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Pollen ultrastructure of the Philydraceae

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Pollen morphology, sculpturing, and wall ultrastructure of the five species in the monocot family Philydraceae were investigated in order to assess phylogenetic relationships. All members of the Philydraceae have monosulcate, heteropolar pollen grains with a tectate-columellate exine having distinctive lamellae inner to the foot-layer. Histochemical tests of *Philydrum lanuginosum* indicate an ektexinous exine composition. The aperture wall of all members of the family consists of a thick, 2-layered intine with exine absent or composed of scattered deposits. The inner intine layer is infused with numerous vesicular or channel-like structures. Histochemical tests of *Philydrum lanuginosum* suggest that the outer intine layer is primarily cellulosic and the inner intine layer is pectic-rich, a trend opposite from that noted in pollen of other monocot taxa. Palynological similarities between the Philydraceae and related families, including monosulcate apertures and a tectate-columellate exine, are hypothesized to represent ancestral features which are of no value in assessing phylogenetic relationships.

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The Philydraceae (sensu Hamann 1966) are a small monocot family of four genera and five species: *Helmholtzia* (2 species; eastern Australia, New Guinea), *Orthothylax* (monotypic; eastern Australia); *Philydrella* (monotypic; western Australia), and *Philydrum* (monotypic; Australia, Malaysia, and eastern Asia). All family members are perennial, rhizomatous or cormose herbs having distichous, ensiform (equitant, unifacial) leaves and a simple to branched spicate, bracteate inflorescence. The Philydraceae probably constitute a monophyletic taxon as evidenced by the occurrence of a distinctive and apparently derived floral structure, consisting of: fusion of the two outer latero-posterior tepals with the inner posterior tepal and presence of a single, anteriorly positioned stamen (Hutchinson 1973, Hamann 1966).

Because of the morphological homogeneity of the Philydraceae, taxonomists have encountered few differences as to the circumscription and intrafamilial classification of the family (see Hamann 1966). Interfamilial classifications of the Philydraceae have varied; most systems place the family "near" to either the Haemodoraceae or Pontederiaceae. For example, Engler & Prantl (1930) placed the Philydraceae adjacent to the Pontederiineae (Pontederiaceae and Cyanastraceae), whereas Hutchinson (1934, 1959, 1973) classified the Philydraceae with five other families (Taccaceae, Apostasiaceae, Velloziaceae, Hypoxidaceae, and Haemodoraceae) in his order Haemodorales. Hamann (1966) suggested close affinities between the Philydraceae and Pontederiaceae and possibly also to the Haemodoraceae and Hypoxidaceae. The Philydraceae was classified by Dahlgren (1980) and Dahlgren & Clifford (1982) as the order Philydrales, closely related to the Haemodorales, Pontederiales, Velloziales, and Commelinales. Thorne (1976) grouped the Philydraceae with the Pontederiaceae in the suborder Pontederiineae (Commeliniflorae), to which Thorne (1981), based on chemical evidence (Harris & Hartley 1980) added the Haemodoraceae. The Philydraceae were thought to share common ancestry with the Pontederiaceae by Takhtajan (1980) because of similarities in anatomy and embryology. Finally, Cronquist (1981) placed the Philydraceae adjacent

