglosseae. In both apertures and pseudoapertures, the ectexine is essentially absent and the endexine is thickened with comparison to the non- (or inter-) apertural regions (fig. 4B). However, the medial region of the true aperture wall consists of a thickened band of intine, where the endexine is essentially absent or makes up only a thin upper layer (figs. 2A, 4A). Elsewhere, the intine is very thin, including

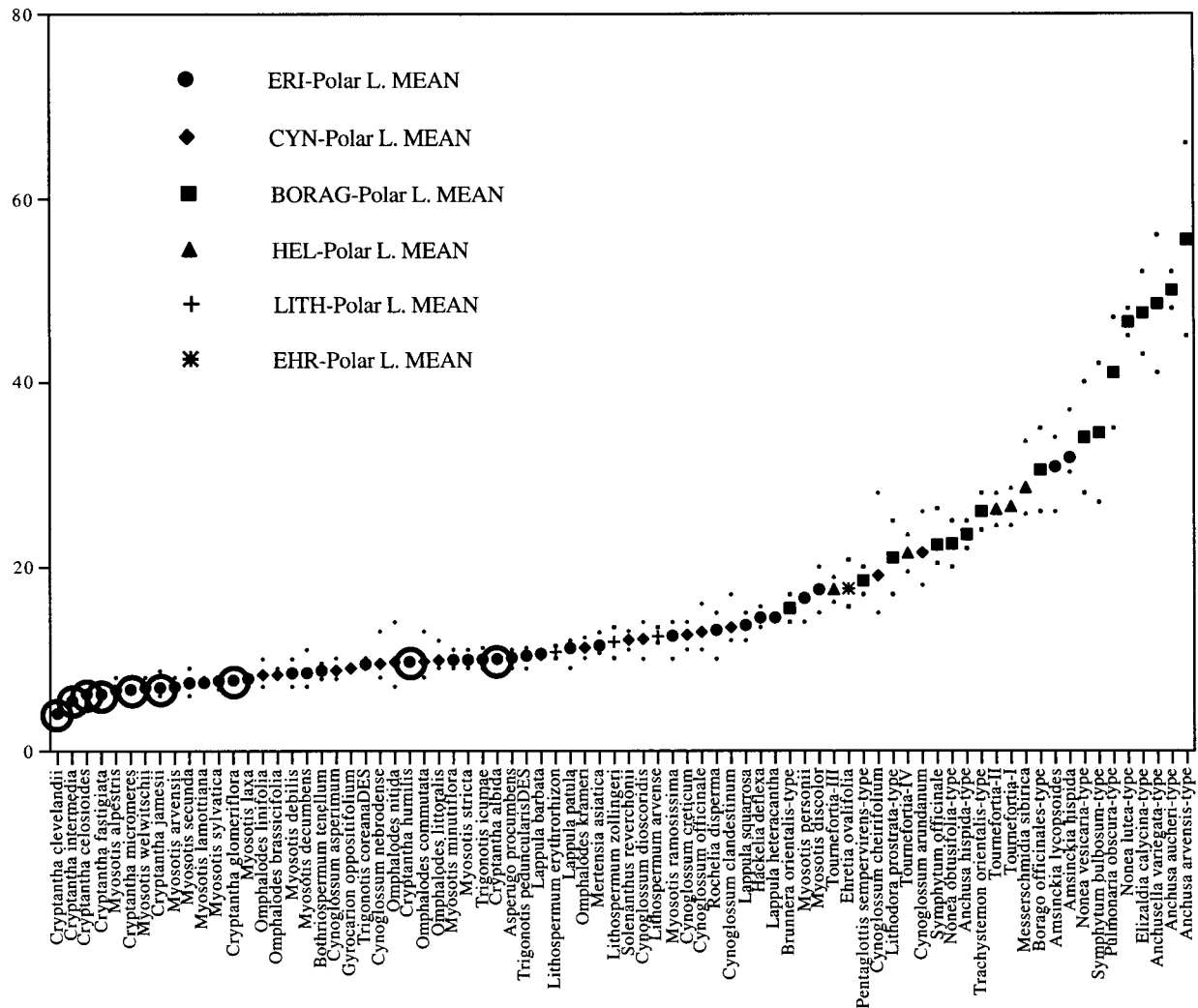


Fig. 7 Pollen polar length (diameter) in micrometers. Symbols for taxonomic groups indicated in legend at upper left. Means = large symbols; minimum and maximum values = small dots. Circles around symbols indicate *Cryptantha* species. Taxon abbreviations are as follows: ERI = Eritricheae, CYN = Cynoglosseae, BORAG = Boragineae, HEL = Heliotropoideae, LITH = Lithospermeae, EHR = Ehretioideae.

