POLLEN MORPHOLOGY OF SAPROPHYTIC TAXA IN THE GENTIANACEAE[1]

Siwert Nilsson[2] and John J. Skvarla[3]

Abstract

Pollen morphology of the saprophytic genera *Bartonia, Cotylanthera, Leiphaimos, Obolaria, Voyria,* and *Voyriella* (Gentianaceae) was studied by light and electron microscopy. *Bartonia, Obolaria,* and *Cotylanthera* are similar in fine structure, although the exine of the first two genera is reticulate and smooth in the latter genus. *Leiphaimos* and *Voyria* are indistinguishable but markedly different from all the other genera. *Voyriella* does not resemble *Voyria* or *Leiphaimos,* but appears similar to genera such as *Curtia* or *Enicostema* of Gilg's subtribe Gentianeae-Erythraeinae. Considerable intraspecific variation was noted within some of the taxa.

The Gentianaceae includes a number of saprophytic genera: *Cotylanthera, Obolaria, Bartonia, Voyria, Voyriella,* and *Leiphaimos* (Gray, 1868[4]; Johow, 1889; Knoblauch, 1894; Gilg, 1895; Figdor, 1896; Holm, 1897; Oehler, 1927). *Cotylanthera* has an Asiatic to Australasiatic distribution, *Bartonia* and *Obolaria* occur in eastern North America, and *Voyria, Leiphaimos,* and *Voyriella* are distributed in Central America, the West Indies, South America and tropical Africa.

The pollen morphology of these genera has been considered in earlier taxonomic, morphological and cytological studies (Gray, 1848; Gilg, 1895; Köhler, 1905; Oehler, 1927; Jonker, 1936*a,* 1936*b;* Erdtman, 1952; Raynal, 1967). Since questions concerning the taxonomic relationships of some of these genera still persist, we have re-evaluated the pollen by light microscopy and extended the study to include electron microscopy.

Materials and Methods

Pollen was removed from herbarium specimens. Most samples were first processed by acetolysis treatment (Erdtman, 1960), while a few were soaked in 70% alcohol prior to further processing. For light microscopy only acetolyzed pollen was utilized. The pollen was mounted in glycerine jelly on microscope slides; coverslips were affixed and sealed with paraffin. Observations were made with a Leitz-Laborlux microscope. Size measurements generally are based on ten pollen grains.

For electron microscopy, acetolyzed and 70% alcohol treated pollen was stained with OsO_4 and uranyl acetate, or staining was omitted. Subsequent incorporation into agar, alcohol dehydration, and embedding in Araldite-Epon resins follows Skvarla (1966). Thin sections were made with diamond knives; section stains used were uranyl acetate, lead citrate, and saturated solutions of $KMnO_4$ in acetone. Observations and electron micrographs were made with a

---

[1] The authors extend their thanks to the Directors of the various herbaria for permission to collect samples of pollen. Special thanks are due to Professor F. P. Jonker for fruitful discussions on taxonomy and for verifying the specimens deposited at Utrecht, and also to Dr. W. Punt (Utrecht) for immeasurable help in supplying material and in some cases preparing slides. We are grateful to Mrs. Carole Pyle for assistance in preparing the manuscript.

[2] Palynological Laboratory, 171 42 Solna, Sweden.

[3] Samuel Roberts Noble Laboratory of Electron Microscopy, Department of Botany and Microbiology, The University of Oklahoma, Norman, Oklahoma 73069. Supported in part by NSF grant GB-6768.

[4] Gray regarded *Eophylon* (*i.e. Cotylanthera*) as parasitic.

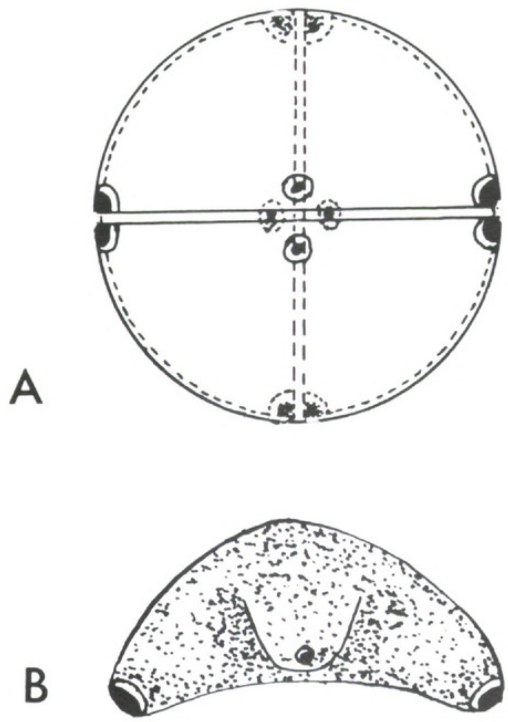


FIGURE 1.—Schematic drawings after photomicrographs of *Voyria clavata*, × 1,500. — A. Tetrad arrangement with 12 pores. — B. Single grain in lateral view, pore seen in face view smaller in size.

Philips EM-200 electron microscope. Acetolyzed, gold-coated pollen of *Voyria clavata* was examined with a Stereoscan Mk IIa scanning reflection electron microscope, at the Swedish Geological Survey, Stockholm.