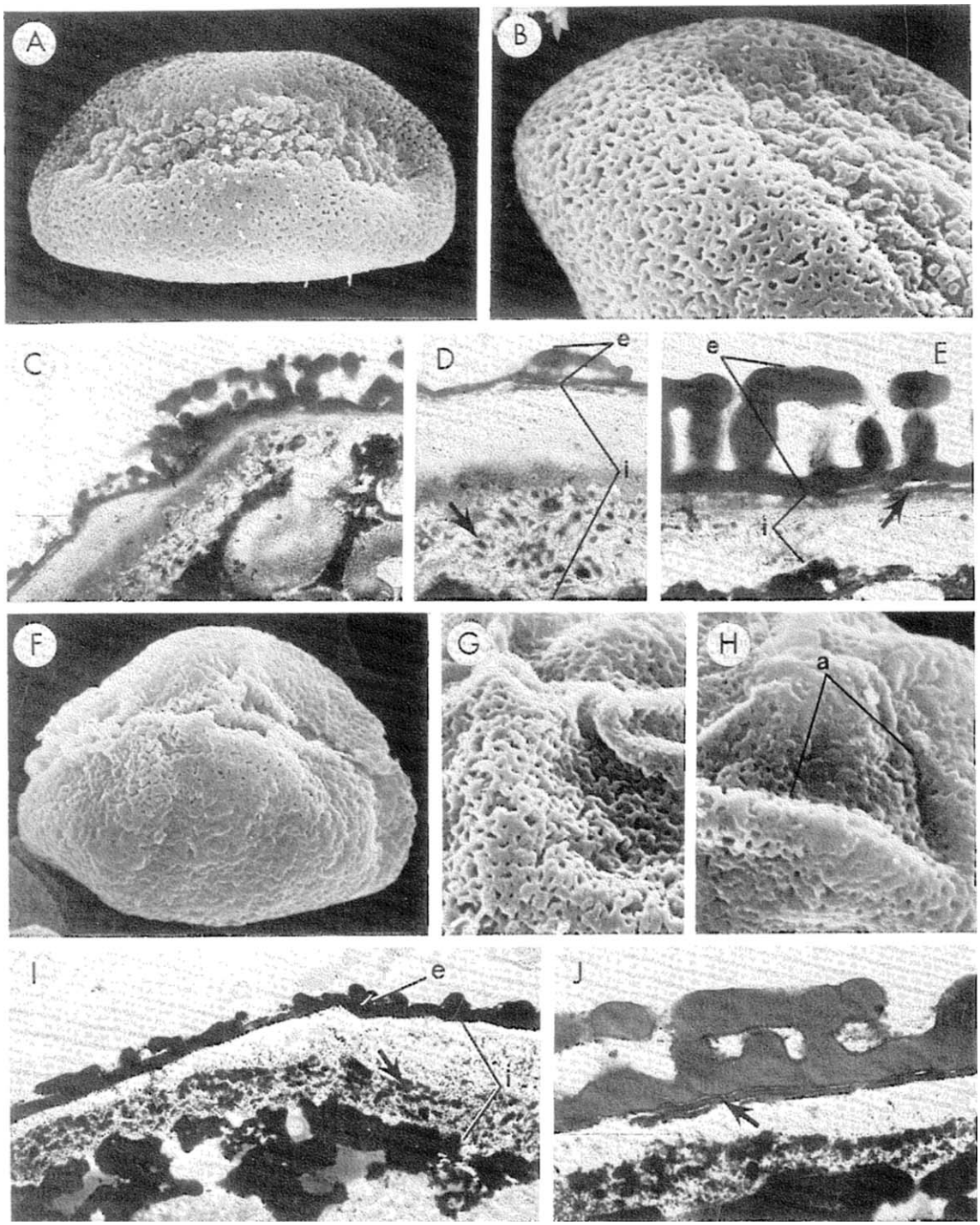

Fig. 1. Helmholtzia. A–E: *H. acorifolia.* (A) Whole grain, aperture above. SEM ×2700. (B) Close-up; note reticulate non-apertural wall and verrucate apertural elements. SEM ×5100. (C) Interface between apertural wall (lower left) and non-apertural wall; note, in apertural region, expansion of the 2-layered intine. TEM ×11000. (D) Apertural wall, showing thick, 2-layered intine (*i*) with numerous vesicles (arrow) of inner intine layer. Note exine (*e*) composed of a continuous thin layer and larger irregular exine elements. ×20000. (E) Nonapertural wall. Note 2-layered intine (*i*), with vesicular inner layer, and tectate-columellate exine (*e*), with lamellate inner foot-

to the Pontederiaceae and Haemodoraceae in the Liliales.

All four genera and five species of the family have been investigated palynologically with bright-field microscopy (Erdtman 1966, Hamann 1966 and references cited therein). Pollen grains of *Philydrum* are distinct in the family in being united as tetragonal tetrads at maturity; grains of the other four species are shed as monads (Erdtman 1966, Hamann 1966). Erdtman (1966) described *Orthothylax glaberrima, Philydrella pygmaea,* and *Philydrum lanuginosum* as monosulcate; *Helmholtzia acorifolia* was described by Erdtman as "probably 3-or zonisulcate." Hamann (1966), however, disagreed with Erdtman, and described both *H. acorifolia* and *H. novo-guineensis* as monosulcate. (Hamann reported that the "3-sulculate" condition described by Erdtman was possibly the result of a shrinkage effect from acetolysis.) With regard to sculpturing, Erdtman (1966) described *Orthothylax* and *Philydrella* as "reticulate (polybrochate)," and *Philydrum* as "reticulate (slightly heterobrochate; muri +/− duplibaculate)." Both species of *Helmholtzia* are reported to have a fine, unresolvable (with light microscopy) exine sculpturing (Hamann 1966). The sexine of all genera in the family is described as thicker than or as thick as the nexine (Erdtman 1966). Erdtman summarizes the Philydraceae as "pollenomorphologically a +/− heterogenous family."

The purpose of the present study is to describe and characterize, using SEM and TEM, the pollen sculpturing and ultrastructure of the Philydraceae in order to assess phylogenetic relationships of the family, particularly with reference to other monocotyledonous taxa. Of the families cited by the above authors to be closely related to the Philydraceae, the Cyanastraceae (Simpson, in press, Haemodoraceae (Simpson 1983*a*), Velloziaceae (Ayensu & Skvarla 1974), and selected members of the Hypoxidaceae, Pontederiaceae and Taccaceae (Simpson 1983*b*) have been investigated with respect to pollen morphology and wall ultrastructure. A tectate-columellate exine wall architecture is found in investigated members of the Cyanastraceae (*Cyanastrum*), Hypoxidaceae (*Hypoxis, Curculigo*), Pontederiaceae (*Heteranthera, Pontederia*), Taccaceae (*Tacca*), and Velloziaceae (*Barbecenia, Vellozia*). In contrast, fourteen genera of the Haemodoraceae have a 1- to 3-layered, non-tectate-columellate exine architecture (see Simpson, 1983*a*). Therefore, a primary goal of the present study is to determine what similarities and differences of pollen sculpturing or wall architecture occur between members of the Philydraceae and these presumed related families. In addition, the determination of whether these palynological features are ancestral or derived within this complex of families is vital in order to assess their value in recognizing monophyletic taxa (sensu Hennig 1966).