both apertural and pseudoapertural regions that are between the equator and poles (fig. 4B) and in nonapertural regions (fig. 4A). In addition, true apertures can be distinguished from the pseudoapertures in two other respects. True apertures have a concentration of cytoplasmic vesicles in the region beneath the intine (figs. 2A, 4A). These vesicles probably function to secrete substances into the cellulosic matrix of the intine that may be involved in pollen tube growth or recognition reactions (Walker and Doyle 1975). Also, apertures tend to have a band of verrucate surface exine (apparently etkexine) deposits. These exine deposits are almost entirely lacking on the pseudoapertures. These three wall characteristics—the presence of a thick intine, cytoplasmic vesicles, and outer etkexinous deposits—define the aperture in *Cryptantha*. Thus, the current study conforms with the definition of a pseudoaperture as “a thinning of the exine which, although superficially resembling an aperture, is not associated with a thickening of the intine

and is presumed not to function as an exitus” (Thanikaimoni 1980; Punt et al. 1994).

Correlating our findings to light microscope terminology, we find that the apertures of *C. intermedia* appear to be compound (colporate), with the absence of etkexine corresponding to an ectoaperture and the absence of endexine and thickening of intine corresponding to an endoaperture. Away from this endoapertural region at the equator, the ectoaperture is virtually indistinguishable from the pseudoapertures (fig. 4B). The medial band of thickened intine of the endoaperture roughly corresponds positionally to the band of verrucate exinous deposits observed with SEM (fig. 1A, 1D), implying a developmental correlation or link between the two features. Because these exine deposits were observed in other *Cryptantha* species with SEM, we infer these other species to have compound apertures as well. We also infer that other species of the Boraginaceae described as having compound apertures

