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Publication details, including instructions for authors and subscription information:
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Pollen Ultrastructure of the Tecophilaeaceae

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Available online: 01 Sep 2009

To cite this article: Michael G. Simpson (1985): Pollen Ultrastructure of the Tecophilaeaceae, Grana, 24:2, 77-92

To link to this article: http://dx.doi.org/10.1080/00173138509429918

Grana 24: 77–92, 1985

Pollen ultrastructure of the Tecophilaeaceae

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Simpson, M. G. 1985. Pollen ultrastructure of the Tecophilaeaceae. – Grana 24: 77–92, 1985. Uppsala 25 September 1985. ISSN 0017-3134.

The classification of the monocot family Tecophilaeaceae has been variable. Pollen ultrastructural studies using SEM and TEM were made in an attempt to resolve taxonomic problems. Six genera of the Tecophilaeaceae: *Conanthera, Cyanella, Odontostomum, Tecophilaea, Walleria,* and *Zephyra,* are palynologically similar in having monosulcate, heteropolar, generally foveolate (rugulose in one genus) pollen grains with a tectate-columellate exine architecture and an operculate aperture. The operculum, which is hypothesized to be a shared derived character for these six genera, consists of a band of tectate-columellate exine positioned median and parallel to the aperture. An electron-dense, apparently endexinous layer occurs inner to the ektexine both in the operculum and along the aperture periphery. *Walleria* differs among these taxa in that the operculum consists of a band of granular ektexine atop the endexinous basal layer. The genus *Cyanastrum*, sometimes classified in the Tecophilaeaceae, has monosulcate, heteropolar, rugulose, tectate-columellate pollen grains which, however, lack an operculum. Thus, the present study tends to support the classification of *Cyanastrum* in a separate, monotypic family, the Cyanastraceae. *Eriospermum*, a genus sometimes placed in or suggested to have close affinities with the Tecophilaeaceae (particularly with *Walleria*), has pollen which is monosulcate, heteropolar, foveolate to reticulate with a tectate-columellate exine. However, grains of *Eriospermum* lack an operculum and an endexinous basal layer, providing no supporting evidence for the close relationship of *Eriospermum* to the Tecophilaeaceae. The monosulcate aperture type and tectate-columellate exine architecture present in all investigated taxa are hypothesized, by outgroup comparison, to be ancestral features, which are of no value in grouping monophyletic taxa. Previous classifications of the Tecophilaeaceae as a tribe (Conanthereae) of the family Haemodoraceae are refuted based on comparative studies.

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(Manuscript received 2 September 1983, revised version accepted 22 March 1984)

The Tecophilaeaceae, as defined by Hutchinson (1934, 1959, 1973) are a monocot family of 7 genera: *Cyanella, Walleria,* and *Cyanastrum* of Africa; *Conanthera, Tecophilaea,* and *Zephyra* of Chile; and *Odontostomum* of U.S.A.(California). Diagnostic characters of the family include the cormose to tuberous, generally "fibrous" rootstocks, bisexual and trimerous flowers, six stamens or combinations of stamens and staminodes, and poricidal anther dehiscence. However, the classification and interfamilial relationships of Hutchinson's assemblage have been questioned. Four or more of the genera have been grouped in the tribe Conanthereae and placed either within the Haemodoraceae (Bentham & Hooker 1883, Melchior 1964) or within the Amaryllidaceae, subfamily Hypoxidoideae (Pax 1888, Pax & Hoffmann 1930). Three genera, *Odontostomum, Walleria,* and *Cyanastrum,* traditionally have been classified in groups distantly related to the Conanthereae. For example, Bentham & Hooker (1883) placed *Walleria* in the Liliaceae, tribe Uvularieae, and Krause (1930) included *Odontostomum* in the Liliaceae, tribe Asphodeleae. Dahlgren & Clifford (1982), who treated *Walleria* in the Tecophilaeaceae, suggest affinities of that genus with *Eriospermum* of their Eriospermaceae. Takhtajan (1980), however, placed *Eriospermum* in his Tecophilaeaceae with the seven genera included in the

family by Hutchinson. *Cyanastrum* was raised to familial rank (Cyanastraceae) by Engler (1930), Cronquist (1981), and Dahlgren & Clifford (1982); the segregation of *Cyanastrum* to familial status has been based primarily on its aberrant possession of broad, cordate leaves and perispermous seeds.

The three genera of the Tecophilaeaceae which have been investigated embryologically, *Cyanastrum, Cyanella,* and *Odontostomum*, are identical with respect to tapetal type, microsporogenesis, and ovular parietal cell development (Cave 1952, Fries 1919, Nietsch 1941, de Vos 1950), thus tending to support the classification of *Cyanastrum* within the family (see de Vos 1961). Additionally, the genus *Lanaria*, which has most often been classified in the Haemodoraceae, is embryologically similar to these three genera of the Tecophilaeaceae (de Vos 1961, 1963), prompting some authors (e.g., de Vos 1961, Willis 1973, Dahlgren & Clifford 1982) to suggest a close affinity between *Lanaria* and members of the Tecophilaeaceae, a suggestion refuted, however, by Simpson (1983*a*).

Light microscope studies of pollen morphology have been made on most genera of the Tecophilaeaceae. Radulescu (1973) described the pollen of *Cyanella* as having a reticulate to foveolate exine appearing pilate-tegillate in optical cross-section. Erdtman (1966) reported the pollen morphology of *Conanthera, Cyanella, Tecophilaea,* and *Zephyra* as monosulcate, operculate, small to medium sized (17.5–40 μm), often biconvex, with a thin exine and "sexine about as thick as nexine". Erdtman stated that the classification of these genera as the family Tecophilaeaceae is supported by palynological evidence. The single species of *Cyanastrum* examined by Erdtman was described as having grains "1-sulcate (24×42×33 μm), occasionally trichotomosulcate, tenuiexinous, sexine about as thick as nexine or slightly thicker, baculate, subreticulate". Erdtman recognized *Cyanastrum* as a member of the Cyanastraceae (after Engler 1930), and argued that *Cyanastrum* is palynologically more similar to *Philydrella* (syn. *Pritzelia*) of the Philydraceae and to *Stemona* of the Stemonaceae.

The purpose of the present study is to assess the palynological similarities and differences of the Tecophilaeaceae (sensu Hutchinson 1973) and of *Eriospermum* using scanning and transmission electron microscopy plus limited brightfield and fluorescence microscope observations. Specific questions considered are: 1) What pollen ultrastructural features characterize the Tecophilaeaceae? 2) What is the ultrastructural nature of the "operculum" of family members? 3) Is the taxonomic placement of *Cyanastrum, Odontostomum, Walleria,* and *Eriospermum* in the Tecophilaeaceae supported by palynological evidence? 4) Does wall architecture support the segregation of the Tecophilaeaceae as a distinct family versus its treatment as the tribe Conanthereae of the Haemodoraceae or Amaryllidaceae?

MATERIALS AND METHODS

Pollen samples were obtained either from herbarium sheets ("DRIED") or were fixed in formalin/acetic acid/alcohol ("FAA"). Dried anthers were re-hydrated in Aerosol OT for 1–4 days, followed by several water rinses. The following eight genera and fourteen species were examined (parentheses indicate herbaria where vouchers are deposited): *Conanthera trimaculata* D. Don. "DRIED"—C. Grandjot (MO), *C. bifolia* Ruiz & Pav. "DRIED"—E. P. Killip & E. Pisano 39690 (US), *Cyanastrum cordifolium* Oliv. "FAA"—J. K. Bowden 141 (K) (Spirit collection no. 33980); *Cyanella alba* L.f. "FAA"—R. Ornduff 7463 (UC), *C. lutea* L.f. var. *lutea* "FAA"—R. Ornduff 7565 (UC), *C. hyacinthoides* L. "FAA"—R. Ornduff 7501 (UC), *C. orchidiformis* Jacq. "FAA"—S. R. Crispin 628 (MSC); *Eriospermum abyssinicum* Bak. "DRIED"— R. Seydel 4158 (US), *E. natalense* Bak. "DRIED"— E. Wedermann & H.-D. Oberdieck 1303 (US), *Odontostomum hartwegii* Torr. "FAA"—UCBG 53.845, *Tecophilaea violaeflora* Bertol ex Colla. "DRIED"—O. Buchtien 10 Sept. 1895 (US); *Walleria mackenzii* J. Kirk. "DRIED"—J. Buchanan 1891 (US), *W. muricata* N. E. B. "DRIED"—N. C. Chase 5182 (MO); *Zephyra elegans* D. Don. "DRIED"—E. Werdermann 776 (US).

For SEM studies whole, dehisced anthers containing mature pollen were placed in a modified capsule between two 5 μm Nucleopore filters. Anthers or free pollen were progressively dehydrated to 100% ethanol, then infiltrated with 100% Freon 113 (intermediate fluid). The material was critical-point dried in a BOMAR SPC 900/EX drier using CO_2 as the transition fluid. Pollen grains were tapped onto a stub covered by double-stick Scotch tape, sputter coated (ca. 200 A thickness) with gold/palladium (60/40), and viewed with a JEOL T20 SEM.