MATERIAL 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 species were examined (parentheses indicate herbaria where vouchers are deposited): *Helmholtzia acorifolia* F. v. Mueller "FAA" — M. G. Simpson 81-16A (DUKE); *H. novo-guineensis* (Krause) Skottsberg "DRIED" — L. J. Brass 12859 (A); *Orthothylax glaberrimus* (Hooker fil.) Skottsberg "FAA" — cult. Hort. Bot. Kew (Herb. U. Hamann, Bochum 1183); *Philydrella pygmaea* (R. Brown) Caruel "FAA" — M. G. Simpson 28IX81A (DUKE); *Philydrum lanuginosum* Banks & Solander ex Gaertner "FAA" — E. F. Constable 4128 (Herb. U. Hamann, Bochum 959, ex. Herb. NSW 58993).

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 of wall architecture, pollen samples were fixed in 4.2% buffered gluteraldehyde for 2 hours, rinsed 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 BEAM 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

layer (arrow). TEM ×21 000. F–J: *H. novo-guineensis*. (F) Whole grain, sulcate aperture above. SEM ×3 100. (G) Close-up, nonapertural wall. SEM ×4 700. (H) Close-up, edge of apertural wall (*a*). SEM ×4 600. (I) Apertural interface, aperture region to right. Note exine deposits (*e*) and thick, 2-layered intine (*i*), with vesicular inner layer (arrow). TEM ×16 000. (J) Non-apertural wall, showing tectate-columellate exine with prominent lamellae (arrow) at inner foot-layer. TEM ×34 000.

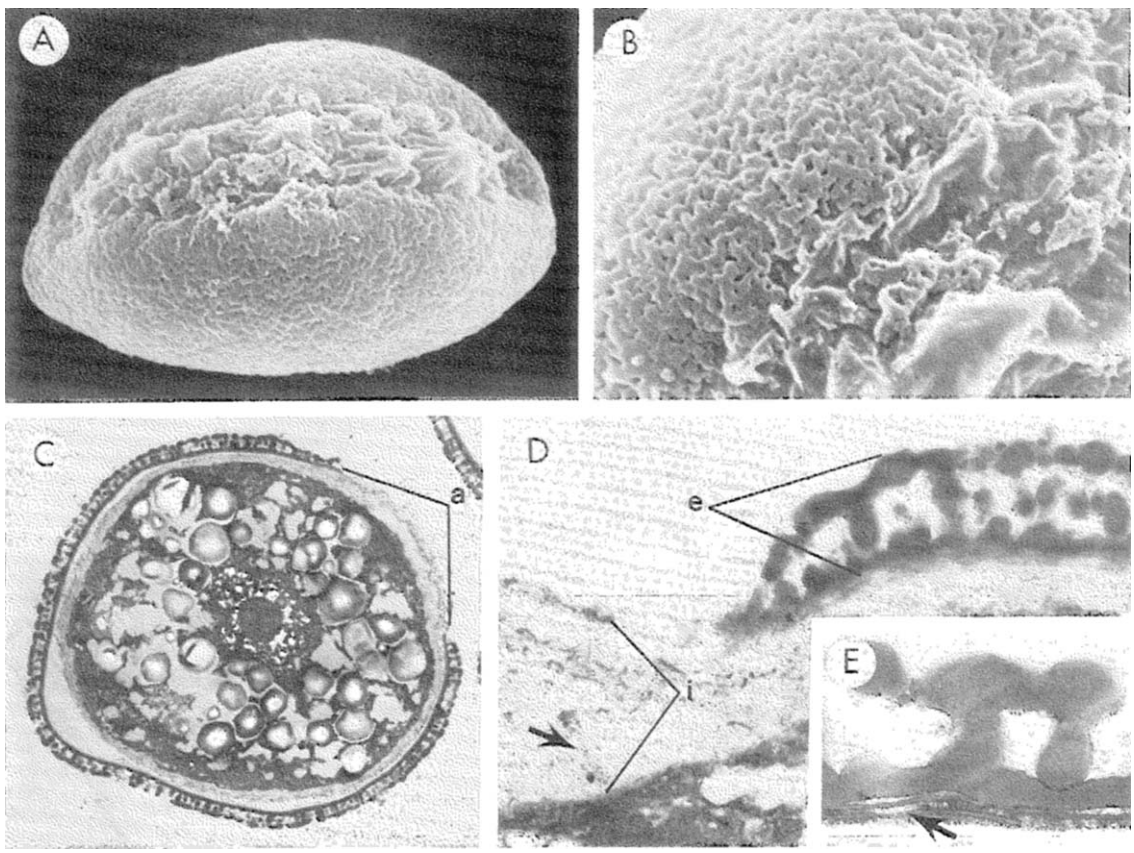

Fig. 2. Orthothylax glaberrimus (A) Whole grain, aperture above. SEM ×3500. (B) Close-up of non-apertural (left) and apertural (right) walls. SEM ×7000. (C) Grain cross-section. Note aperture (*a*). TEM ×4200. (D) Interface between apertural wall (left) and nonapertural wall (right). Note exine (*e*) and thick, 2-layered intine (*i*) of aperture, with obscure channels of inner layer (arrow). TEM ×10000. (E) Non-apertural wall with tectate-columellate exine, having lamellate inner foot-layer (arrow). TEM ×39000.