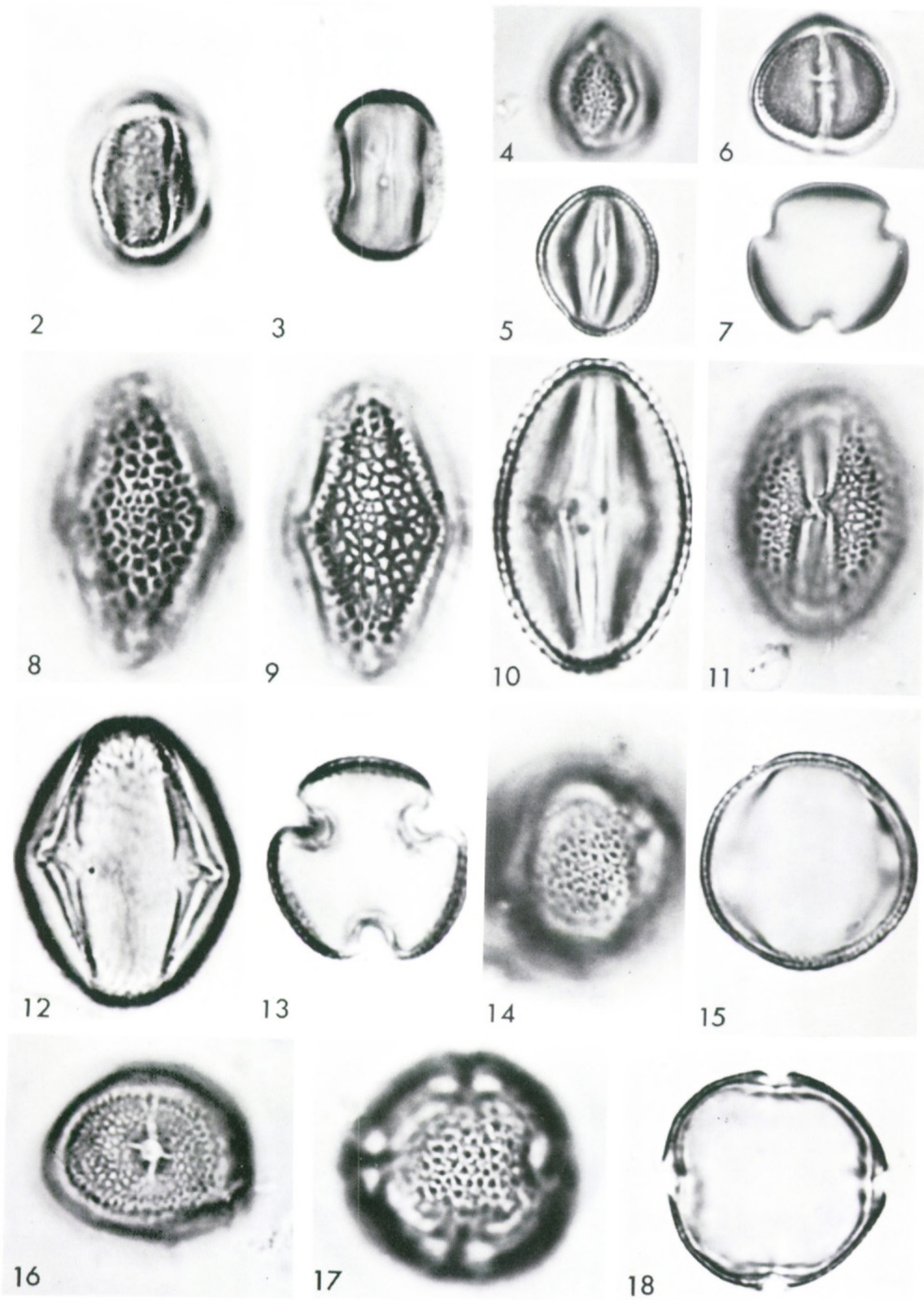
RESULTS

Surface morphology was observed by light microscopy and occasionally by scanning electron microscopy, and fine structural characters were analyzed by transmission electron microscopy. Descriptions of surface morphology follow Erdtman (1943, 1952, 1966). Electron micrographs of thin sections are interpreted on the basis of exine stainability, and the terminology of Faegri (1956) is used. Accordingly the ektexine corresponds to sexine and nexine 1, and nexine 2 is equivalent to endexine.

Cotylanthera Blume. Fig. 2–3.

Pollen grains radially symmetrical, isopolar, oblate spheroidal to prolate (-perprolate), outline oval or rhomboid in lateral view, rounded-triangular and trilobate in polar view; 11–15 × 5–13μ; tricolporate, colpi comparatively wide, slightly constricted at equator, ora traceable in side view only, diameter of apocolpia 4–7μ; exine ca. 0.5μ thick, sexine appears to be as thick as nexine, smooth to granulate (LO-pattern), baculate.

Specimens examined: *Cotylanthera tenuis* Blume.—NEW GUINEA: Demta near Hollandia *BW 4121* (L); Island of Japen, Samberi, *Aët & Idjan 151* (L). SUMATRA: Korthals, *L. 909-57-418* (L). PHILIPPINES: Rizal, Luzon, *Loher 14701* (UPS).


2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Intraspecific variation. One specimen *(Loher 14701)* differs by having extremely prolate (perprolate) pollen grains which are comparatively narrow in lateral view.

Fine structure (Fig. 49–50). The endexine is sharply differentiated from the ektexine by electron density. The foot layer is thicker than the endexine in mesocolpia but highly reduced or absent at the colpi; bacules are short and broad.

Bartonia Mühl. *ex* Willd. Fig. 4–7.

Pollen grains radially symmetrical, isopolar, oblate spheroidal to prolate, outline oval or rhomboid in lateral view, rounded-triangular and trilobate in polar view; $11–19 \times 10–15\mu$; tricolporate, colpi ± constricted at equator, ora small, ca. $1 \times 1\mu$, or traceable in side view only, diameter of apocolpia $5–8\mu$; exine 1μ thick, sexine as thick as nexine or thicker, reticulate or with OL-pattern, less often striato-reticulate, reticulum delicate, lumina ca. 0.5μ in diameter (rarely up to 1μ), muri ca. 0.5μ wide, baculate.

Specimens examined: *Bartonia paniculata* (Michx.) Mühl. subsp. *paniculata*.—NOVA SCOTIA: Lower Argyle, *Fernald, Bissell, Graves, Long & Linder* 22288 (S); subsp. *iodandra* (Robins) Gillett. NOVA SCOTIA: Île Madame, Arichat, *Rousseau* 35563 (S).

Bartonia verna (Michx.) Mühl.—FLORIDA: Riverview, *Blanton* 6927 (S); 3 mi. W of Bithlo, *Moldenke* 205 (S); Braidentown, *Tracy* 7540 (S).

Bartonia virginica (L.) BSP.—NOVA SCOTIA: Louis Lake, Port Joli, *Fernald, Long & Linder* 22295 (S). MAINE: *s. loc., Chickering,* 1875 (US). MASSACHUSETTS: Pelham, Mt. Lincoln, *Seymor* 3789 (MASS); Amherst, *Torrey,* 1945 (MASS). NEW JERSEY: Along Maurice River, W of Vineland, *Adams* 977 (S).

Interspecific variation. *Bartonia paniculata* has pollen grains with a very fine reticulum or OL-pattern (lumina less than 0.5μ in diameter) and closely spaced bacules as compared with *B. verna* and *B. virginica* which have a coarser reticulum (lumina up to 1μ) and less closely spaced bacules.