For TEM analysis pollen samples were fixed in 4.2% buffered gluteraldehyde for 2 hours, rinsed several times in 0.1 M Sorensen's phosphate buffer, and post-fixed in 2% OsO_4 for 1 hour. After two rapid water rinses and progressive dehydration to 100% ethanol, the material was infiltrated in a series of increasing concentrations of Spurr's resin (Spurr 1969). Fully infiltrated grains were placed in an obconical BEEM capsule and polymerized 8–12 hours in a 65 C oven. Sections ca 500–800 A thick were prepared using a Dupont diamond knife on a Cambridge-Huxley ultramicrotome, and mounted on uncoated 200 mesh copper grids. Preparations were post-stained

with uranyl acetate (saturated in 95% ethanol, 15 minutes) and lead citrate (0.2% aq., 7 minutes) and viewed with a Siemens Elmiskop 101 TEM.

For cytochemical tests of *Cyanella lutea*, unacetolyzed, FAA-fixed pollen grains were stained, mounted whole in glycerin jelly, and photographed in optical cross-section with brightfield or UV fluorescence microscopy, using a Leitz Labolux compound microscope (see Kress & Stone 1982, for a review of pollen cytochemistry methods). Pectic-rich intine was detected by positive staining with alcian blue (1% in 3% acetic acid, 5 minutes). Intine containing polysaccharides with 1,2-glycol groups (presumed to be predominantly cellulosic) was identified by red staining using the periodic acid-Schiff (PAS) reagent (after Jensen 1962). Ektexine was identified by bright red staining with basic fuchsin (1% in 95% ethanol, 5 minutes) and by bright yellow fluorescence with auramine O (0.01% in 0.05 M tris-HCl buffer, pH 7.2, 5 minutes). Fluorescence illumination was achieved with an Osram HBO 200 watt super pressure Hg lamp; filters used were a BG-38 heat filter, UG-1 UV excitation filter, BG-23 red suppression filter, and a blue or UV barrier filter.

Mean maximum pollen lenghts were measured from fixed or rehydrated grains mounted in 70% ethanol. Pollen terminology follows that of Walker & Doyle (1975).

RESULTS

POLLEN ULTRASTRUCTURE

Conanthera (2 of 5 species examined)

C. trimaculata.—Grains 17 μm, monosulcate, heteropolar (Fig. 1A). Aperture with a prominent median, exinous operculum. Sculpturing of exine (including operculum) foveolate, that of aperture membrane verrucose (Fig. 1A). Non-apertural intine relatively thin (Fig. 1D). Apertural intine thick and 2-layered (Fig. 1B), the outer layer having radially oriented channels or vesicles (Fig. 1C), the inner layer continuous with the non-apertural intine (Fig. 1B). Non-apertural exine tectate-columellate (tectate-perforate), apparently ektexinous, with a continuous foot-layer (Fig. 1D). Operculum consisting of a band of tectate-columellate ektexine having a thin foot-layer, atop an electron-dense, endexinous basal layer (Fig. 1C, E). Exine of aperture membrane composed of verrucose to clavate ektexinous elements atop a thin, electron-dense, apparently endexinous basal layer (Fig. 1C).

C. bifolia.—Grains 17 μm, monosulcate, heteropolar, with a prominent exinous operculum (Fig. 1F). Non-apertural and opercular surface foveolate, aperture membrane verrucose (Fig. 1G). Non-apertural intine thin (Fig. 1I). Apertural intine thick (thinner directly beneath operculum), 2-layered, with radially oriented channels in the thick outer layer (Fig. 1H). Non-apertural exine tectate-columellate (tectate-perforate) with a homogeneous and continuous foot-layer, columellae, and tectum (Fig. 1I). Exine of aperture, including operculum, similar to that of *C. trimaculata*, with apparent outer ektexinous layer and inner, electron-dense endexinous basal layer (Fig. 1H).

Cyanella (4 of 8 species examined)

C. lutea var. *lutea*.—Grains 37μm, monosulcate, heteropolar with operculate apertures (Fig. 2A). Exine sculpturing (including operculum) foveolate, that of aperture membrane psilate to gemmate (Fig. 2B). Non-apertural intine thin, obscurely 2-layered (Fig. 2D). Apertural intine 2-layered and thick, particularly the inner intine layer; outer apertural intine with thin, sinuous channel-like structures (Fig. 2E, F). Intine beneath operculum thin, forming a cavity (Fig. 2C). Non-apertural exine tectate-

Fig. 1. Conanthera. A–E: *C. trimaculata.* (A) Pollen grain, transverse equatorial view, showing aperture (*a*) with operculum (*o*). SEM ×4300. (B) Whole grain, sectioned along polar axis. Note operculum (*o*) and apertural intine (*i*). TEM ×4500. (C) Aperture region at periphery of operculum. Note ektexine (*ek*), endexinous basal layer (*en*), and 2-layered intine (*i*) with channel-like vesicles (*arrow*). TEM ×27000. (D) Non-apertural region, showing thin intine (*i*) and tectate-columellate exine (*e*). TEM ×24000. (E) Operculum wall, showing tectate-columellate ektexine (*ek*) and apparent endexinous basal layer (*en*). TEM ×26000. F–I: *C. bifolia.* (F) Whole grain, polar view, aperture facing. SEM ×3800. (G) Close-up of aperture with operculum (*o*). SEM ×8400. (H) Apertural region, showing operculum atop 2-layered intine (*i*), with prominent channel-like vesicles in outer intine layer. Note, peripheral to operculum, exine composed of outer ektexine (*ek*) and an inner endexinous basal layer (*en*). TEM ×12000. (I) Non-apertural, tectate-columellate exine atop fibrillar intine. TEM ×36000.

Fig. 2. Cyanella. A–F: *C. lutea* var. *lutea.* (A) Whole grains, aperture facing. SEM ×2000. (B) Close-up of wall, showing operculum (*o*). SEM ×3300. (C) Cross-section of grain. Note thick, 2-layered intine (*i*) and operculum (*o*) of aperture (*a*). TEM ×2900. (D) Non-apertural wall, showing thin intine (*i*) and tectate-columellate exine (*e*). TEM ×14000. (E) Periphery of apertural wall. Note ektexine (*ek*), basal endexinous layer (*en*) at edge of aperture, and thick 2-layered intine (*i*) with channel-like vesicles in outer wall (*arrow*). TEM ×9200. (F) Operculum, showing 2-layered intine and exine composed of a tectate-columellate ektexine (*ek*) and an inner, electron-dense basal layer of endexine (*en*). TEM ×7300. (G): *C. hyacinthoides.* Pollen grain, showing apertural operculum. SEM ×4300. (H) *C. orchidiformis.* Pollen grain. Note prominent apertural operculum. SEM ×3300.

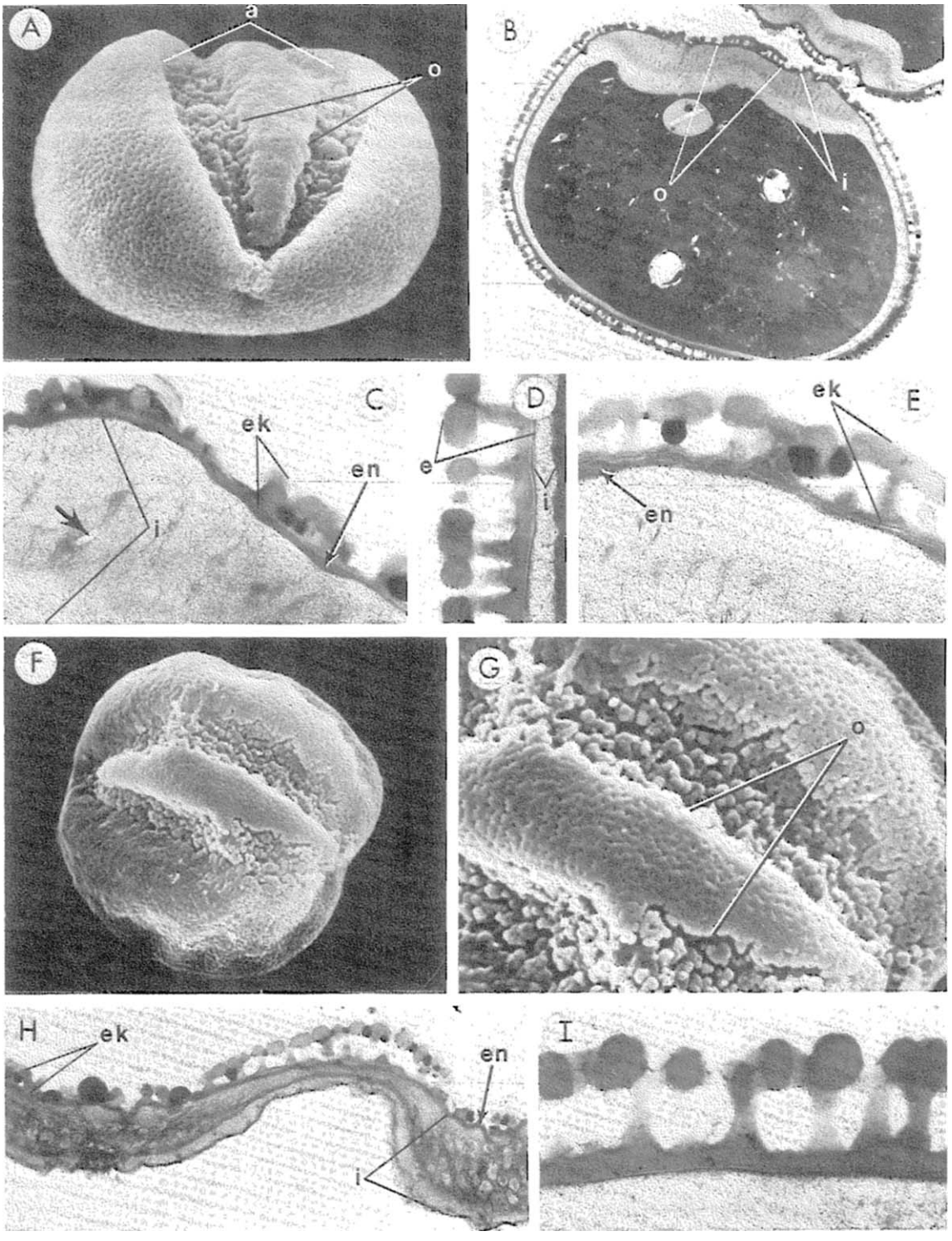


Fig. 1.