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

For cytochemical tests unacetolyzed, FAA-fixed pollen grains were stained, mounted whole in glycerol, and photographed in optical cross-section with brightfield or UV fluorescence microscopy, using a Leitz Laborlux 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 min). 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 min) and by bright yellow fluorescence with auramine O (0.01% in 0.05M tris-HCl buffer, pH 7.2, 5 min). 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 lengths (preceeding pollen descriptions) were measured from fixed or rehydrated grains mounted in 70% ethanol. Pollen terminology follows that of Walker & Doyle (1975).

RESULTS

Pollen Ultrastructure

Helmholtzia (2 of 2 species investigated)

H. acorifolia. — Grains 26 µm, monosulcate, heteropolar (Fig. 1A). Sculpturing of non-apertural wall reticulate, apertural surface with scattered verrucose or irregular elements (Fig. 1B). Non-apertural initine 2-layered, with vesicles in the inner layer (Fig. 1E). Apertural intine thick, 2-layered (Fig. 1C, D) with numerous contorted, vesiculare structures comprising the inner layer (Fig. 1D). Non-

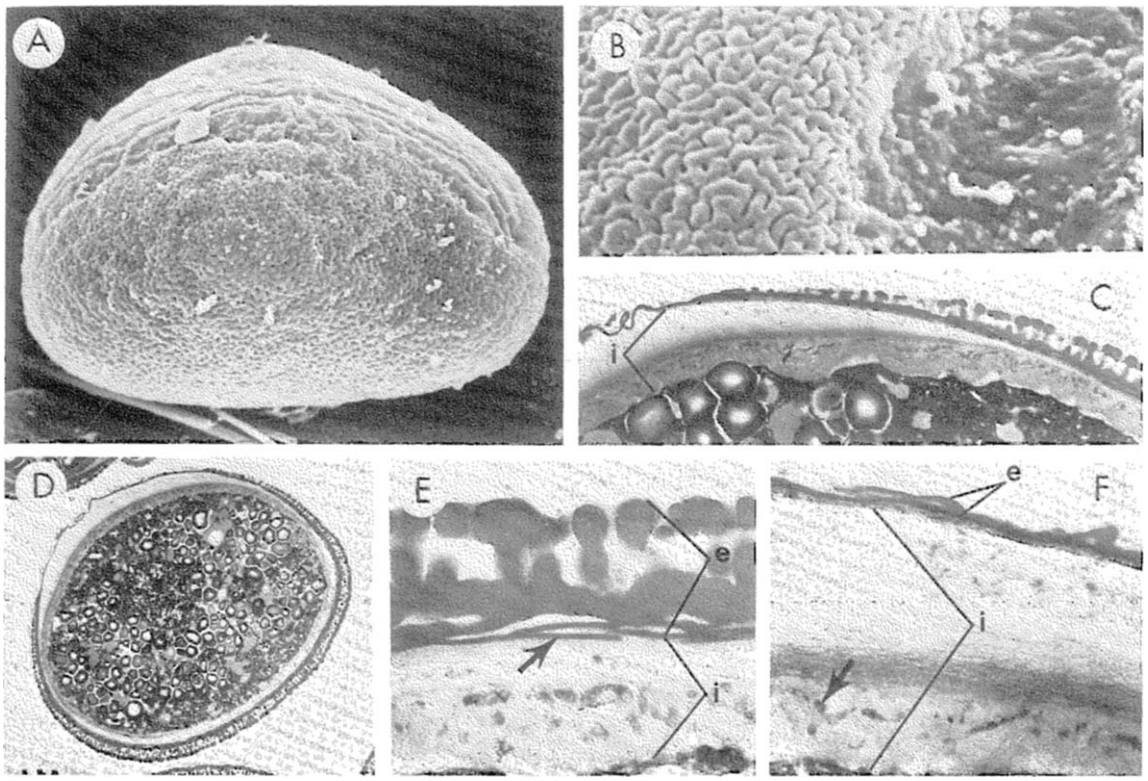

Fig. 3. Philydrella pygmaea. (A) Whole grain, aperture above. SEM ×2400. (B) Close-up of nonapertural (left) and apertural (right) walls. SEM ×8100. (C) Interface between apertural wall (left) and nonapertural wall (right). Note thick, 2-layered intine (*i*). TEM ×5600. (D) Whole grain cross-section, showing aperture (upper left). TEM ×1600. (E) Non-apetural wall. Note 2-layered intine (*i*), with vesicular inner layer, and tectate-columellate exine (*e*) with lamellate inner foot-layer (arrow). TEM ×22400. (F) Apertural wall. Note outer exine layer (*e*) and thick, 2-layered intine (*i*), with vesicles (arrow) in inner layer. TEM ×17800.

apertural exine tectate-columellate (semi-tectate), with a continuous, homogeneous foot-layer; lamellate, apparently ektexinous deposits present at inner foot-layer (Fig. 1E). Exine of aperture region composed of scattered, irregular, apparently ektexinous elements atop a thin, continuous nexine of uncertain composition (Fig. 1C, D).

H. novo-guineensis. — Grains 25 µm, monosulcate, heteropolar (Fig. 1F). Non-apertural wall reticulate (Fig. 1G), apertural wall obscure in material examined (Fig. 1H). Intine 2-layered, becoming thickened in apertural region, with electron-dense, irregular vesicles in inner layer (Fig. 1I). Exine of non-apertural region tectate-columellate, ektexinous, with prominent, apparently ektexinous lamellae inner to the homogeneous foot-layer (Fig. 1J). Apertural exine composed of a verrucose sexine and a thin, continuous nexine, the composition of either obscure in material examined (Fig. 1I).

