ON THE TAXONOMIC STATUS OF *LOPHIOLA AUREA* KER-GAWLER

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Familial and tribal treatments of the Haemodoraceae have been inconsistent, and there has been disagreement among various authorities over the inclusion of *Lophiola aurea* in the family (Geerinck, 1969).

Lophiola aurea = *(L. americana* (Pursh) Wood; see Robertson, 1976), which is the only species of *Lophiola,* grows in acid, pine-barren bogs from New Jersey to Florida, with a disjunct Nova Scotian population. The species has been variously treated, and placed in the tribe Conostyleae of the Amaryllidaceae (Pax, 1930), in the tribe Haemodoreae (Geerinck, 1969), and the Conostyleae of the Haemodoraceae (Hutchinson, 1973). Ornduff (1979), basing his conclusion on gross morphology and chromosome numbers, suggested that *L. aurea* is more closely allied with the tribe Haemodoreae and possibly with the genus *Lachnanthes,* the only other North American member of the Haemodoreae. However, Robertson (1976) investigated vegetative morphology, Simpson and Dickison (1981) investigated anatomy, and Simpson (1981) embryology, of *Lachnanthes* and *Lophiola,* and they found few similarities between these taxa. Our phytochemical and palynological studies of various taxa of the Haemodoraceae further suggest that *Lophiola aurea* is not closely related to other genera of Hutchinson's Haemodoreaceae.

MATERIALS AND METHODS

Pollen from living and dried herbarium material was used in this study. Live pollen was prepared for transmission electron microscopy (TEM) by fixation in cacodylate-buffered gluteraldehyde-formaldehyde followed by fixation with osmium tetroxide, dehydration in an ethanol series, and embedding in Dow Epoxy Resin-334 (DER-334). Pollen removed from herbarium specimens was acetolyzed and prepared for TEM by dehydration in an ethanol series, and embedded in DER-334. Sectioning was done on an LKB-1 ultramicrotome; the sections were post-stained in uranyl acetate-lead citrate,

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and viewed on a Philips EM-300. Pollen was prepared for scanning electron microscopy (SEM) by mounting the pollen on stubs with the high vacuum wax Apiezon W-100, coated with gold-palladium, and viewed on a Coates and Welter Field Emission Electron Microscope.

Dried, defatted, above ground parts of *Lophiola aurea* (Edwards, *s.n.*, New Jersey, CONN) were extracted with 95% ethanol. Thin-layer chromatography of the extract (Silica gel, ethyl acetate-benzene, 1:2) indicated the presence of two phenolic compounds (positive color reaction with diazotized *p*-nitroaniline) having R_f s 0.3 and 0.1. Column chromatography of the mixture over silica gel with ethyl acetate and increasing amounts of ethanol (95%) resulted in the isolation of the less polar compound as a pale yellow solid of mp 219–223° (ethyl acetate-petrol ether).

RESULTS

Palynology

Hutchinson (1973) includes ten genera in the tribe Haemodoreae. Three genera and three species were investigated palynologically; *Lachnanthes caroliana* (Lam.) Dandy, (*Weatherley s.n.*, Connecticut, CONN); *Wachendorfia paniculata* Burm., (*Zavada 501*, Cult., CONN); and *Xiphidium caeruleum* Aubl., (*Wolfe 234*, Surinam, CONN). Pollen of this tribe is monosulcate (Erdtman, 1952; Radulescu, 1973; present study). Pollen of *Lachnanthes caroliana* averages 36–44 μm along its long axis and sculpturing is scabrate to verrucate (fig. 1). Wall structure is atectate and infrequently traversed by minute channels (fig. 2). No endexine is evident. Pollen of *Wachendorfia paniculata* averages 55–58 μm along its long axis and sculpturing on the proximal and distal faces of the pollen grain is scabrate to pustulate (fig. 5). Separating the proximal and distal faces of the pollen grain is a psilate ridge (figs. 4, 5). Wall structure on the proximal and distal faces is granular with large and small spherical to irregularly shaped granules closely appressed to one another (fig. 3; cf. "structure grenue" of Van Campo and Lugardon, 1973). The wall structure of the psilate ridge differs in having a thick tectum with a granular layer beneath (fig. 4). No foot layer or endexine is evident. Pollen of *Xiphidium caeruleum* averages 40–55 μm along its long axis and pollen grains are psilate (figs. 6, 7). Wall structure is tectate with the tectum fused to irregularly shaped rod-like structures which are often interspersed with granules (fig. 7).

The tectum is occasionally traversed by minute channels (fig. 7). No foot layer or endexine is evident.