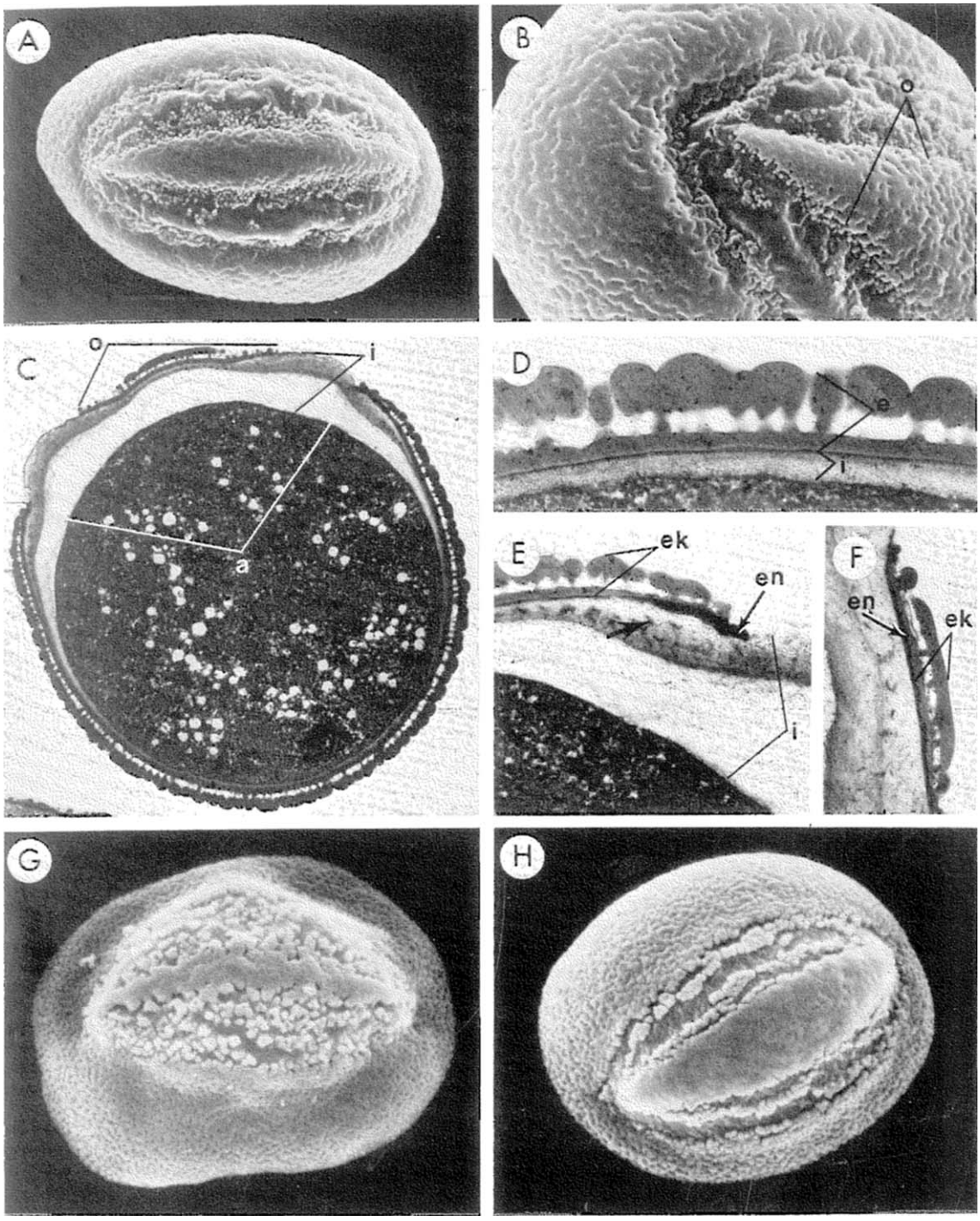


Fig. 2.

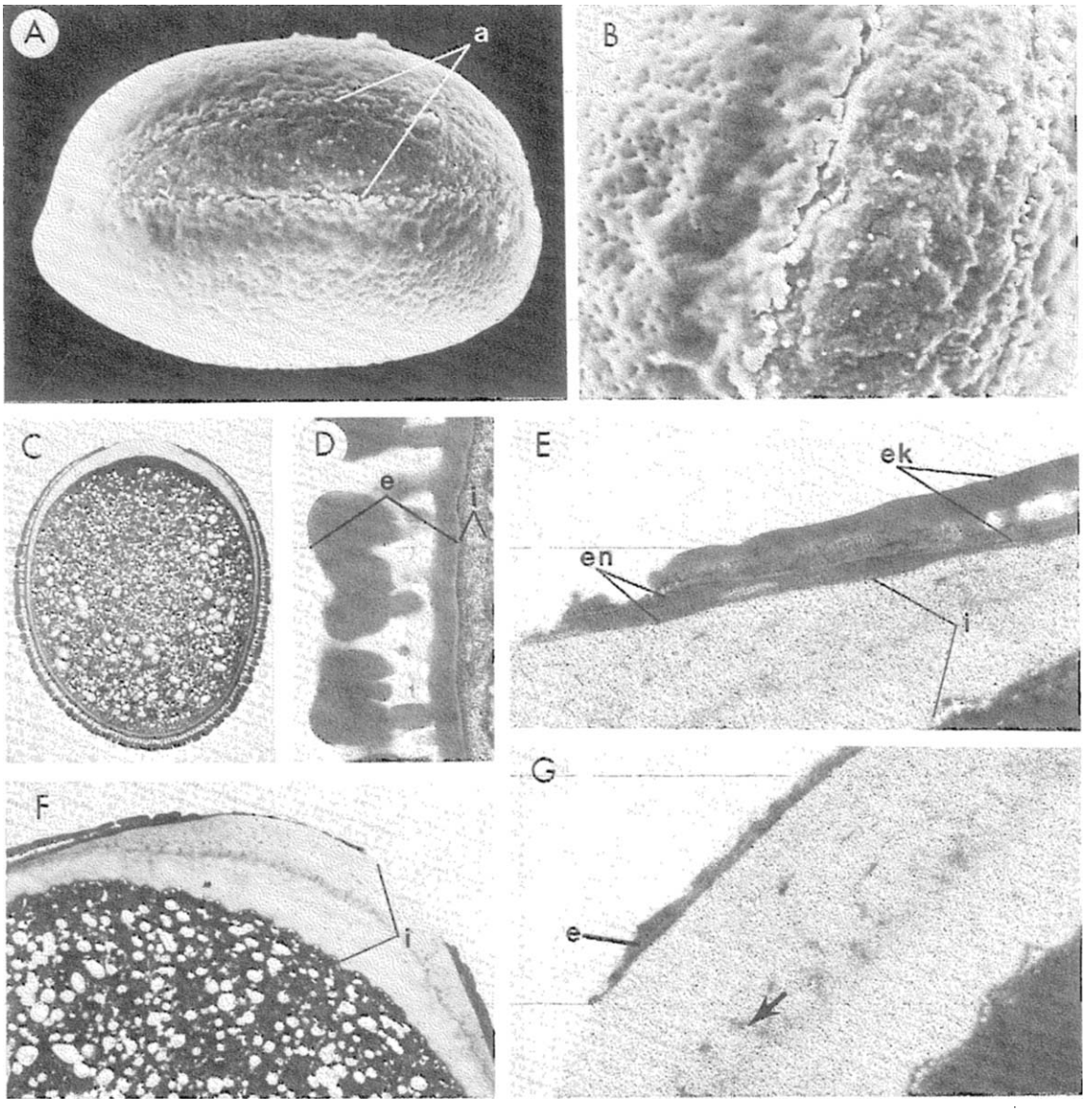


Fig. 3. Cyanella alba. (A) Whole grain, aperture (*a*) above. SEM ×1800. (B) Close-up, showing foveolate non-apertural exine (left) and non-operculate aperture (right). SEM ×3300. (C) Whole grain cross-section, aperture region above. TEM ×1600. (D) Non-apertural region, showing intine (*i*) and tectate-columellate exine (*e*). TEM ×15000. (E) Wall at edge of aperture. Note intine (*i*), ektexine (*ek*), and apparent endexinous basal layer (*en*). TEM ×20000. (F) Aperture region. Note thick, 2-layered intine (*i*). TEM ×4100. (G) Close-up of aperture, showing channel-like vesicles of outer intine (*arrow*) and thin layer of exine (*e*). TEM ×19000.

columellate (tectate-perforate) (Fig. 2D). Operculum composed of an outer median band of tectate-columellate exine (Fig. 2C). Exine along periphery of aperture and operculum intectate (Fig. 2C, E, F), the upright columellae comprising the gemmate sculpturing. A thin layer of electron-dense, endexine present beneath ektexine at periphery of aperture membrane (Fig. 2E) and operculum (Fig. 2F).