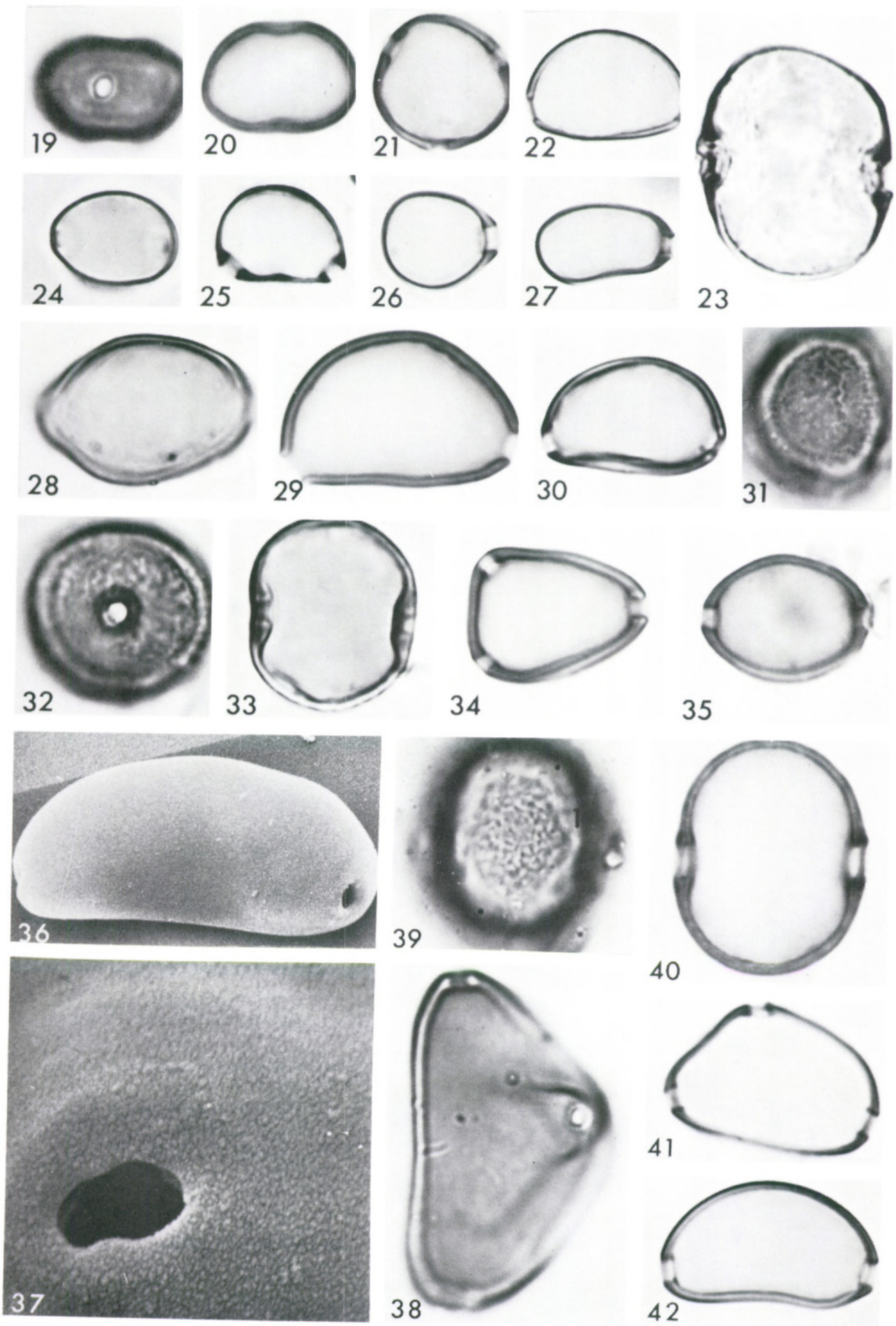
Fine structure (Fig. 43–45). Fine structure similar to *Cotylanthera;* the upper surfaces of bacules are expanded and fused with adjacent bacules to form a reticulate to striato-reticulate pattern.

Obolaria L. Fig. 8–13.

Pollen grains radially symmetrical, isopolar, spheroidal to prolate, outline oval or rhomboid in lateral view, rounded triangular and trilobate in polar view; $19–28 \times 17–21\mu$; tricolporate, colpi distinctly constricted at equator, ora traceable in side view only, diameter of apocolpia $6–8\mu$; exine 2μ thick, sexine as

←

FIGURES 2–18.—Pollen of saprophytic Gentianaceae, $\times$ 1,500. — 2–3. *Cotylanthera tenuis.*—2. Finely granulate sexine (LO-pattern).—3. Almost optical cross-section. — 4–7. *Bartonia paniculata* subsp. *paniculata.*—4. Finely reticulate sexine (OL-pattern).—5. Optical cross-section.—6. Colpus with small os in face view.—7. Polar view, optical cross-section. — 8–13. *Obolaria virginica.*—8. Reticulate sexine, at higher focal plane.—9. Reticulate sexine, at lower focal plane.—10. Optical cross-section.—11. Constricted colpus in face view.—12. Near optical cross-section, two colpi with ora in lateral view.—13. Polar view, optical cross-section. — 14–18. *Voyriella parviflora.*—14. Finely reticulate sexine (OL-pattern).—15. Optical cross-section.—16. Colpus with distinct os having lateral extensions.—17. Polar view of tetracolporate grain, sexine reticulate.—18. Polar view, optical cross-section.


19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

thick as nexine or thicker, reticulate, lumina ca. 0.5–1.5μ in diameter, muri ca. 0.5μ wide, baculate.

Specimens examined: *Obolaria virginica* L.—WASHINGTON, D.C.: *Lenander,* 1926 (S). INDIANA: Posey Co., *Deam 27035* (NY, US). NORTH CAROLINA: SW of Hillsboro, *Ahles & Haesloop 53200* (S).

Fine structure (Fig. 46–48). Fine structure similar to *Bartonia.*

Voyriella (Miq.) Miq. Fig. 14–18.

Pollen grains radially symmetrical, isopolar, suboblate to prolate spheroidal, outline circular to oval in lateral view, ± circular and tri- to tetralobate in polar view; 13–20 $\times$ 14–20μ; tri- to tetracolporate, colpi meridional, or when four obliquely converging into pairs, ora usually lalongate (ca. 2 $\times$ 6μ) with lateral extensions, diameter of apocolpia 7–8μ; exine 1–1.5μ thick, sexine as thick as nexine, or thicker, reticulate or with OL-pattern, lumina ca. 0.5(–1.0)μ in diameter, muri ca. 0.5μ wide, baculate.

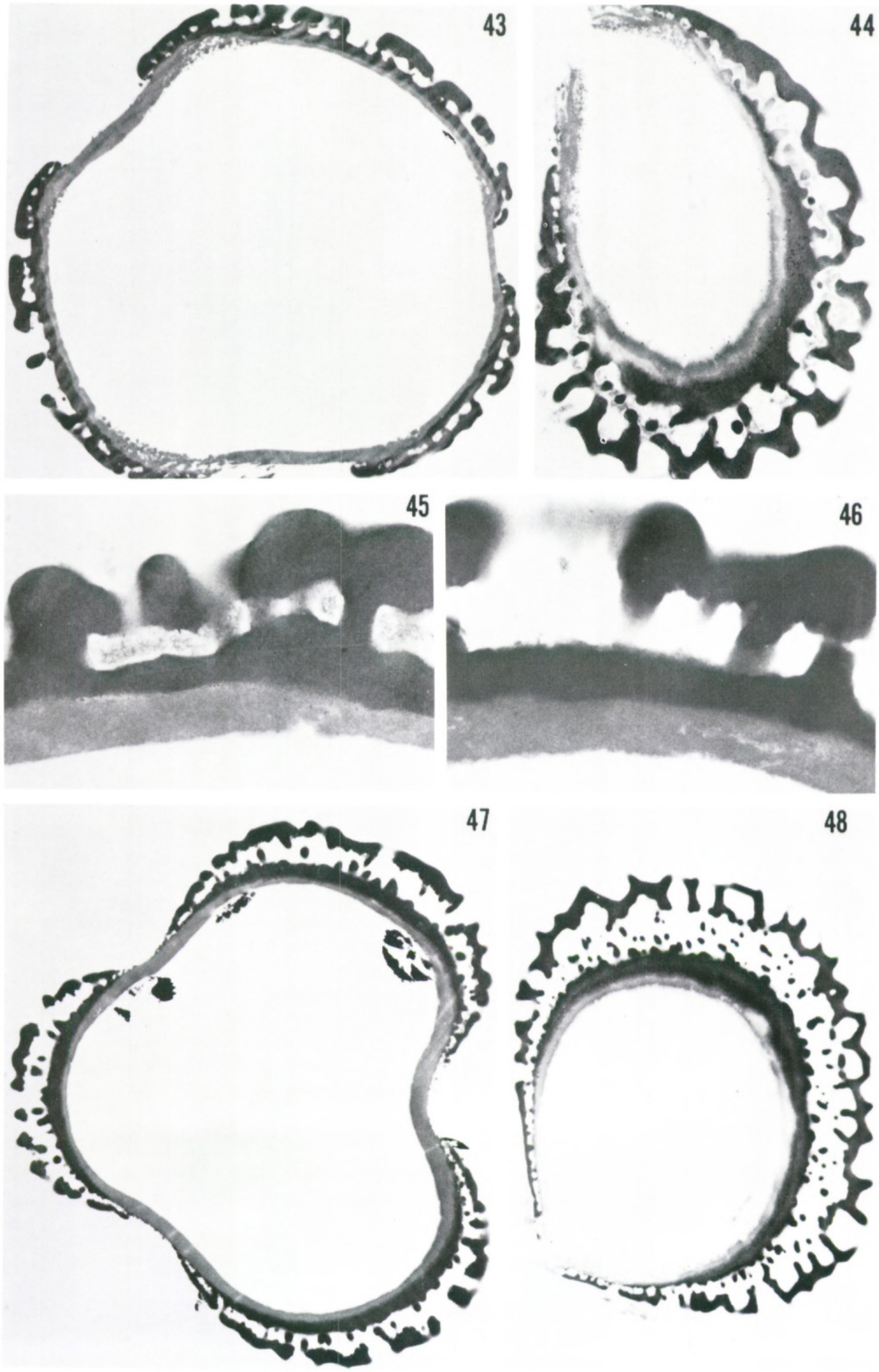
Specimens examined: *Voyriella oxycarpha* Sandw.—SURINAM: Maratakka River, Snake Creek, *L. B. B. 10767* (U).