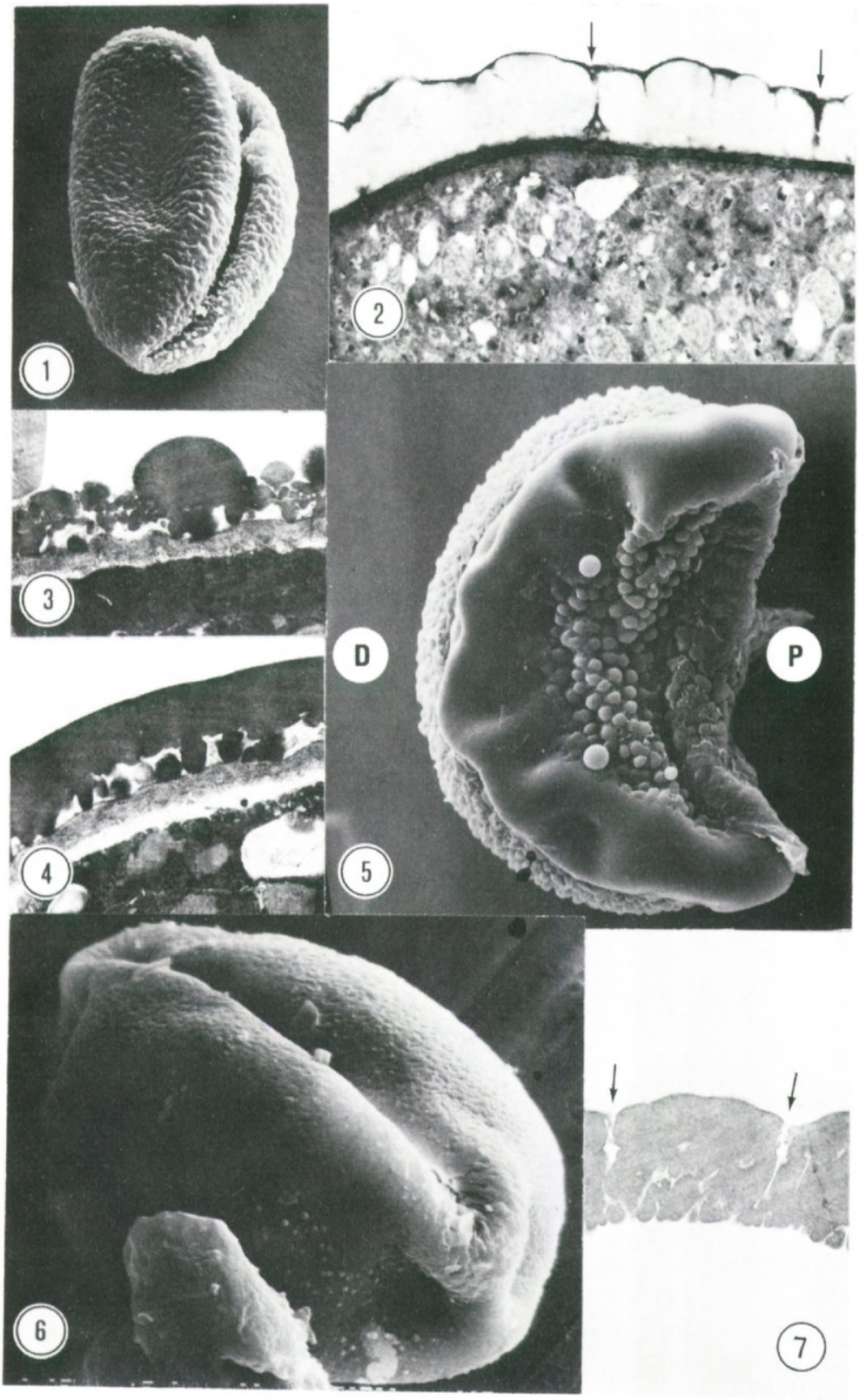
Hutchinson (1973) includes six genera in the tribe Conostyleae. Three genera and three species were investigated palynologically; *Anigozanthos flavus* D.C. ex Red., (*Edwards s.n.*, "1977", CONN); *Conostylis setosa* Lindl., (*Wolfe s.n.*, Australia, CONN); and *Lophiola aurea* Ker-Gawler, (*Edwards s.n.*, New Jersey, CONN). Pollen of this tribe is 2–8-porate, except *Lophiola aurea* which is monosulcate (Erdtman, 1952; Radulescu, 1973; present study). Pollen of *Anigozanthos flavus* averages 36–44 μm along its long axis and is diporate with the pores located opposite one another along its long axis (fig. 8). Sculpturing is roughly scabrate and wall structure is atectate (figs. 8, 9). No endexine is present. Pollen of *Conostylis setosa* is triporate averaging 22–29 μm along its long axis and pollen wall sculpturing is roughly scabrate (fig. 10). Wall structure is atectate and no endexine is present (fig. 11). Pollen of *Lophiola aurea* is monosulcate averaging 29 μm along its long axis. Pollen grains are finely reticulate (fig. 13). Wall structure is tectate-columellate with a thin foot layer and no endexine (fig. 12).