C. hyacinthoides.—Grains 19 μm, monosulcate, heteropolar, with a median, rather irregular apertural operculum (Fig. 2G). Sculpturing of non-apertural exine surface foveolate, that of operculum

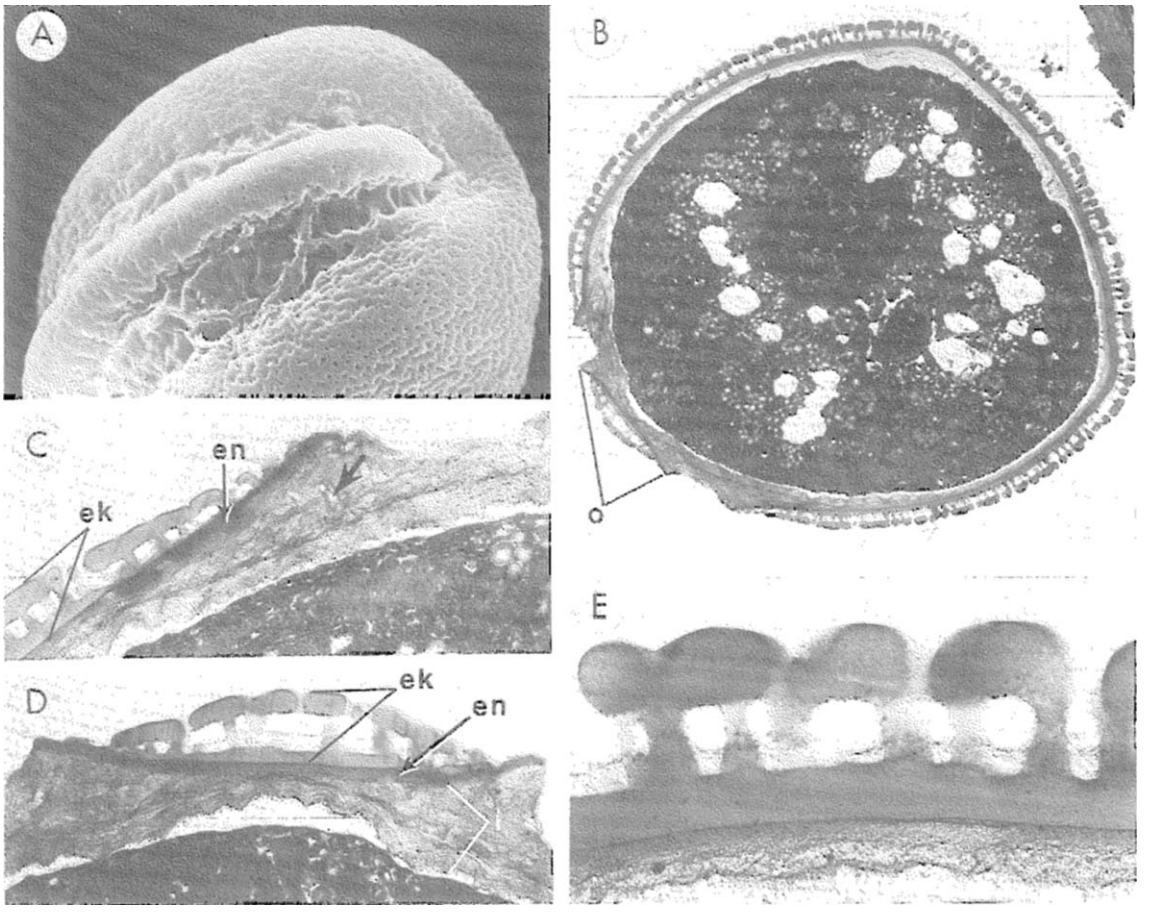


Fig. 4. Odontostomum hartwegii. (A) Whole grain. Note ridged operculum. SEM ×4000. (B) Grain in cross-section, showing operculum (*o*). TEM ×3200. (C) Interface of non-apertural (left) and apertural regions, showing 2-layered apertural intine with channel-like vesicles in outer wall (*arrow*). Note reduction of both foot-layer and columellae of ektexine (*ek*), replaced by electron-dense endexinous basal layer (*en*). TEM ×11000. (D) Operculum cross-section. Note 2-layered intine (*i*), tectate-columellate ektexine (*ek*) and endexine (*en*). TEM ×12000. (E) Cross-section, non-apertural region, showing thin, 2-layered intine and tectate-columellate exine. TEM ×31000.

foveolate to granular; apertural membrane surrounding operculum gemmate to verrucose (Fig. 2G). TEM observations not made.

C. orchidiformis.—Grains 23 μm, monosulcate, heteropolar, with a wide and prominent operculum (Fig. 2H). Non-apertural exine foveolate. Operculum psilate, pitted with micropores; aperture membrane flanking operculum with numerous gemmate exine elements (Fig. 2H). TEM observations not made.

C. alba.—Grains 42 μm, monosulcate, heteropolar; operculum absent (Fig. 3A, C, F). Non-apertural surface foveolate, that of aperture membrane psilate to scabrate (Fig. 3B). Non-apertural intine thin (Fig. 3C, D). Apertural intine thick and 2-layered, with channel-like vesicles in the outer intine layer (Fig. 3G); inner intine layer of aperture region continuous with non-apertural intine (Fig. 3F). Non-apertural exine tectate-columellate (tectate-perforate) (Fig. 3C, D). Exine at edge of aperture with reduced foot-layer and columellae, replaced by an electron-dense, apparently endexinous basal layer (Fig. 3E). Apertural exine composed of a thin, continuous outer layer (Fig. 3G).

Odontostomum (monotypic)