Voyriella parviflora (Miq.) Miq.—SURINAM: Brokopondo district, *Donselaar 2469, 3165* (both U); Nassau Mountains, *Lanjouw & Lindeman 2229* (NY, U); Kabel, *Lindeman 4482* (U); Moengo, *Lindeman 5768* (U).

Fine structure (Fig. 51–52). Endexine is approximately the same thickness as foot layer. Short bacules support a thick, slightly perforate tectum. Similar to *Bartonia.*

←

FIGURES 19–42.—Pollen of saprophytic Gentianaceae, 19–35, 38–42, $\times$ 1,500; 36, $\times$ 2,100; 37, $\times$ 6,200.—19–22. *Leiphaimos corymbosus* (19–21, *Lanjouw & Lindeman 2396;* 22, *Sandwith 1065*).—19. Radially symmetrical, isopolar grain with one of three pores in face view.—20. Optical cross-section.—21. Polar view, optical cross-section.—22. Bilateral, heteropolar grain, depressed ovate, diporate.—23. *Leiphaimos spruceanus,* radially symmetrical, isopolar, diporate grain in optical cross-section, pores with prominent annular thickening.—24–25. *Leiphaimos aphyllus.*—24. Bilateral, heteropolar and diporate grain in polar view.—25. Same, convexo-plane, pores with annular thickening.—26–27. *Leiphaimos flavescens.*—26. Monoporate grain in lateral (?) view, pore with prominent annulus.—27. Monoporate, elongated grain in lateral (?) view, pore with annular thickening. — 28–29. *Leiphaimos aurantiacus.*—28. Bilateral, heteropolar, diporate grain in polar view.—29. Same, convexo-plane, pores without annular thickening. — 30. *Leiphaimos tenuiflorus,* bilateral, heteropolar, diporate grain, convexo-concave. — 31–35. *Leiphaimos calycinus* (31–33, *Versteeg 154;* 34–35, *Donselaar 2463*).—31. Radially symmetrical, isopolar, diporate grain, sexine surface with coarse texture.—32. Same, one pore with annular thickening in face view.—33. Same in optical cross-section, prominent annular thickening.—34. Bilateral, heteropolar, triporate grain in polar view.—35. Same, diporate grain in polar view. — 36–38. *Voyria clavata* (36–37, Brownsberg, *Stahel s.n.;* 38, *Donselaar 1982*).—36. Bilateral, heteropolar, diporate grain, convexo-concave (reniform), scanning electron micrograph.—37. Pore with slightly protruding rim and finely granular sexine surface at higher magnification, scanning electron micrograph. — 38. Triporate grain in slightly oblique position, lateral view, convexo-concave. — 39–42. *Voyria rosea* (39–40, *B.W. 941;* 41–42, *Florschütz & Maas 2834*).—39. Radially symmetrical, isopolar, diporate grain, sexine surface with coarse texture.—40. Same, optical cross-section, pores with annular thickening.—41. Bilateral, heteropolar, triporate grain in polar view.—42. Bilateral, heteropolar, diporate grain, convexo-concave.


43
44
45
46
47
48

Leiphaimos Cham. & Schlechtd. Fig. 19–35.

Pollen grains bilateral, heteropolar, reniform, semi-ovoidal to unsymmetrically biconvex, or radially symmetrical, isopolar, oblate to spheroidal, or irregular, outline in lateral view convexo-concave,[5] convexo-plane[5] to depressed ovate, or ± circular, in polar view circular to oval, or irregular; 7–13 × 11–24 × 8–15μ ($P \times E_1 \times E_2$) or 9–22 × 11–18μ ($P \times E$) or diameter 11–20μ; one to six-porate, pore diameter 1–3μ, pore margin reinforced by an annular thickening ca. 0.5–1μ wide, 1–2μ high, consisting of thickened sexine or nexine or both, annulus occasionally absent; exine ca. 0.5–1μ thick, at the apertures up to 2μ thick, stratification obscure although the exine appears divided into sexine and nexine at the apertures, sexine smooth or rough (scabrous), non-baculate.

The pollen of *Leiphaimos* has been grouped into a number of types which are not sharply defined from each other. The grouping is to be considered an attempt to assort the variation observed within the genus.

1. *Leiphaimos parasiticus*-type. Pollen grains radially symmetrical (rarely bilateral), isopolar, suboblate to spheroidal or irregular; 3- to 4-porate, pores of varying size, diameter 1–2μ, annular thickening usually relatively faint; sexine surface smooth. Fig. 19–22.

Specimens examined: *Leiphaimos albus* Standl.—PANAMA: Barro Colorado Island, *Dodge,* 1934 (MO); Colón, *Dodge, Steyermark & Allen 16927* (MO).

Leiphaimos corymbosus (Splitg.) Gilg.—GUYANA: Mazaruni Station, *Sandwith 1065* (U). SURINAM: Nassau Mountains, *Cowan & Lindeman 39184* (NY); *Lanjouw & Lindeman 2396* (U); Maratakka River, Snake Creek, *Maas 3279* (U); Brokopondo district, *Donselaar 1688* (U).

Leiphaimos montanus Jonk.—SURINAM: Wilhelmina Range, *B.W. 6982 p.p.* (type, U).

Leiphaimos parasiticus Schlechtd. & Cham.—MEXICO: Chiapas, *Dressler 1410* (US). CUBA: Pinar del Río, *Ekman 17333* (S); Oriente, *Ekman 2085* (S). HAITI: Mountains Terre-Nueve, *Ekman 5036* (S). BRAZIL: Pernambuco, *Pickel 4011* (S).