Orthothylax (monotypic)

O. glaberrimus. — Grains 22 µm, monosulcate, heteropolar (Fig. 2A, C). Non-apertural wall foveolate to finely reticulate; apertural region with a few scattered, irregular exine elements (Fig. 2A, B). Intine of non-apertural and apertural regions faintly 2-layered (Fig. 2D), with obscure channel-like structures present in the inner apertural intine (Fig. 2D). Non-apertural exine tectate-columellate, apparently ektexinous, with a lamellated inner foot-layer (Fig. 2E). Apertural exine largely absent (Fig. 2A, C, D).

Philydrella (monotypic)

P. pygmaea. — Grains 39 µm, monosulcate, heteropolar (Fig. 3A, D). Non-apertural wall finely rugulose, apertural wall psilate to scabrate (Fig. 3B). Non-apertural intine 2-layered, with vesicular structures in the inner layer (Fig. 3E). Apertural

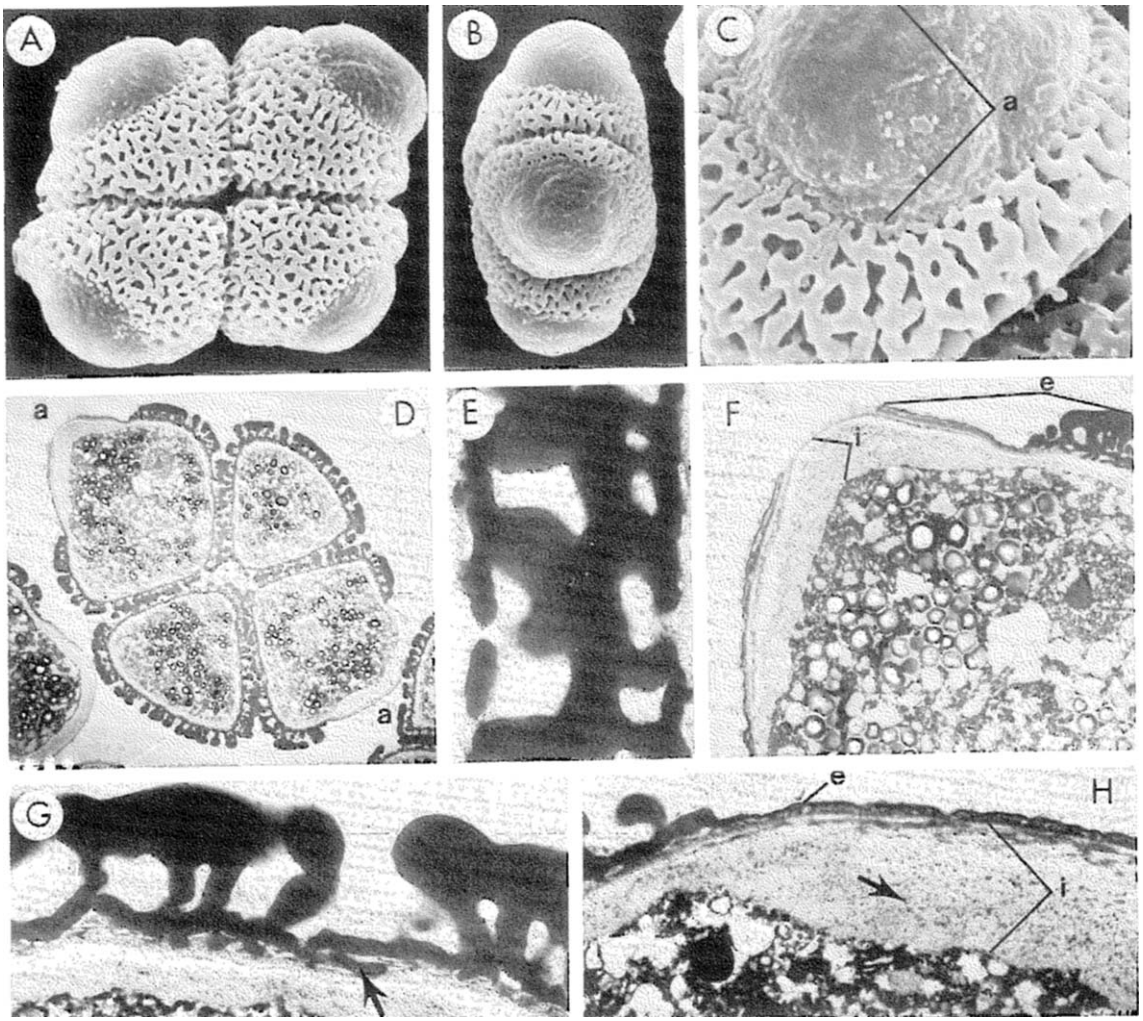

Fig. 4. Philydrum lanuginosum. (A) Tetragonal tetrad of grains, equatorial view. Note distal apertures. SEM ×1200. (B) Tetrad, polar view. SEM ×900. (C) Close-up of one grain. Note coarsely reticulate nonapertural wall and psilate, hemispheric aperture (*a*). SEM ×3100. (D) Tetrad, in slightly oblique sagittal section. Note apertures (*a*), visible in two grains. TEM ×900. (E) Exine of two contacting pollen grains. Note fusion in tectal region. TEM ×14000. (F) Aperture interface. Note exine (*e*), which is tectate-columellate in non-apertural wall (right), forming apparent thin, outer layer at aperture wall (left). Note thick, 2-layered intine (*i*) of aperture. TEM ×2900. (G) Non-apertural wall. Note lamellate inner foot-layer (arrow) of exine (*e*). TEM ×11000. (H) Apertural wall, showing thin, discontinuous exine layer (*e*) and thick, 2-layered intine (*i*), with vesicles (arrow) in the inner intine layer. TEM ×7400.