O. hartwegii.—Grains 26 μm, monosulcate, heteropolar, with a narrow, ridged operculum (Fig. 4A);

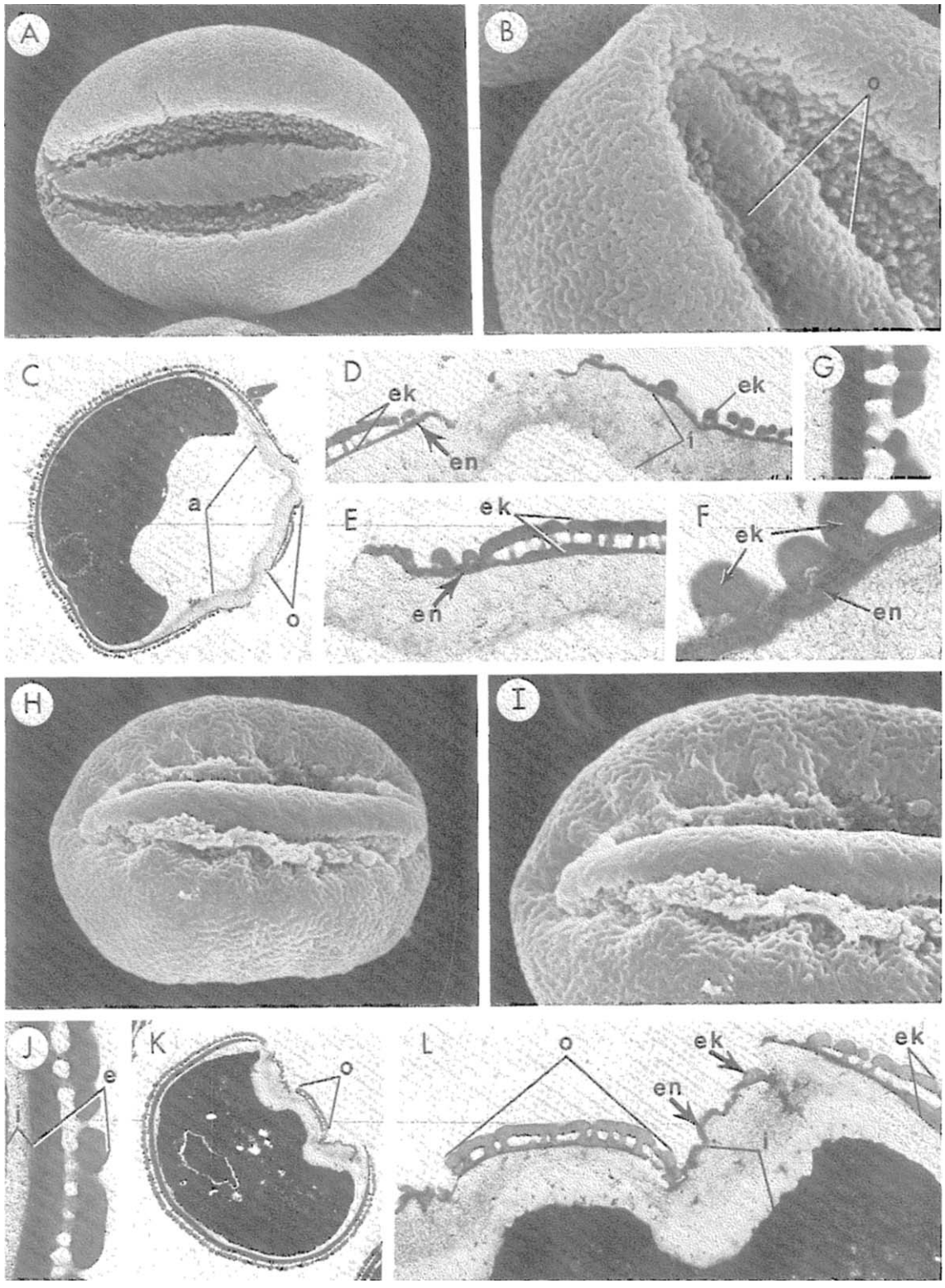
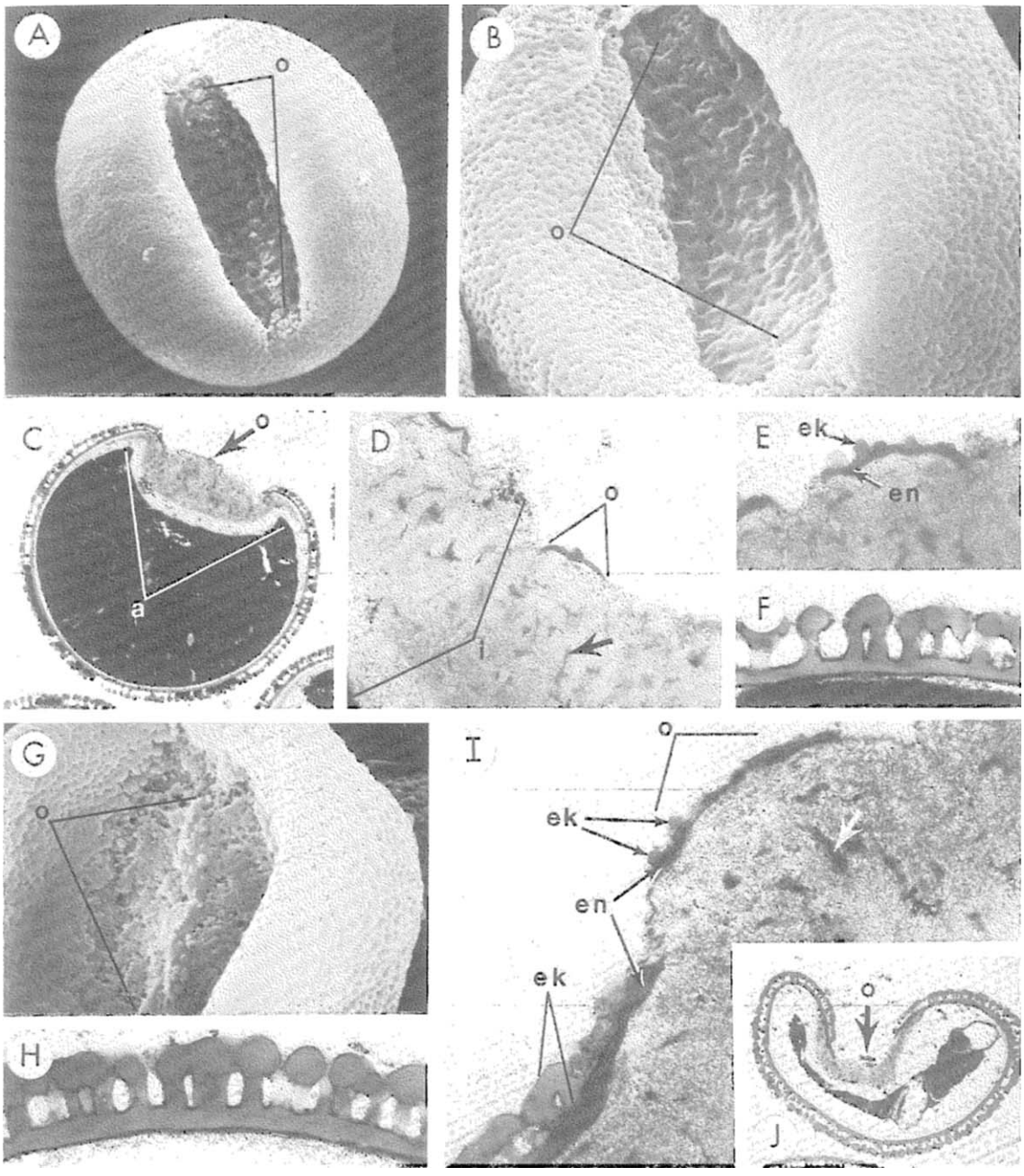


Fig. 5. A–G: *Tecophilaea violaeflora*. (A) Whole grain, aperture facing. SEM ×2900. (B) Close-up, showing rugulose non-apertural exine and operculum (*o*). SEM ×6400. (C) Grain in cross-section. Note aperture (*a*) and operculum (*o*). TEM ×2500. (D) Aperture wall periphery. Note intine (*i*), endexinous basal layer (*en*), and outer ektexinous elements (*ek*). TEM ×9900. (E) Operculum, showing intine, tectate-columellate ektexine (*ek*), and endexine (*en*). TEM ×12000. (F) Close-up of edge of operculum, showing outer ektexine (*ek*) and inner electron-dense, lamellated endexine (*en*). TEM ×41000. (G) Tectate-columellate exine and thin intine of non-apertural region.



TEM ×23 000. H–L: *Zephyra elegans*. (H) Whole grain, aperture facing; note operculum. SEM ×3 700. (I) Close-up of aperture wall surrounding operculum. SEM ×5 400. (J) Non-apertural region, showing fibrillar intine (*i*) and tectate-columellate exine (*e*). TEM ×20 000. (K) Grain in cross-section, showing operculum (*o*). TEM ×2 800. (L) Apertural region. Note thick, 2-layered intine (*i*), operculum (*o*), ektexine (*ek*), and thin, scanty basal layer of endexine (*en*). TEM ×9 900.

Fig. 6. Walleria. A–F: *W. muricata*. (A) Whole grain, aperture facing. Note thin, verrucose, median operculum (*o*). SEM ×3 900. (B) Close-up. Note operculum (*o*). SEM ×7 600. (C) Grain in cross-section, showing thick, 2-layered intine of aperture (*a*) and thin operculum (*o*). TEM ×3 300. (D) Aperture region. Note exinous operculum (*o*) and 2-layered intine (*i*) with channel-like vesicles (*arrow*) in outer intine layer. TEM ×18 000. (E) Close-up of operculum. Note outer verrucose ektexinous elements (*ek*) atop thin, basal endexine (*en*). TEM ×17 000. (F) Non-apertural wall, with tectate-columellate exine. TEM ×16 000. G–J: *W. mackenzii* (G) Close-up of pollen grain, with collapsed aperture. Note ridged operculum (*o*). SEM ×4 800. (H) Non-apertural, tectate-columellate exine. TEM ×19 000. (I) Interface between non-apertural region (left) and aperture wall, showing operculum (*o*) and thick intine with channel-like vesicles (*arrow*). Note ektexine (*ek*) and electron-dense, endexinous basal layer (*en*). TEM ×20 300. (J) Cross-section of whole, collapsed grain. Note aperture and operculum. TEM ×2 900.