Inter- and intraspecific variation. One specimen of *L. corymbosus (Sandwith 1065)* has bilateral, heteropolar pollen grains instead of the generally radially symmetrical ones and is better referred to the *Leiphaimos tenuiflorus*-type of pollen. Another collection *(Maas 3279)* has curved or more or less irregular pollen grains with a large number of pores and a scabrous sexine surface. In *L. parasiticus* itself there are specimens that tend to have slightly curved pollen grains.

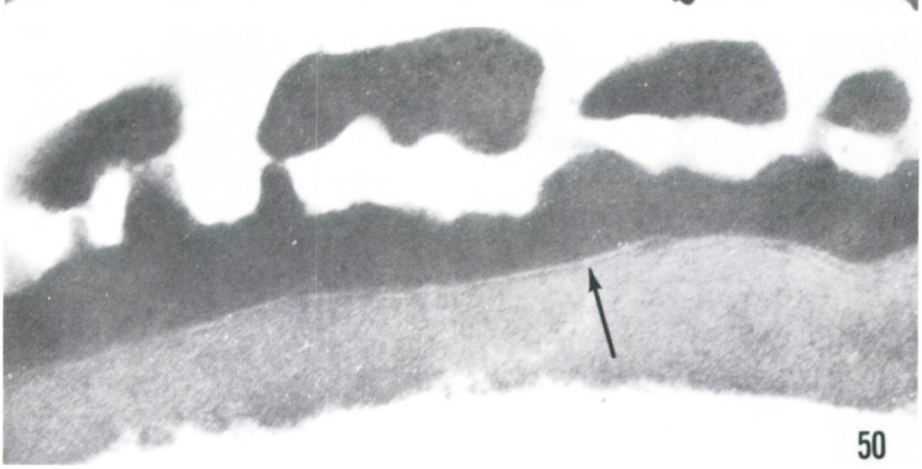
Fine structure (Fig. 56–57). Observations are for *L. parasiticus.* Endexine is considerably thicker than ektexine; at aperture margins endexine is prominently thickened. Upper surface of endexine (or foot layer) is slightly lamellate. Foot

[5] Outline in lateral view according to Straka, 1964. (*Cf.* also *Voyria.*)

←

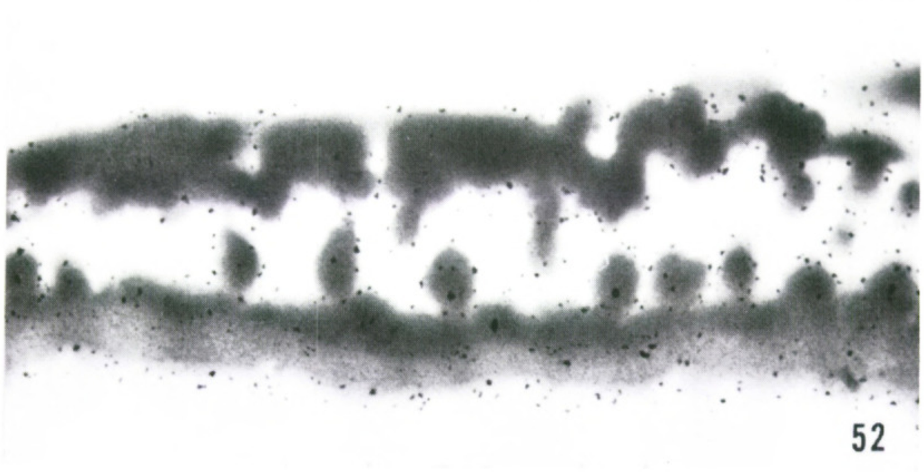
FIGURES 43–48.—Electronmicrographs of pollen of saprophytic Gentianaceae. — 43–45. *Bartonia paniculata* (Michx.) Mühl. subsp. *paniculata.*—43. Near median section, × 7,200. —44. Oblique view emphasizing reticulate nature of sexine, × 11,400.—45. View through apocolpium, × 39,200. — 46–48. *Obolaria virginica* L.—46. View through apocolpium, × 34,000. Note similarity to Fig. 45.—47. Near median section, × 7,200.—48. Oblique view, × 11,000. Note similarity to Fig. 44.

49



50

51



52

layer reduced and supports minute and fragile bacules. A markedly thickened tectum, which is slightly perforate, extends over the bacules.

2. *L. spruceanus*-type. Pollen grains radially symmetrical, isopolar, ± spheroidal 2- to 6-porate, pores 1–2μ in diameter, with a prominent annular thickening, ca. 0.5–1μ wide, 1μ high; sexine smooth or scabrous. Fig. 23, 31–33.

Specimens examined: *Leiphaimos calycinus* (Griseb.) Miq.—SURINAM: Nickerie River, *L. B. B. 11045* (U); Maratakka River, Snake Creek, *Maas 3272* (U); Gonini River, *Versteeg 154* (U). The other collections examined resemble *e.g., Voyria caerulea.*

Leiphaimos spruceanus (Benth.) Gilg.—SURINAM: Brownsweg, *Donselaar 3000* (U); Brokopondo district, *Donselaar 3153* (U); without locality, *Stahel 402* (U).

Voyria rosea p.p. (Fig. 39–40) has the same type of pollen as *L. spruceanus.*

Intraspecific variation. *L. calycinus* has pollen of the *Leiphaimos spruceanus*-type, but some specimens (*Donselaar 2463, 3045*) resemble those of *Voyria caerulea.*

3. *L. aphyllus*-type. Pollen grains bilateral, heteropolar or rounded to irregular in shape; 1- to 2-porate, pores 1–2μ in diameter, with a prominent annulus, ca. 0.5μ wide, 1μ or more high; exine ca. 0.5(–1.0)μ thick, sexine smooth. Fig. 24–27.

Specimens examined: *Leiphaimos aphyllus* (Jacq.) Gilg.—CUBA: Sierra Maestra, *Ekman 5353* (S). HAITI: Port Margot, *Ekman 2820* (S); Massif du Nord, *Ekman 4837* (NY). SURINAM: Without locality, *Stahel 403* (U); Donderkreek-Wayambo River, *Stahel 1915* (U); Brownsweg, *Wessels-Boer 600* (U).

Leiphaimos flavescens (Griseb.) Gilg.—SURINAM: Coppename district, *Florschütz & Maas 2639* (U).

Voyria primuloides cited by Raynal (1967) has a similar type of pollen.

4. *L. tenuiflorus*-type. Pollen grains bilateral, heteropolar or irregular; 1- to 2-porate, pores 2–4μ in diameter, devoid of annular thickening, pore margins often folded outwards; exine 0.5–1μ thick, sexine smooth. Fig. 28–30.

Specimens examined: *Leiphaimos aurantiacus* (Splitg.) Miq.—SURINAM: Maratakka River, Snake Creek, *L. B. B. 10768* (U); Jande Creek near Kabel Station, *Lindeman 4430* (U). GUYANA: Mazaruni Station, *Davis*, 1937 (S, U). BRAZIL: Maracassume, Mato do Caxoeira, *Moses 57* (U).