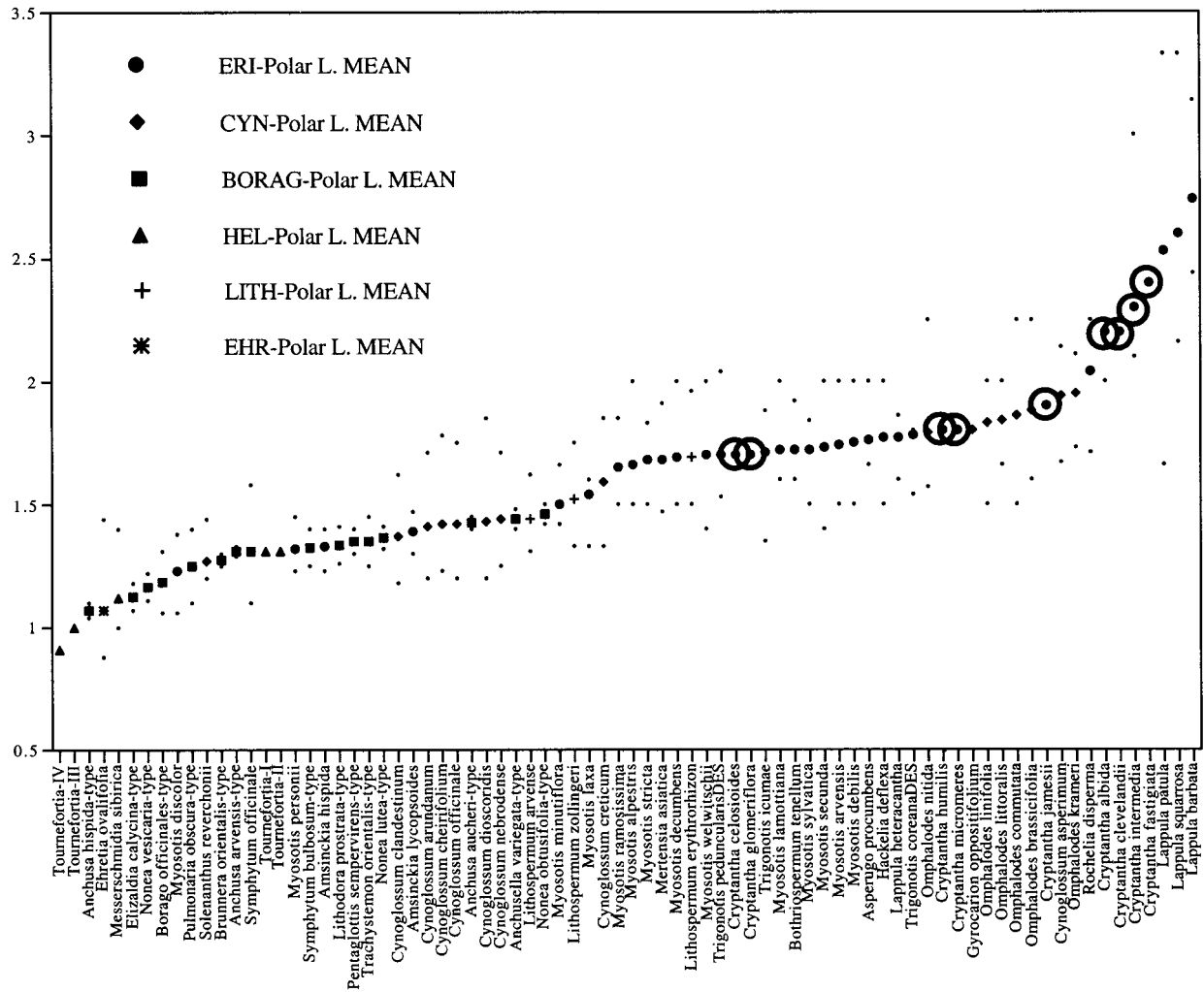


Fig. 8 Pollen polar : equatorial diameter (P : E) ratio; symbols and abbreviations are as in fig. 7

(table 1) have a similar ultrastructure to that observed here for *C. intermedia*.

In addition, we note the presence of curious triangular structures at both poles of *C. intermedia* pollen, which we term “polar pseudoapertures.” As with the pseudoapertures, polar pseudoapertures essentially lack ectexine (fig. 2B), although they sometimes have a single verrucate deposit (fig. 1C). Polar pseudoapertures also have a thickened endexine layer and lack a thickened intine layer (fig. 2A, 2B). There is no evidence that polar apertures function as the site of pollen tube exitus, nor do the polar apertures likely function in harmomegathy. Their presence may be a structural remnant of sporogenesis. Structures similar to the polar apertures in *C. intermedia* were described by Gentry and Carr (1976, p. 130) as “irregular spaced shallow depressions at the poles” in *Hackelia* species (relatively close relatives to *Cryptantha*) and are probably homologous; however, these workers did not cite their aperture-like nature.

Pollen shape, medial constriction, aperture type, aperture number, polar length, P : E ratio, and exine thickness for investigated members of the Boraginaceae, s.l., are cited in table 1. Certain trends can be noted within and among tribes and

subfamilies. With regard to pollen shape, the Boragineae, Heliotropoideae, and Ehretioideae have mostly spheroidal to subprolate (with some prolate) grains. The Cynoglosseae and Eritricheae have grains that are subprolate to perprolate, with Eritricheae being mostly prolate. The Lithospermeae have prolate grains but with a unique rectangular outline. In addition, the Boragineae, Heliotropoideae, and Ehretioideae have no medial, equatorial constriction, with the exception of one *Nonea* species of the Boragineae. In the Eritricheae, the great majority of taxa have a medial constriction; in the Cynoglosseae, about half the taxa have a constriction; and in the Lithospermeae, two of three investigated taxa have this feature.