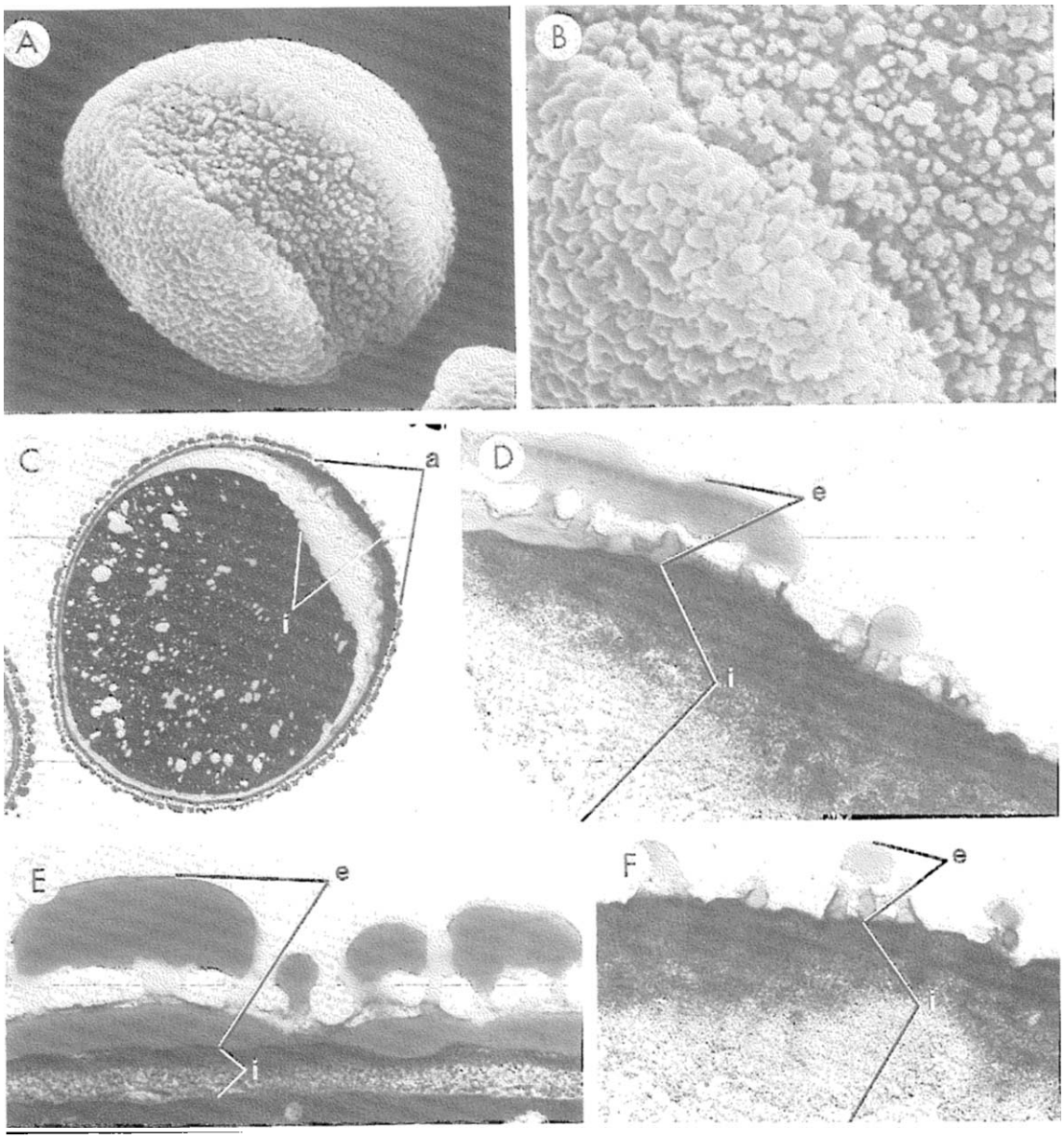


Fig. 7. Cyanastrum cordifolium. (A) Whole grain, showing monosulcate aperture. SEM ×1700. (B) Close up of grain. Note rugulose non-apertural exine (lower left) and scattered exinous elements of aperture region (upper right). SEM ×4200. (C) Grain in cross-section, along polar axis, showing aperture (*a*). Note 3-layered apertural intine (i) with osmiophilic outer layer. TEM ×2100. (D) Interface between non-apertural (left) and apertural regions. Note exine (*e*) and outer 2 layers of intine (*i*), the outermost osmiophilic. TEM ×22000. (E) Non-apertural region, showing tectate-columellate exine (*e*) and thin, 2-layered intine (*i*). TEM ×27000. (F) Apertural wall. Note verrucose to tectate-columellate exinous elements (*e*) and two outer intine layers (*i*). TEM ×16000.

sculpturing of non-apertural exine and operculum foveolate (Fig. 4A). Non-apertural intine thin, fibrillar, 2-layered (Fig. 4B, E). Apertural intine relatively thick and 2-layered; sinuous channel-like structures present in the thicker, outer intine layer (Fig. 4C, D). Non-apertural exine tectate-columellate with a homogeneous foot-layer (Fig. 4B, E). Opercular ektexine tectate-columellate with an

electron-dense endexinous basal layer inner to the foot-layer (Fig. 4B, D). Exine along periphery of aperture lacking an ektexinous foot-layer, having an electron-dense apparently endexinous layer (Fig. 4C). Fibrillar deposit apparent in the lower tectal cavities of both non-apertural and opercular exine walls (Fig. 4D, E).

Tecophilaea (1 of 2 species examined)

T. violaeflora.—Grains 26 µm, monosulcate, heteropolar, operculate (fig. 5A, C). Sculpturing of non-apertural and opercular exine somewhat rugulose, that of aperture bordering the operculum verrucose (Fig. 5B). Non-apertural intine thin, fibrillar (Fig. 5G). Intine of apertural wall relatively thick and faintly 2-layered (Fig. 5C, D). Non-apertural exine tectate-columellate; foot-layer homogeneous (Fig. 5G). Opercular exine tectate-columellate, with a thin, electron-dense, often lamellated endexine beneath the ektexinous foot-layer (Fig. 5E, F).

Zephyra (monotypic)

Z. elegans.—Grains 22 µm, monosulcate, heteropolar, and operculate (Fig. 5H, K). Sculpturing of non-apertural exine foveolate to rugulose, that of the operculum striate, and that of the aperture membrane surrounding operculum verrucose (Fig. 5I). Non-apertural intine thin (Fig. 5J). Apertural intine thick, 2-layered (Fig. 5K, L). Exine of non-apertural region tectate-columellate, with a thick, continuous foot-layer (Fig. 5J). Opercular exine tectate-columellate, with a thin ektexinous foot-layer and a very thin electron-dense layer of endexine (Fig. 5L). Apertural exine surrounding operculum composed of a thin layer of electron-dense endexine beneath scattered ektexinous verrucose elements (Fig. 5 K, L).

Walleria (2 of ca. 3 species examined)

W. muricata.—Grains 16 µm, monosulcate, heteropolar (Fig. 6A, C). Non-apertural surface foveolate, aperture membrane with a median line of verrucose exinous elements (apparent operculum) along aperture length (Fig. 6A, B, D). Non-apertural intine thin (Fig. 6F). Apertural intine thick, 2-layered, with channel-like vesicles in the outer layer (Fig. 6C, D). Non-apertural exine tectate-columellate, homogeneous (Fig. 6F). Median band of apertural exine 2-layered, composed of a verrucose ektexinous outer layer and a thin, electron-dense endexinous basal layer (Fig. 6D, E).

W. mackinzii.—Grains 28 µm, monosulcate, heteropolar, with a finely foveolate non-apertural surface and an apparent apertural operculum composed of a thin, median, verrucose ridge (Fig. 6G, J). Non-apertural intine thin, fibrillar (Fig. 6H). Apertural intine thick, containing radially oriented, channel-like vesicles throughout (Fig. 6I). Non-apertural exine tectate-columellate with a perforate tectum and a continuous foot-layer (Fig. 6H). Exine along periphery of aperture lacking a foot-layer, the latter replaced by a prominent, electron-dense, apparently endexinous basal layer (Fig. 6I). Apparent operculum composed of verrucose ektexinous elements atop a thin, electron-dense endexinous layer, resembling the basal layer along the aperture periphery (Fig. 6I).

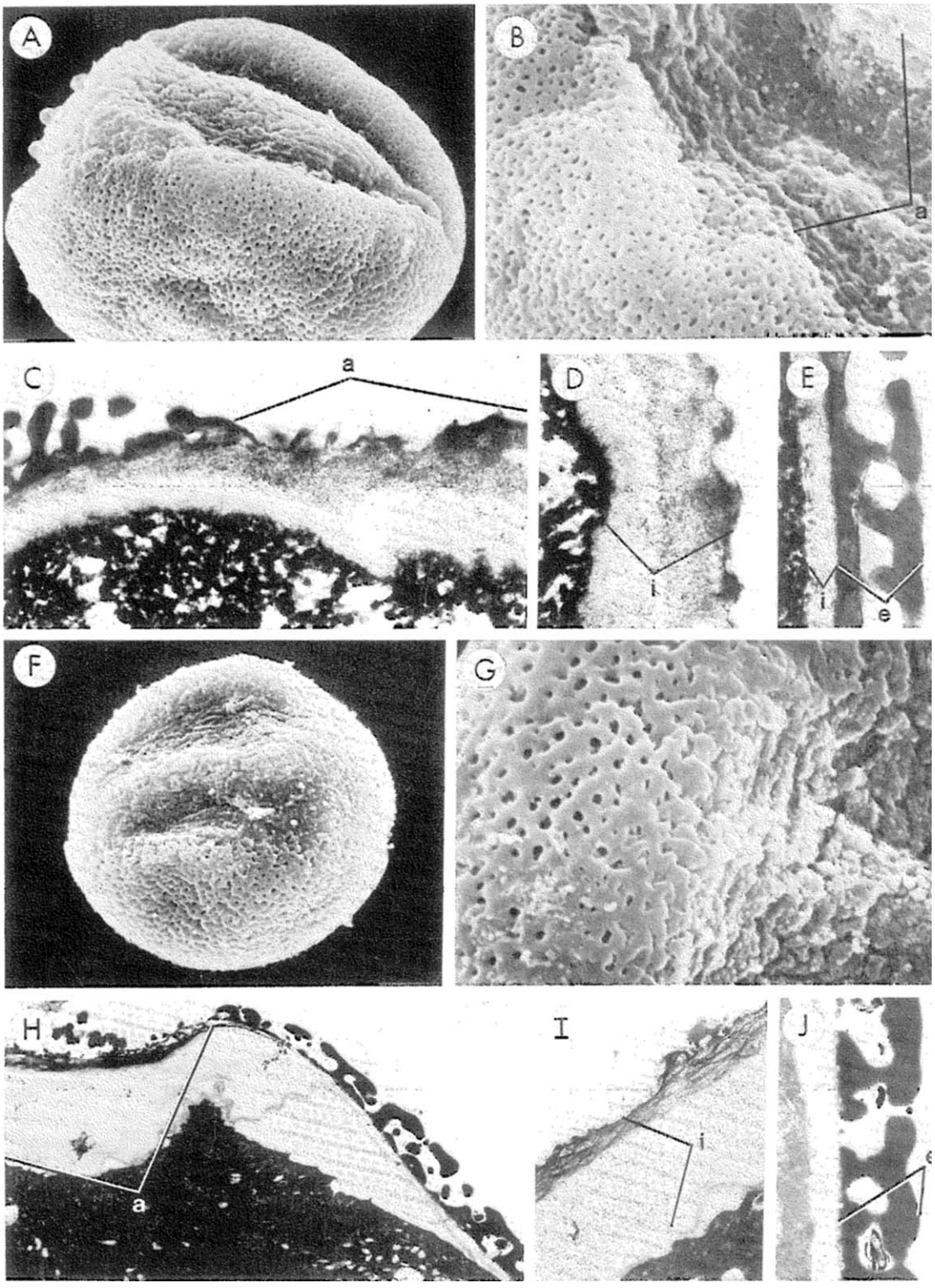
Cyanastrum (1 of 6 species examined)