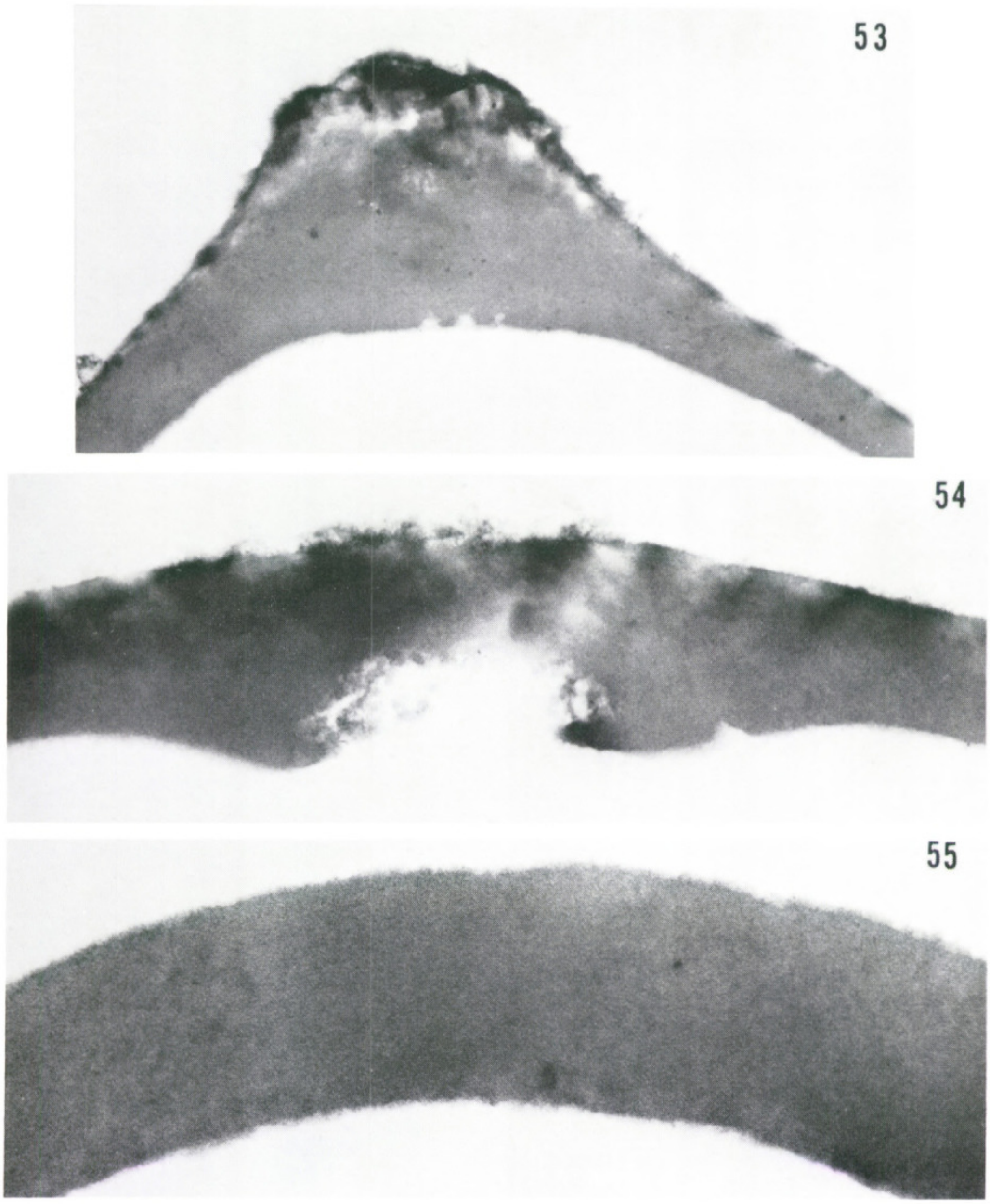
Leiphaimos leucanthus (Miq.) Gilg.—SURINAM: Commevijne River, Tempatikreek, *Focke 1253* (U).

Leiphaimos tenellus (Hook.) Miq.—PANAMA: Without locality, *Dodge, Steyermark & Allen 16926* (U); Darien, Puerto St. Dorotea, *Dwyer 2284* (MO). COLOMBIA: La Jagua, Magdalena Valley, *Allen 581* (U).

Leiphaimos tenuiflorus (Griseb.) Miq.—SURINAM: Linker-Coppename River, *Florschütz & Maas 3156* (U); Nassau Mountains, *Lanjouw & Lindeman 2722* (U).

←

FIGURES 49–52.—Electronmicrographs of pollen of saprophytic Gentianaceae. — 49–50. *Cotylanthera tenuis* Bl.—49. View across colpus showing reduction of ektexine and thick endexine. × 13,050.—50. View along apocolpium, × 58,800. Note lamellae near upper surface of endexine (arrow). — 51–52. *Voyriella parviflora* (Miq.) Miq.—51. View near margin of colpus, × 26,000. Black dots are OsO_4 precipitate.—52. View along apocolpium, × 26,000. Black dots are OsO_4 precipitate.



Figures 53–55.—Electronmicrographs of *Voyria rosea* Aubl.—53. View oblique to aperture margin, × 22,750. Note thin, electron dense cap over otherwise homogeneous exine.—54. View oblique to pore, × 50,400.—55. View along apoporium, × 97,000. Note that electron dense cap is not evident.

Interspecific variation. *L. aurantiacus* has larger grains than the other species referred to of this type. The pore margins are distinctly folded outwards, the pollen wall appears to be thicker, and the shape more stable than in the other species.

Fine structure (Fig. 58–62). Exine stratification is difficult to interpret. The exine appears to consist of a single layer; however, fine lamellae are present on the upper part of the exine. These lamellae are most clearly observed in unacetolyzed material. An intine which approaches the exine in thickness is visible in the unacetolyzed pollen. Numerous dense inclusions are present within the intine.

Voyria Aubl. Fig. 36–42.

Pollen grains bilateral, heteropolar, reniform, semi-ovoidal to unsymmetrically biconvex or radially symmetrical, isopolar, ± spheroidal or irregular, outline in lateral view convexo-concave, convexo-plane to depressed ovate, or ± circular, in polar view circular to oval, or irregular; 9–20 $\times$ 17–31 $\times$ 10–18μ (P $\times$ E_1 $\times$ E_2) or 10–18 $\times$ 15–18μ (P $\times$ E) or diameter 10–15μ; one- to six-porate, pore diameter 2–3μ, pore margin reinforced by an annular thickening ca. 0.5μ wide and 0.5–1μ high, consisting of thickened sexine or nexine or both; exine 0.5–1μ thick, at the apertures up to 2μ thick, stratification obscure although the exine appears divided into sexine and nexine at the apetures, sexine smooth or rough (*cf.* Fig. 36), non-baculate.

The pollen grains of *Voyria* are basically the same as those of *Leiphaimos*.

Specimens examined: *Voyria clavata* Splitg.—Surinam: Brokopondo district, *Donselaar 1982* (U); Brownsberg, *Stahel* (U); without locality, *Florschütz 1828;* without locality, *Stahel* (U).