Clear trends are evident with regard to aperture type and number (table 1). The Boragineae, Lithospermeae, and Ehretioideae have isocolpate grains exclusively, while all members of the Cynoglosseae and Eritricheae have heterocolpate pollen, i.e., with pseudoapertures alternating with true colporate apertures. Members of the Heliotropoideae have either isocolpate or heterocolpate grains. With regard to aperture number, the Boragineae often have more than three apertures (ranging from three to 13), the Lithospermeae have four or six apertures,

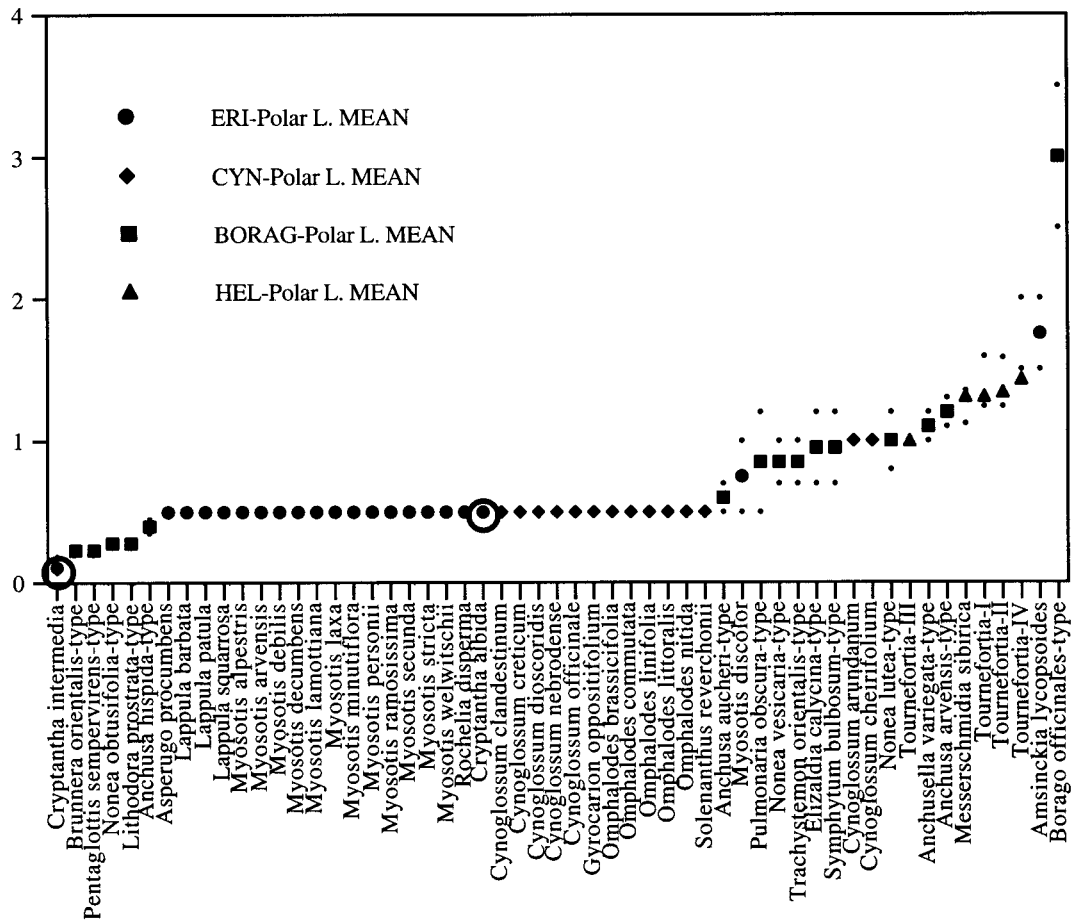


Fig. 9 Pollen exine thickness (ektexine plus endexine) in micrometers. Symbols and abbreviations are as in fig. 7. Data unavailable for members of Heliotropoideae and Lithospermeae.

and the Ehretioideae taxa have three apertures. Among the heterocolpate taxa, all Cynoglosseae have three apertures and three pseudoapertures. All Eritricheae also have three apertures and three pseudoapertures, with the exception of two *Myosotis* species, which have four to six true apertures alternating with four to six pseudoapertures. In the Heliotropoideae, the heterocolpate taxa have the common pattern of three apertures and three pseudoapertures, whereas the isocolpate taxa have three or four apertures and no pseudoapertures.