Phytochemistry

The yellow-colored compound isolated from silica gel chromatography had λmax (EtOH) 227 (Logϵ 4.49), 293 (4.51), and 330 sh (3.90) nm. $\sqrt{}$max 3270, 1650, and 1520 cm.$^{-1}$ δ (persilyl ether in $CDCl_3$ 60MHz) 7.22 (2H, d, 9Hz), 7.12 (2H, d, 9Hz), 6.76 (2H, d, 9Hz), 6.61 (2H, d, 9Hz), 5.94 (2H, dd, 2.5Hz), 5.76 (1H, s), 5.66 (1H, d, 2Hz), 5.22 (1H, q, 12 and 5Hz), 4.36 (1H, d, 12Hz), 3.00–2.78 (2H, m). M^+ (%) 542 (.02); 416.885, $C_{24}H_{16}O_7$ (65); 296.0317, $C_{16}H_8O_6$ (52); 270.0520, $C_{15}H_{10}O_5$ (3); 126 (40) and 94 (100). The MS of the persilyl ether had M^+ 974 indicating the presence of six hydroxyl groups in the molecule. Comparison of these spectral data with those published for the biflavanone GB–1a (Jackson et al., 1967 and Bandaranayake et al. 1975) indicated that the compound was 5, 7, 4′, 5″, 4‴ -hexahydroxy [3,8″] biflavanone.

DISCUSSION

The pollen data underscore the unique status of *Lophiola aurea* in the Haemodoraceae. *Lophiola aurea* is the only species investigated exhibiting the tectate-columellate wall structure and reticulate exine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sculpturing. The five other genera investigated have atectate or tectate-granular wall structure. The monotypic species *Lanaria plumosa* Ait., placed in the Haemodoraceae by Hutchinson (1973), is the only other genus reported to have reticulate exine sculpturing (Erdtman, 1952). The reticulate exine pattern is usually accompanied by the tectate-columellate wall structure. Thus, *Lophiola* and *Lanaria* are the only genera of the Haemodoraceae, as assigned by Hutchinson, exhibiting palynological features not found among the other genera of the family (Erdtman, 1952; present study). Their palynological features are more similar to those of some members of the closely related family Tecophilaeaceae (sensu Hutchinson). However, data on vegetative morphology (Robertson, 1976), anatomy (Simpson and Dickison, 1981), embryology (Simpson, 1981), and palynology and chemistry (present study) more firmly establish the differences between *L. aurea* and other members of the Haemodoraceae than the taxonomic affinities of *L. aurea*. This is due to the paucity of detailed morphological, anatomical, palynological and chemical data on the Tecophilaeaceae and other closely related families to the Haemodoraceae. Placement of *L. aurea* in any of these families would make that designation as tenuous as its present status.

Excluding *Lophiola aurea* (and *Lanaria plumosa*) from the Haemodoraceae highlights the stenopalynous nature of the remaining genera in each tribe. This lends support to a treatment of the Haemodoraceae similar to Hutchinson's, save the inclusion of *Lophiola* and *Lanaria*. The tribe Haemodoreae then only includes taxa with monosulcate pollen, a more primitive situation than the 2–8-porate