intine thick, 2-layered, with an electron-dense region between layers and with vesicles present in the inner layer (Fig. 3C, F). Exine of non-apertural region tectate-columellate, apparently ektexinous, with a prominently lamellate inner foot-layer (Fig. 3E). Apertural exine composed of a thin, continuous layer of uncertain composition, with occasional, tangentially flattened, apparently ektexinous outer elements (Fig. 3F).

Philydrum (monotypic)

P. lanuginosum. — Grains 34 μm, monosulcate, heteropolar (apertures distal; Fig. 4D), shed in tetragonal tetrad units (Fig. 4A, B). Wall of non-apertural region coarsely reticulate (Fig. 4A, C), apertural wall psilate, somewhat hemispheric in shape (Fig. 4A, B, C). Non-apertural intine thin, 2-layered (Fig. 4G). Apertural intine thick, 2-layered (Fig. 4F), the inner intine layer with fine vesicular

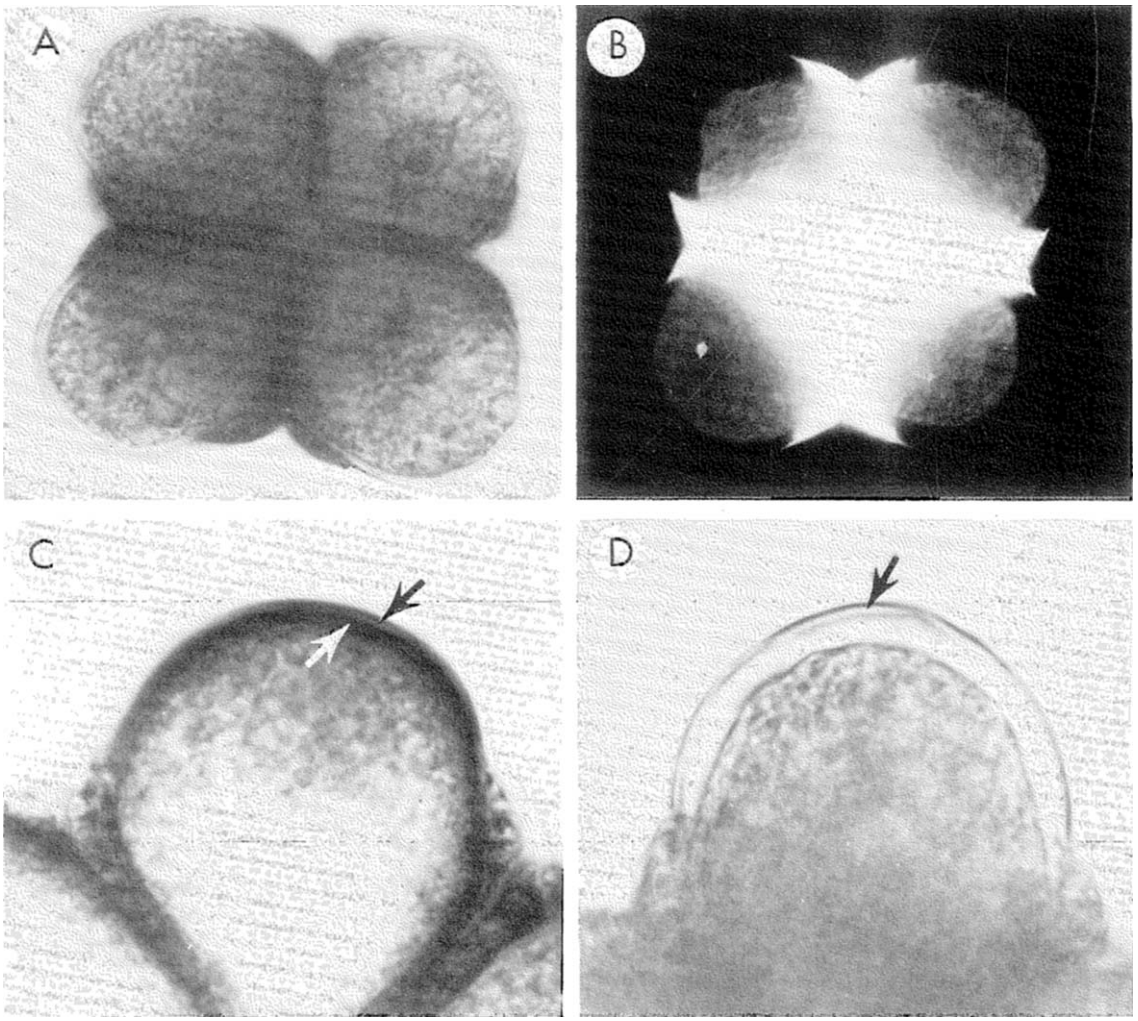

Fig. 5. Philydrum lanuginosum. (A) Basic fuchsin staining. Note positive (dark red) reaction of exine. ×1000. (B) Auramine O staining, showing positive fluorescence of exine. ×800. (C) Alcian blue staining. Note dense staining (dark blue, at arrow) of inner apertural intine and moderate (light blue) staining of outer apertural intine. ×1700. (D) PAS treated, showing staining (light pink) of outer intine layer (arrow). ×1700.