There are also trends with respect to pollen size features. The Eritricheae and Cynoglosseae have the smallest pollen grains (fig. 7). The polar diameter of most investigated Eritricheae ranges from 4.1 to 14.5 μm , exceptions being species of *Myosotis* and *Amsinckia*. The two investigated species of *Myosotis* that have four to six true apertures alternating with four to six pseudoapertures are slightly larger, ranging from 16.6 to 17.5 μm ; within this genus, this appears to represent a correlation between pollen size and aperture number. The two species of *Amsinckia* are exceptionally large for the tribe, with polar lengths of 30.8 and 31.8 μm . Most members of the Cynoglosseae, ranging in size from 8.3 to 13.4 μm , overlap in size with the bulk of the Eritricheae. Two *Cynoglossum* species are larger, ranging from 19.0 to 21.5 μm . The three investi-

gated members of the Lithospermeae fall within the upper end of the Eritricheae-Cynoglosseae range, at 10.7–12.4 μm . The Boragineae have the largest pollen grains in the family, ranging from 15.5 to 55.5 μm . Finally, the Heliotropoideae (17.6–28.6 μm) and Ehretioideae (17.6 μm) are also relatively large, falling within the lower range of the Boragineae (fig. 7).

The ratio of polar to equatorial diameter (P : E ratio) is correlated with pollen shape. Not surprisingly, members of the Eritricheae and Cynoglosseae, many of which have prolate or perprolate pollen grains, have the highest P : E ratios (Eritricheae 1.23–2.74 and Cynoglosseae 1.27–1.95), although their total range overlaps with other investigated members of the family (fig. 8). The Boragineae, Heliotropoideae, and Ehretioideae, which have mostly spheroidal to prolate grains, have a P : E ratio on the lower end of the range for the family as a whole (Boragineae 1.07–1.46, Heliotropoideae 0.91–1.31, and Ehretioideae 1.07). The Lithospermeae, which have the unique rectangular prolate grains, fall into an intermediate position (1.44–1.69) along the range for the Boraginaceae, s.l.

For exine thickness, data are more scanty and less precise. The available data show that members of the Eritricheae and Cynoglosseae, with a few exceptions, tend to be at the lower range of exine thickness for the family as a whole, with C.

intermedia (Eritricheae) having the smallest reported exine thickness at 0.11 μm . The Heliotropoideae tend to have a thicker exine (1.00–1.43 μm). The Boragineae range from having some of the smallest values to the largest (*Borago officinalis* type at 3.00 μm).

From the above summaries, some higher-level palynological trends can be noted within the Boraginaceae. The palynological distinctiveness of some of the tribes and subfamilies noted may correspond with recent higher-level analyses of the Boraginaceae, s.l. (Böhle and Hilger 1998; Gottschling et al. 2001). These analyses support the conclusion that the traditional Boraginaceae, s.l., is paraphyletic and that the subfamilies Heliotropoideae, Cordioideae, and Ehretioideae are best classified as separate families, as suggested by Gottschling et al. (2001). Within a more restricted Boraginaceae, s.s., the tribes Eritricheae and Cynoglosseae together are quite distinctive palynologically in having smaller, more elongate heterocolpate pollen grains, with almost all having three apertures alternating with three pseudoapertures. Although some of these features occur in some other members of the Boraginaceae, they may, as a whole, represent palynological apomorphies for the two tribes, particularly the heterocolpate condition. In addition, almost all members of the Eritricheae and many of the Cynoglosseae have pollen grains with a medial, equatorial constriction. This may represent a clear derived palynological feature within a complex of these two tribes. The

above-cited analyses also appear to support the conclusion that heterocolpate pollen grains in some Heliotropoideae (the only other group of the Boraginaceae, s.l., having this condition) evolved independently of those in the Eritricheae and Cynoglosseae.

In conclusion, we now have a better understanding of how wall ultrastructure corresponds to aperture morphology in the heterocolpate species *C. intermedia*. Clear differences in the ultrastructure of exine, intine, and cytoplasm are evident between the true apertures and pseudoapertures in this species. We assume that such features are probably present in many other heterocolpate taxa, at least in the closely related Eritricheae and Cynoglosseae. However, further comparative studies within these two tribes are needed to assess definitively the extent of this palynological feature as a derived feature. Such studies may be further useful in demonstrating variation in pollen shape, wall sculpturing, aperture shape and number, and pseudoaperture shape and number; all of these features undoubtedly will be systematically informative at a lower level.

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