Voyria caerulea Aubl.—Surinam: Emma Keten, *Jonker & Daniels 732* (U); Saramacca River, *Maguire 24130* (U); Rechter-Coppename River, *Wessels-Boer 1391* (U).

Voyria rosea Aubl.—Surinam: Maratakka River, *B. W. 941* (U); Bakhuisgebirge, *Florschütz & Maas 2834* (U); Nickerie River, *L. B. B. 10913* (U). Guyana: Kaieteur Plateau, *Maguire & Fanshawe 23140* (U).

Intraspecific variation. One collection of *Voyria rosea (B.W. 941)* has radially symmetrical, 2-porate, pollen grains (=*Leiphaimos spruceanus*-type) while the other specimens have grains of the elongated, curved type (*cf. Leiphaimos calycinus p.p.,* Fig. 34–35) or are irregular in shape.

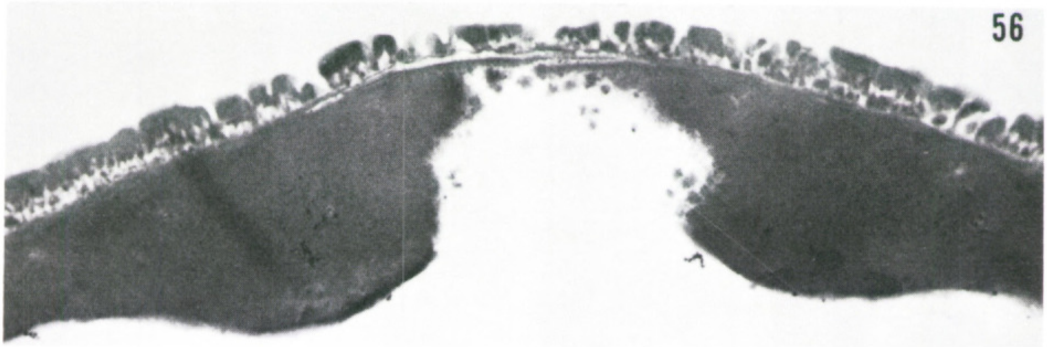
Fine structure (Fig. 53–55). Exine is thin, and differentiation into ektexine and endexine is extremely difficult. Ektexine is most clearly observed at pores and consists of an electron dense band. In other sections an ektexine is not recognized.

Discussion of Surface Morphology

Polarity. Previous interpretations of polarity in *Voyria* do not agree with our study. While the pores have been considered polar (Gilg, 1895; Köhler, 1905; Oehler, 1927), it seems more likely that they are equatorial (Fig. 1).

In one collection of *Voyria clavata* some of the pollen grains are united in tetrads. The grains are arranged in pairs and each pair is in a different plane. The polar axes of each pair cross those of the other pair at right angles or nearly so (Fig. 1A). The pores meet in pairs at six places or at four places. A similar tetrad arrangement has been reported for other families, *e.g.* Balsaminaceae, Saxifragaceae, Tropaeolaceae, (Huynh, 1968, Fig. 37, 39, 41, 47–49).

In one specimen of *Leiphaimos spruceanus (Donselaar 3153)* square, rhomboidal and linear tetrads are found. Isopolar grains occur in all genera examined, but in *Voyria* and *Leiphaimos* they are prevailingly heteropolar.



FIGURES 56–57.—Electronmicrographs of *Leiphaimos parasiticus* Schlechdt. & Cham.—56. View oblique to pore, × 29,300. Note highly reduced ektexine with thick tectum and foot layer composed of fine lamellae.—57. View through entire pollen grain, × 9,900. Note granular appearance of endexine under pore.

Symmetry and Shape. Radially symmetrical grains occur in all taxa, although *Voyria* and *Leiphaimos* have predominantly bilateral, elongated, more or less curved pollen. In the Gentianaceae the latter type of pollen is found exclusively in these two genera, but it has also been reported in Proteaceae (Erdtman, 1952; Smith, 1968), Tropaeolaceae and Saxifragaceae (Huynh, 1968). Since the rounded tri- or more -porate grains appear to be radially symmetrical with an equatorial arrangement of pores, it seems that those of elongated grains are also in an equatorial plane. Consequently one might consider the curved pollen of *Leiphaimos parasiticus* or *L. corymbosus* as being derived from radially symmetrical ones that occur within or between single collections. Oehler (1927) considered young, one-celled pollen of *Voyria* to be spheroidal and older pollen to be curved, possibly due to uneven growth of the surface. Oehler's conclusion seems plausible when considering the variable outlines of pollen grains. In accordance with Oehler, younger grains of *Cotylanthera* are spheroidal; older grains more or less triangular.

Size. Most genera examined have relatively small pollen grains (longest axis less than 20μ). *Obolaria* has relatively large grains, and *Voyria* has larger grains on the average than *Leiphaimos*.

Apertures. The colpi of pollen of *Bartonia* and *Obolaria* are usually constricted at the equator (*cf.* Iverson & Troels-Smith, 1950, Pl. 15, 3d, 3e). The number of apertures varies considerably. Porate grains occur in *Leiphaimos* and *Voyria*. According to Gilg (1895) *Leiphaimos* has ovoidal pollen with one apical pore, while *Voyria* has "Einzelpollen schwach wurstförmig gebogen" (pollen slightly curved, sausage-like) with two apical pores. We have found that neither the number of apertures nor the pollen shape are stable enough characters to differentiate the two genera. It is not unusual for one of the pores to be smaller than the others, an observation in agreement with Erdtman (1952). The reduction of pore size and number of pores suggests that the monoporate grains may be derived from polyporate (diporate) ones as exemplified by the monoporate and elongated pollen of *Leiphaimos flavescens* (Fig. 27).

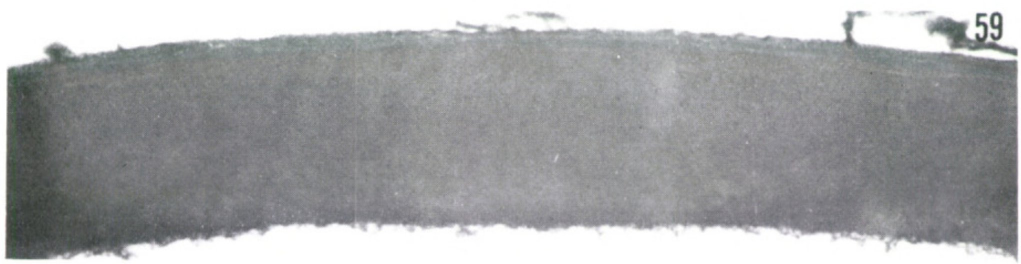
The other genera studied, *Bartonia, Cotylanthera, Obolaria,* and *Voyriella,* all have three- (to four-) colporate pollen grains. The ora are usually indistinct except for *Voyriella* where they are sharply delimited and often provided with lateral extensions. The latter is a common feature in other groups of Gentianaceae (Nilsson, 1967).

Discussion of Fine Structure

Pollen fine structure of the saprophytic taxa shows a diversity in morphology. To an extent this diversity was consistent with the morphological differences noted by light microscopy. *Bartonia, Obolaria,* and *Cotylanthera* were practically indistinguishable with electron microscopy, and *Voyriella* was very similar to these genera.