C. cordifolium.—Grains 35 µm, monosulcate, heteropolar (Fig. 7A). Non-apertural sculpturing somewhat rugulose, aperture membrane with numerous scattered verrucose exine elements (Fig. 7B, F). Operculum absent. Intine of non-apertural region thin (Fig. 7E), becoming thick and 3-layered in apertural region (Fig. 7D), the innermost layer loosely fibrillar and probably artifactual. Outer intine layer fibrillar, osmiophilic, with some channels (Fig. 7C, D, F). Non-apertural exine tectate-columellate, with a perforate tectum and a rather thick, continuous foot-layer (Fig. 7E). Exine at aperture periphery with a reduced foot-layer (Fig. 7D). Apertural exine composed of verrucose to tectate-columellate elements with foot-layer absent (Fig. 7D, F). Endexine absent or possibly extremely thin (Fig. 7D, F).

Eriospermum (2 of ca. 80 species examined)

E. abyssinicum.—Grains 42 µm, monosulcate, heteropolar (Fig. 8A). Exine foveolate to reticulate; aperture membrane somewhat scabrate in material examined (Fig. 8A, B). Non-apertural intine thin (Fig. 8E). Apertural intine thick, 2-layered; channels not evident (Fig. 8D). Non-apertural exine tectate-columellate, with a rather continuous foot-layer (Fig. 8E). Apertural exine lacking (Fig. 8C, D); endexinous layer at aperture periphery absent (Fig. 8C).

E. natalense.—Grains 39 µm monosulcate, heteropolar (Fig. 8F), with a foveolate to reticulate non-apertural exine and a somewhat scabrate apertural surface (Fig. 8G). Non-apertural intine thin



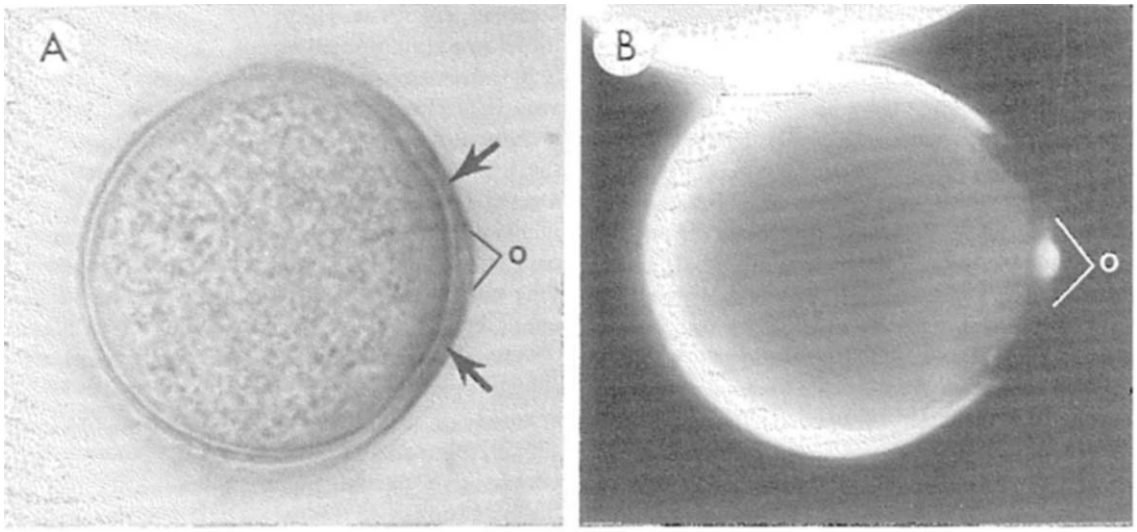


Fig. 9. Cyanella lutea pollen grains in optical cross-section. (A) Alcian blue, brightfield microscopy. Note dense (dark blue) staining of outer apertural intine (*arrows*). Note operculum (*o*). ×1900. (B) Auramine O, fluorescence microscopy. Note bright (yellow) fluorescence of exine, including operculum (*o*). ×1900.

(Fig. 8H, J). Apertural intine thick, 2-layered; channels not evident (Fig. 8I). Non-apertural exine tectate-columellate with a perforate tectum and a discontinuous foot-layer (Fig. 8J); foot-layer reduced at aperture periphery (Fig. 8H). Apertural exine absent (Fig. 8I). Endexinous layer at aperture periphery absent (Fig. 8H).

Wall chemistry

Cyanella lutea var. *lutea*.—Outer apertural intine alcian blue positive (staining dark blue), indicative of pectic-rich region (Fig. 9A). Inner intine layer PAS positive (staining pink), indicative of probable

Fig. 8. Eriospermum. A–E: *E. abyssinicum*. (A) Whole grain, aperture above. SEM ×2400. (B) Close-up, showing foveolate to reticulate non-apertural wall (left) and scabrate aperture region (right). SEM ×5100. (C) Interface between non-apertural region (left) and aperture (*a*). TEM ×13000. (D) Aperture region, composed of thick 2-layered intine (*i*). Note apparent absence of exine. TEM ×13000. (E) Non-apertural region. Note intine (*i*) and tectate-columellate exine (*e*). TEM ×21000. F–J: *E. natalense*. (F) Whole grain, aperture above. SEM ×1800. (G) Close-up, showing foveolate to reticulate non-apertural exine (left) and scabrate aperture (right). SEM ×5400. (H) Interface between aperture (*a*) and non-apertural region (right). TEM ×6000. (I) Aperture region, composed of 2-layered intine (*i*). TEM ×10000. (J) Non-apertural region. Note tectate-columellate exine (*e*) with discontinuous foot-layer. TEM ×11000.

DISCUSSION

All investigated taxa are palynologically similar in possessing monosulcate, heteropolar pollen grains with a tectate-columellate exine having continuous and homogeneous foot-layer, columellae, and tectum. However, the monosulcate aperture type, which is widespread among monocots, "primitive" dicots, and gymnosperms, is very probably ancestral within the angiosperms as a whole (see, e.g., Walker & Doyle 1975). A tectate-columellate exine wall architecture, similar to that in the Tecophilaeaceae, occurs in virtually all investigated families presumed to be closely related to the Tecophilaeaceae, including members of the Velloziaceae (Ayensu & Skvarla 1974), Hypoxidaceae, Liliaceae, Taccaceae, Philydraceae, and Pontederiaceae (Simpson 1983*b*). It is likely, by outgroup comparison, that the tectate-columellate wall architecture is an ancestral feature for all these taxa possessing it. Thus, the similarities in aperture type and non-apertural exine architecture among all investigated taxa of the present study are probably primitive for the complex as a whole and can provide no infor-

mation as to the recognition of monophyletic taxa (sensu Hennig 1966).