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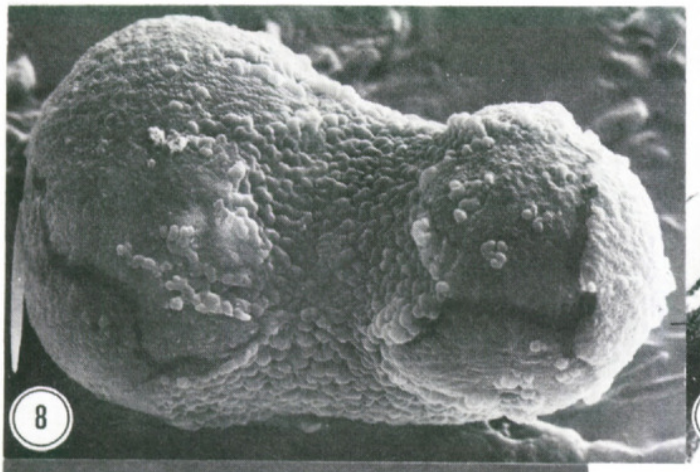
Figures 1–7. **Pollen of the Haemodoreae.** Figure **1.** *Lachnanthes caroliana*, acetolyzed, SEM × 1,530. Figure **2.** *L. caroliana*, unacetolyzed, TEM showing atectate wall structure, minute channels (arrows), and thin intine, × 17,000. Figure **3.** *Wachendorfia paniculata*, unacetolyzed, TEM showing spherical to irregularly shaped granules comprising the wall of the distal face of the pollen grain, note thin intine, ×14,200. Figure **4.** *W. paniculata*, unacetolyzed, TEM showing wall structure in the region of the psilate ridge, note thick tectum and granular layer beneath resting on a thin intine, ×14,200. Figure **5.** *W. paniculata*, acetolyzed, SEM showing scabrate and pustulate exine sculpturing on the proximal (P) and distal (D) faces of the pollen grain, note psilate ridge separating these regions, × 2,850. Figure **6.** *Xiphidium caeruleum*, acetolyzed, SEM showing sulcus, × 2,850. Figure **7.** *X. caeruleum*, acetolyzed, TEM showing granular infrastructure, also with irregularly shaped rods, note the minute channels (arrows) traversing the relatively thick tectum, ×25,000.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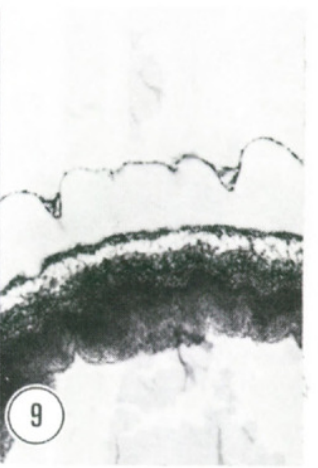
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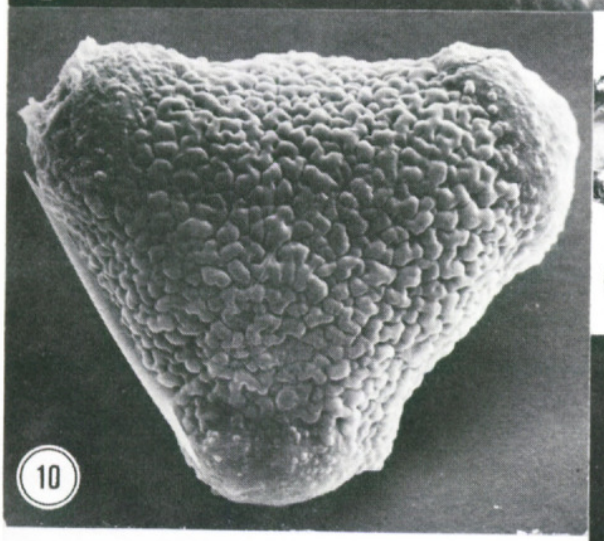
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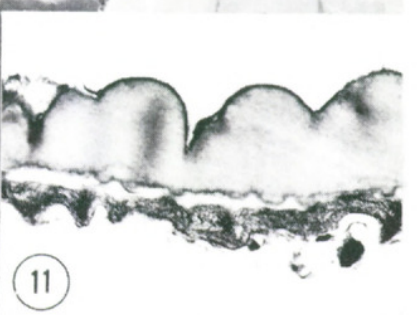
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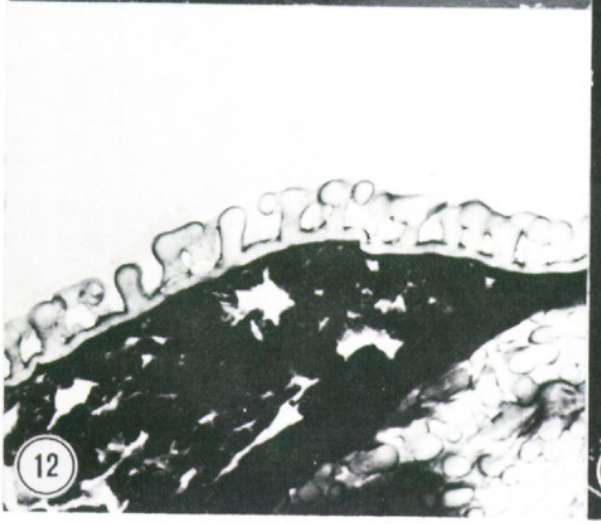
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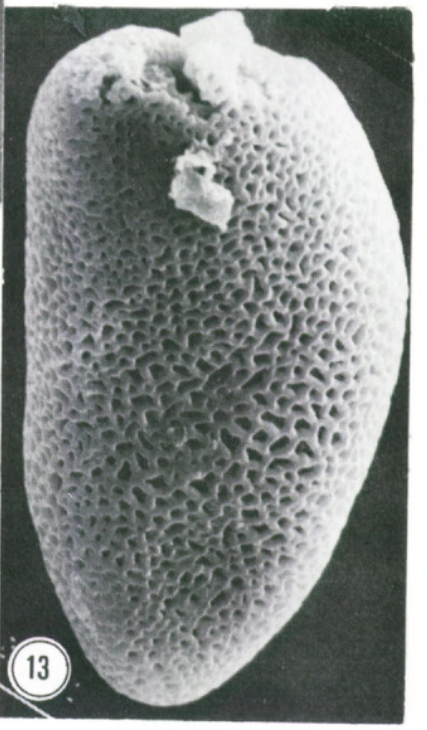
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pollen characteristic of the tribe Conostyleae. The evolutionarily advanced status of the entire family is supported by the occurrence of the atectate and granular wall structure: wall structural types occurring in the more advanced taxa of other monocot orders (Walker & Doyle, 1975; Zavada, manuscript in preparation).