structures (Fig. 4H). Exine of non-apertural wall tectate-columellate, apparently ektexinous, with a lamellate inner foot-layer (Fig. 4G). Exine tectal region of adjacent grains fused at point of contact (Fig. 4E). Apertural exine generally confined only to peripheral region of aperture (Fig. 4F), composed of a thin, apparently ektexinous layer and 1–2 subtending, electron-dense layers (Fig. 4H).

Wall Chemistry

Philydrum lanuginosum. — Exine of non-apertural wall with positive basic fuchsin reaction (staining dark red; Fig. 5A) and positive auramine O fluorescence (fluorescing bright yellow; Fig. 5B). Inner apertural intine alcian blue positive (staining dark blue; Fig. 5C). Outer apertural intine layer PAS positive (staining pink; Fig. 5D).

DISCUSSION

All five species of the Philydraceae are palynologically similar in having monosulcate, heteropolar pollen grains. The present study, thus, agrees with the observations of Hamann (1966), who described

Helmholtzia acorifolia as monosulcate and not 3-sulculate as reported by Erdtman (1966). More importantly, all members of the Philydraceae show basic similarities in pollen wall architecture. For all five species the exine of the non-apertural region is tectate-columellate with distinctive lamellae attached at the inner surface of the homogeneous foot-layer. In *Philydrum lanuginosum* this exine wall stains positively with basic fuchsin and fluoresces positively with auramine O, both tests indicative of an ektexinous wall composition (Kress & Stone 1982). Preliminary histochemical studies by the author indicate that the exine of the other four species is similarly ektexinous. The composition of the inner exinous lamellae could not be resolved from these histochemical tests. Based on TEM micrographs, however, the lamellae appear similar in structural composition and identical in staining properties to the foot-layer, columellae, and tectum of known ektexinous composition. Because endexine generally has different TEM staining properties from ektexine (Kress & Stone 1982), it is likely that the lamellate structures of these taxa is ektexinous in chemical make up.

All family members show similarities in the composition of the aperture wall. The apertural intine in all species is thick and 2-layered. Numerous vesicular structures are present in the inner intine layer; an electron-dense fibrillar region usually occurs at the interface between inner and outer intine layers. In *Philydrum lanuginosum* this inner, vesicular intine layer is pectic-rich (alcian blue positive) and the outer intine layer is predominantly cellulosic (PAS positive). The exine of the apertural region is variable among members of the Philydraceae and may be absent (as in *Orthothylax glaberrimus*) or consist of scattered, verrucose elements overlying a thin, continuous nexine (all other species). From the present studies it is unclear as to the composition of these layers; both layers appear to have TEM staining properties similar to the ektexine of the non-apertural region. However, developmental studies may be needed to determine whether one or more of these layers might be endexinous instead.

Few significant palynological similarities are noted between the Philydraceae and investigated members of presumed relatives, thus providing no firm evidence for the interfamilial relationships as suggested, e.g., by Hutchinson 1973, Thorne 1981, and Dahlgren 1980. Members of the Haemodoraceae are distinct from the Philydraceae in having a 1- to 3-layered exine wall architecture which lacks a typical tectate-columellate architecture (Simpson 1983*a*). Although members of the Cyanastraceae, Hypoxidaceae, Taccaceae, Pontederiaceae, and Velloziaceae have a tectate-columellate exine wall architecture (similar to that of the Philydraceae), this wall architectural type is common among angiosperms as a whole (Walker & Doyle 1975) and probably primitive for this complex of monocotyledons (Simpson 1983*a*, *b*, 1985).

In addition, the apertural intine of investigated members of the Cyanastraceae, Haemodoraceae, Hypoxidaceae, Taccaceae, and Pontederiaceae consists of an inner fibrillar layer and an *outer* layer infused with channels or vesicles, thus being opposite to that of the Philydraceae. Although the intine of all members of the Philydraceae shows structural resemblance (in location of vesicles) to that of some monocotyledonous taxa (e.g., grasses, such as *Zea mays*; see Skvarla & Larson 1966, Kress & Stone 1982), the pattern of staining is opposite to the common trend encountered among monocotyledons, including *Zea* (i.e., an outer pectic-rich "exintine" and an inner, predominantly cellulosic "endintine"; Kress & Stone 1982). The significance of this "inversion" of inner wall chemistry in the Philydraceae is not at present known.

Of presumed Philydraceae relatives investigated to date, only certain species of *Vellozia* (Ayensu & Skvarla 1974) show some similarity to the Philydraceae in having a lamellate inner exine foot-layer and in having grains shed as tetragonal tetrads, as in *Philydrum*. However, species of *Vellozia* differ from *Philydrum* and other Philydraceae in having an irregular to verrucate sculpturing, in lacking well defined apertures, and in having a very irregular tectate-columellate architecture in several species (Ayensu & Skvarla 1974). Thus, in view of the differences in pollen grain sculpturing and aperture type between *Philydrum* and *Vellozia* and considering the numerous morphological differences between the two families, it is questionable that the tetragonal tetrad condition and inner lamellation in the two genera represent a shared evolutionary event.