Of the investigated taxa, only six genera, *Conanthera*, *Cyanella* (excepting *C. alba*, see below), *Odontostomum*, *Tecophilaea*, *Walleria*, and *Zephyra*, possess an operculum. Thus, the present study confirms the observations of Erdtman (1966) and provides new data for the genera *Odontostomum* and *Walleria*. The operculum of these six genera consists of an outer band of exine (generally resembling the nonapertural exine in sculpturing) situated parallel and median to the aperture. Based on TEM staining properties, the operculum appears to consist of an outer, usually tectate-columellate ektexine and an inner basal layer of more electron-dense endexine. The opercular ektexine usually resembles that of the non-apertural wall in sculptural features. Additionally, in all species of these six genera, the non-apertural exine, where it joins the aperture region, has a reduced foot-layer and columellae, which are replaced by a basal layer of electron-opaque endexine. Cytochemical tests of *Cyanella lutea*, using basic fuchsin and auramine O, indicate that the non-apertural exine and the bulk of the operculum is indeed ektexinous in composition. Thus, the structural distinctiveness of the operculum in these six genera lends support to the hypothesis that they are homologous and arose by a common evolutionary history. Furthermore, because such operculate apertures are not found in any taxa presumed to be closely related to the Tecophilaeaceae, it is proposed that the presence of this operculum constitutes a shared derived character (synapomorphy), providing evidence for the monophylesis (sensu Hennig 1966) of these six family genera.

As reviewed in a previous paper (Simpson 1983*a*), the classification of the Tecophilaeaceae as the tribe Conanthereae of the Haemodoraceae is not supported based on evidence from pollen wall architecture. The occurrence of a tectate-columellate exine architecture in the Tecophilaeaceae distinguishes it from the fourteen genera of the Haemodoraceae which possess a 1- to 3-layered, non-tectate-columellate exine architecture. Thus, the classifications of Bentham & Hooker (1883) and Melchior (1964), which treat the Conanthereae as a tribe of the Haemodoraceae, are refuted based on the present study and on embryological (de Vos 1961) and chemical (Harris & Hartley 1980) evidence. *Lanaria* and *Lophiola*, which Erdtman (1966) cited as similar to members of the Tecophilaeaceae, are similar to that family (and aberrant in the Haemodoraceae; Simpson 1983*a*) in having a tectate-columellate exine wall architecture. As previously discussed, however, the tectate-columellate architecture is probably an ancestral feature for these taxa and provides no evidence regarding phylogenetic relationships. Because *Lanaria* and *Lophiola* differ from the Tecophilaeaceae in lacking an operculum, there is no pollen evidence supporting their classification in that family. Additionally, the operculum of the Tecophilaeaceae approximates a "pontoperculate" aperture type, one in which two sulcate apertures are oriented in the polar hemisphere (e.g., as in the genus *Pauridia*; see Simpson 1983*a*). Both pontoperculate and operculate types consist of a median polar band of exinous wall material (similar to that of the non-apertural exine) which is flanked on both sides by a region of thick intine, essentially devoid of continuous exine. The operculate grains of the Tecophilaeaceae differ from the pontoperculate type essentially in that an operculum does not merge with the non-apertural exine at the equatorial "ends" of the pollen aperture. The similarities between operculate and pontoperculate aperture types may indicate the likelihood for one having been an intermediate stage to the evolution of the other; such a consideration may be fruitful in considering the interfamilial relationships of the Tecophilaeaceae.

The intine wall of the six operculate genera in the Tecophilaeaceae is 2-layered, as based on staining properties and fibrillar composition. One or both intine layers becomes greatly thickened in the apertural region, with radially oriented channels or vesicles occurring in the outer intine layer in almost all taxa. Interestingly, the apertural intine directly inner to the operculum is significantly thinner (sometimes forming a possibly artifactual apertural cavity, e.g., in *Cyanella lutea*) than that surrounding the operculum. Cytochemical studies of *Cyanella lutea* indicate that the outer, channeled layer of apertural intine is rich in pectic compounds (alcian blue positive), and the inner, fibrillar intine layer is primarily cellulosic in composition (PAS positive; see Kress & Stone 1982), thus substantiating the general observation in monocot pollen grains of a 2-layered intine, consisting of an outer, pectic-rich "exintine" and an inner, cellulosic "endintine" (terminology after Kress & Stone 1982).

Of the four species of *Cyanella* investigated, only *C. alba* lacks an operculum. The apertural exine of

this species consists instead of a very thin, rather electron-dense, often discontinuous layer (or layers) atop the thick intine. However, *C. alba* is similar to the operculate family members in having a basal layer of electron-dense endexine at the junction of non-apertural and apertural regions. Further studies are needed to determine if the absence of an operculum in *C. alba* is (with reference to other species of *Cyanella* and other genera of the family) an ancestral or a derived feature. If the lack of an operculum is a derived feature, then an ancestor of *C. alba* presumably possessed an operculum which became evolutionarily reduced to a thin apertural exine layer. If the lack of an operculum is a primitive feature (and if the operculum of other family members had a common origin), then the monophylesis (sensu Hennig 1966) of the genus *Cyanella* may be questionable.

Unlike the five genera of the Tecophilaeaceae with prominent opercula, the genus *Walleria* has an apertural membrane with an outer ridge of exinous deposits situated median and parallel to the aperture. This apertural exine is 2-layered, consisting of an inner, thin layer and an outer layer of verrucate to gemmate elements. In both examined species the inner, thin layer of apertural exine is clearly electron-dense and apparently endexinous. Endexine also was observed in *W. mackenzii* as a basal layer at the junction of nonapertural wall with aperture, resembling in all respects the other genera of the Tecophilaeaceae. Thus, based on structure and staining properties, the apertural exine of *Walleria* is probably homologous with the opercula of other family members. However, further research (developmental studies and formal phylogenetic analyses) is needed to confirm the homology between the operculum of *Walleria* and that of other family members and, if confirmed, to assess whether the "operculum" of *Walleria* is an ancestral (intermediate to a tectate-columellate operculate exine) or a derived (by reduction of operculate ektexine) feature.

The single species of *Cyanastrum* investigated differs from other members of the Tecophilaeaceae (except *Cyanella alba*) in lacking an operculum or a line of apertural exine (as in *Walleria*) which may be homologous to an operculum. The apertural exine of *Cyanastrum* is composed of groups of tectate-columellate deposits which are irregularly scattered on the aperture face. Because the presence of monosulcate grains and tectate-columellate exine structure are probably ancestral features (see above), the present palynological study provides no evidence for the inclusion of *Cyanastrum* in the Tecophilaeaceae, sensu Hutchinson (1973). As cited earlier, Erdtman (1966) remarked that pollen of *Cyanastrum* is more similar to that of *Philydrella* (Philydraceae) and *Stemona* (Stemonaceae) than to members of the Tecophilaeaceae. However, ultrastructural studies of the Philydraceae (Simpson 1983*b*) do not confirm any significant palynological similarities with *Cyanastrum*. It seems best at this time to retain the designation of the monogeneric family Cyanastraceae (sensu Engler 1930, Cronquist 1981, Dahlgren & Clifford 1982).

No evidence of any major palynological similarity is to be seen between *Eriospermum* and the six operculate members of the Tecophilaeaceae. Pollen grains of the two examined species of *Eriospermum* lack an operculum or, in fact, any apertural exine at all. In addition, no evidence of endexine, which occurs at the aperture periphery in all operculate genera of the Tecophilaeaceae, was observed in *Eriospermum*. Although *Eriospermum* pollen does resemble all other examined taxa in having a tectate-columellate exine wall and has a foveolate sculpturing similar to some operculate Tecophilaeaceae, these features are of wide occurrence among several monocot families of this general complex and are presumed to be ancestral (see above). Therefore, this palynological study provides no support for the classification of Takhtajan (1980), which grouped *Eriospermum* within the Tecophilaeaceae, nor for the suggestion by Dahlgren & Clifford (1982) of a"close" relationship between *Eriospermum* and *Walleria*.

In conclusion, the present pollen ultrastructural study supports the inclusion of six genera, *Conanthera, Cyanella, Odontostomum, Tecophilaea, Walleria*, and *Zephyra*, in the family Tecophilaeaceae, as suggested by Dahlgren & Clifford (1982). I hypothesize that the common possession of an operculate aperture, found in *no other* taxa presumed related to the complex (Erdtman 1966, Simpson 1983*a, b*, work in progress) constitutes a unique, shared derived character for these six genera. The absence of an operculum in *Cyanella alba* is hypothesized to be by reduction within the family. The inclusion of *Odontostomum* or *Walleria* in the Liliaceae (Bentham & Hooker 1883, Willis 1975) is refuted based on the occurrence of an operculum in these genera. The operculum of *Wal-*

leria, although aberrant, is nevertheless viewed as homologous, based on structural and staining properties, to that of other family members. Pollen grains of the genus *Cyanastrum*, which show no indication of an operculum, provide support for the classification of that genus as a separate family, the Cyanastraceae (sensu Engler 1930, Cronquist 1981, Dahlgren & Clifford 1982). Finally, the absence of an operculum in pollen of *Eriospermum* tends to refute the classification of that genus in the Tecophilaeaceae (sensu Takhtajan 1980) and provides no evidence for a close taxonomic relationship with *Walleria* (sensu Dahlgren & Clifford 1982).

ACKNOWLEDGEMENTS

This study was supported in part by United States National Science Foundation dissertation improvement award grant, DEB-81-09909, and is largely derived from a Ph.D. dissertation submitted to the Department of Botany, Duke University. I wish to thank Robert Ornduff and Roy E. Gereau for providing FAA fixed plant materials; Gwen C. Roney for helpful suggestions with the first drafts; W. John Kress and Donald E. Stone for reviewing the manuscript; and herbaria at K, MO, MSC, and US for supplying dried and fixed materials or for housing vouchers.

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