Investigations of the yellow, orange, brown, and purple pigments present in the colorful root systems, flowers, and seed capsules of eight genera of the Haemodoraceae *(Anigozanthos, Conostylis, Haemodorum, Lachnanthes, Macropidia, Phlebocarya, Wachendorfia,* and *Xiphidium)* have shown them to be derivatives of either 9-phenalenone or naphthoxanthenone. Furthermore, secondary metabolites containing the phenalenone nucleus, or having structures which can reasonably be presumed to have been derived from an intact phenalenone, are rare in nature; their occurrence seems to be restricted to four genera of hyphomycetes (Fungi Imperfecti), one genus within the class Discomycetes, and one family of higher plants. These chemical compounds seem to be chemotaxonomic markers for the Haemodoraceae (Cooke & Edwards, 1981).

Lophiola aurea lacks obvious pigmentation except for its pale yellow flowers, and previous investigation of the plant (Edwards, et al., 1970) has shown it to be devoid of phenalenone and related pigments. The present study, while confirming the absence of phenalenones, has identified the biflavanoid 5, 7, 4′, 5″, 7″, 4‴-hexahydroxy [3,8″] biflavanone (GB 1a) in extracts of *L. aurea,* a compound which has been isolated previously from species of *Garcinia* (Guttiferae) (Jackson, et al., 1967; Bandaranayake, et al., 1975). Biflavonoid compounds have been isolated from some 20 plant families, none of which is monocotyledonous (Geiger & Quinn, 1975); furthermore, no flavonoids have been isolated from the Haemodoraceae. There is an account of the chromatographic

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Figures 8–13. **Pollen of the Conostyleae.** Figure **8.** *Anigozanthos flavus,* acetolyzed, SEM showing the two opposing pores, ×1,760. Figure **9.** *A. flavus,* unacetolyzed, TEM showing atectate wall structure and thick intine, × 17,100. Figure **10.** *Conostylis setosa,* acetolyzed, SEM showing triporate condition, × 2,370. Figure **11.** *C. setosa,* unacetolyzed, TEM showing atectate wall structure and thin intine, × 20,100. Figure **12.** *Lophiola aurea,* unacetolyzed, TEM showing tectate-columellate wall structure, × 20,100. Figure **13.** *L. aurea,* acetolyzed, SEM showing reticulate exine sculpturing on the distal face of the pollen grain, a sculpturing type found in the monotypic genus *Lanaria plumosa* of the Haemodoraceae, × 4,220.

identification of flavonoids in a *Haemodorum* sp. (Bate-Smith, 1968; Gornall, et al., 1979), but no flavonoids have been isolated; the phenalenone pigments are so abundant in *Haemodorum* that the identification of flavonoids by chromatographic methods alone must be regarded as tentative at best.

We feel that the chemical findings reported here: absence of phenalenone and naphthoxanthenone pigments and the presence of a biflavonoid, in conjunction with the palynological data, provide good chemotaxonomic and palynological evidence against the inclusion of *Lophiola aurea* in the Haemodoraceae.

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