Within the Philydraceae some minor palynological differences can be recognized between genera and species. The monotypic *Philydrum* is unique in the family in having grains shed in tetragonal tetrad units, rather than as monads. Four species in three genera (*Helmholtzia acorifolia, H. novo-guineen-*

sis, Orthothylax glaberrimus, and *Philydrum lanuginosum*) have a reticulate nonapertural wall sculpturing, that of *Philydrum* being very coarsely so. *Philydrella*, however, is distinguished from other family members in having a rugulate nonapertural sculpturing. With regard to pollen size, *Helmholtzia* (25–26 μm) and *Orthothylax* (22 μm) are similar, as are *Philydrum* (34 μm) and *Philydrella* (39 μm). The similarity in size between these pairs of genera corresponds to the generic phylogeny of the Philydraceae proposed by Hamann (1966).

In conclusion, the present study affirms the distinctiveness and homogeneity of the Philydraceae with respect to pollen ultrastructural characteristics. Thus, although Erdtman (1966) described the family as heterogeneous with regard to pollen unit and sculpturing, the wall ultrastructure of all family members is identical in having: 1) ektexine with distinctive lamellae at the inner surface of the foot-layer; and 2) a 2-layered apertural intine with vesicular structures concentrated in the *inner* (apparently pectic-rich) layer. These features, together with the characteristic and apparently derived floral structure in the Philydraceae (Hamann 1966) argue strongly for the monophylesis of the family. The palynological characters in common between the Philydraceae and most presumed related families (including monosulcate, heteropolar grains and a tectate-columellate exine architecture) are hypothesized to represent ancestral features, of no value in assessing phylogenetic history. Thus, a clarification of interfamilial relationships must await additional data or future studies (especially rigorous cladistic analyses) of existing data.

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REFERENCES

Ayensu, E. S. & Skvarla, J. J. 1974. Fine structure of Velloziaceae pollen. — Bull. Torrey Bot. Club 101: 250–266.

Cronquist, A. 1981. An integrated system of classification of flowering plants. — Columbia Univ. Press, New York.

Dahlgren, R. 1980. A revised system of classification of the angiosperms. — Bot. J. Linn. Soc. 80: 91–124.

Dahlgren, R. & Clifford, H. T. 1982. The Monocotyledons, a comparative study. — In: Botanical systematics: An occasional series of monographs. Vol. 2. — Academic Press, New York.

Engler, A. & Prantl, K. 1930. Die natürlichen Pflanzenfamilien. 2. Aufl. 15a: 190–191. — W. Engelmann, Leipzig.

Erdtman, G. 1966. Pollen morphology and plant taxonomy. Angiosperms. Corrected reprint and new addendum. — Hafner Publ. Co., New York.

Hamann, U. 1966. Embryologische, morphologisch-anatomische und systematische Untersuchungen an Philydraceen. — Willdenowia Beiheft 4: 1–178.

Harris, P. J. & Hartley, R. D. 1980. Phenolic constituents of the cell walls of monocotyledons. — Biochem. Syst. Ecol. 8: 153–160.

Hennig, W. 1966. Phylogenetic systematics. — The University of Illionois Press, Urbana.

Hutchinson, J. 1934. The families of flowering plants, 1st ed. vol 2, Monocotyledons. — Oxford Univ. Press, London.

Hutchinson, J. 1959. The families of flowering plants, 2nd ed., 2, vol. 2, Monocotyledons. — Oxford Univ. Press, London.

Hutchinson, J. 1973. The families of flowering plants, 3rd ed. — Oxford Univ. Press, London.

Jensen, W. A. 1962. Botanical histochemistry — principles and practice. — W. H. Freeman & Co., San Francisco.

Kress, W. J. & Stone, D. E. 1982. Nature of the sporoderm in monocotyledons, with special reference to the pollen grains of Canna and Heliconia. — Grana 21: 129–148.

Simpson, M. G. 1983*a*. Pollen ultrastructure of the Haemodoraceae and its taxonomic significance. — Grana 22: 79–103.

Simpson, M. G. 1983*b*. Systematics of the Haemodoraceae: Evidence from pollen ultrastructure, embryology, and anatomy. — PhD diss., Duke Univ., Durham, North Carolina.

Skvarla, J. J. & Larson, D. A. 1966. Fine structural studies of Zea mays pollen. I. Cell membranes and exine ontogeny. — Am. J. Bot. 53: 1112–1125.

Spurr, A. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. — J. Ultrastruct. Res. 26: 31–43.

Takhtajan, A. 1980. Outline of the classification of flowering plants (Magnoliophyta). — Bot. Rev. 46: 225–283.

Thorne, R. F. 1976. A phylogenetic classification of the Angiospermae. — Evol. Biol. 9: 35–106.

Thorne, R. F. 1981. New aspects and data in angiosperm classification, I. — XIII Intern. Bot. Congr., Sydney, Australia. Abstracts: 121.

Walker, J. W. & Doyle, J. A. 1975. The bases of angiosperm phylogeny: palynology. — Ann. Mo. Bot. Gard. 62: 664–723.