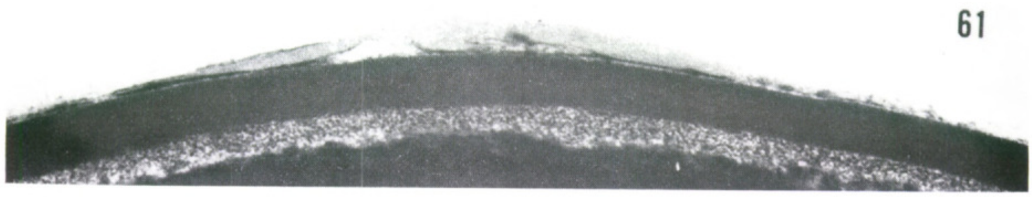
Stratification in *Voyria rosea* was difficult to interpret. The exine appeared to be one layer except for a fine, electron dense band covering the outer surface (Fig. 53–54). This band was not always obvious (Fig. 55), and therefore exine

58

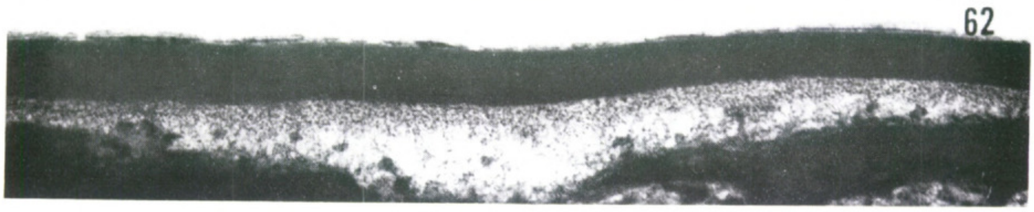


59

60



61



62

interpretation must be somewhat tentative. However, we feel that the band is real and probably is a highly reduced ektexine. We hope to do further work which will clarify this.

Fine structure of *Leiphaimos* indicated considerable interspecific variation. In *L. parasiticus* a reduced tectate ektexine was clearly observed, but in *L. aurantiacus* and *L. tenellus* the ektexine was not evident. Contrary to Gilg (1895) and Köhler (1905) we have found an intine in *L. tenellus* which substantiates some past interpretations for the genus (Oehler, 1927; Raynal, 1967). However, the intine of *L. tenellus* was considerably thicker than that described for *L. parasiticus* (Raynal, 1967). Although the electron microscopic sampling was limited, the fine structure of *Leiphaimos* is considered close to that of *Voyria rosea*.

Taxonomic Evaluation of the Pollen Morphological Data

Bentham and Hooker (1876) placed *Cotylanthera* in the tribe Exaceae together with *Exacum, Sebaea, Belmontia,* and *Tachiadenus*. They considered *Cotylanthera* closely allied to *Exacum* but differing by reduced leaves and anthers with one apical locule and pore. This staminal feature induced Gray (1868) to refer *Eophylon, i.e., Cotylanthera, to* Grisebach's subtribe Chironieae, but also influenced Baillon (1891) to reduce *Cotylanthera* to a section of *Exacum*. Gilg (1895) kept *Cotylanthera* as a genus in the Gentianeae-Exacinae, which was characterized by small pollen grains with a smooth exine surface, barely traceable furrows, and indistinguishable exine and intine. Köhler (1905) described the pollen of *Cotylanthera tenella* as triporate with a smooth exine.

In our study only *Cotylanthera tenuis* was available. The pollen morphology agrees with earlier interpretations of a smooth exine surface (Gilg, 1895; Köhler, 1905); however, a fine granulation was also evident. With the light microscope we have not been able to distinguish pores (ora) with certainty; with electron microscopy the ora were readily apparent.

We have not found any palynological evidence to suggest a close relationship between *Cotylanthera* and *Exacum*. In contrast to *Cotylanthera, Exacum* has striate to striato-reticulate pollen. The small pollen grains which characterize Gilg's subtribe Exacinae also occur in some genera of the Erythraeinae (*e.g., Bartonia, Schinziella, Curtia*).

Grisebach (1839, 1845) placed *Centaurella* (*i.e., Bartonia*) in the Swertieae near *Pleurogyne* (*i.e., Lomatogonium*). Bentham and Hooker (1876) also placed *Bartonia* and *Obolaria* (the latter genus was omitted by Grisebach) in the Swertieae, while Gilg (1895) placed them in the Gentianeae-Erythraeinae (charac-

←

Figures 58–62.—Electronmicrographs of pollen of *Leiphaimos*. — 58–59. *Leiphaimos aurantiacus* (Splitg.) Miq.—58. View through pollen, pores not included, × 6,300. Note similarity of exine to Fig. 55.—59. Homogeneous appearance of exine except for fine lamellae near upper surface, × 50,400. — 60–62. Unacetolyzed pollen of *Leiphaimos tenellus* (Hook.) Miq.—60. View through pollen, pore not included, × 26,000.—61. Fine lamellar band appearing to partially split from upper surface, × 73,500.—62. View oblique to pore, × 58,800. Note intine.

terized by fairly large pollen grains with deep furrows and a smooth or granulated exine differentiated from the intine). Köhler (1905) described the pollen grains of *Bartonia verna* as reticulate with three furrows and three pores, while those of *B. tenella* (*B. virginica sensu* Gillett) had a smooth exine. Our investigation has shown a reticulate sexine for both species. The pollen of *Obolaria virginica* was described as "globosum, membrana tenuissima laevissima" (globose with a smooth and very thin wall) by Gray (1848) and as "oblong" with a "distinctly granulate exine" by Holm (1897). The latter author concluded from his morphological and anatomical studies that *O. virginica* was to be considered a connecting link between the autophytic and saprophytic genera of the Gentianaceae and that its systematic position was close to *Swertia* and *Lomatogonium*. It is at present impossible to assign *Obolaria* either to the Swertieae (Grisebach, 1839, 1845; Bentham & Hooker, 1876) or to the Gentianeae-Erythraeinae (Gilg, 1895). Neither our pollen morphological evidence nor gross morphology (Gillett, 1959) suggest a direct relationship between *Bartonia* and *Obolaria*. Our study may support Holm's idea (1897) of a relationship between *Obolaria* and *Swertia*, but it does not substantiate a close affinity between *Obolaria* and *Lomatogonium* with its different and characteristic type of pollen (Nilsson, 1964, 1967).

The taxonomic treatment of *Voyria*, *Leiphaimos*, and *Voyriella* has varied with time. Grisebach (1839) placed *Voyria* in the Lisiantheae and divided *Voyria* into two sections, *Leiphaimos* and *Lita*. In 1845 he added two more sections, *Leianthostemon* and *Pneumanthopsis*. Miquel (1849) described *Voyriella* as a new section of *Voyria* and in 1850 divided the Gentianeae parasiticae into a number of genera—*Voyria*, *Voyriella*, *Leiphaimos*, and others. Progel (1865) treated *Voyriella* and *Voyria* as genera, dividing the latter into a number of sections. Bentham and Hooker (1876) placed *Voyriella* and *Voyria sens. lat.* in the tribe Chironieae. Gilg (1895) placed *Voyria* in a tribe of its own, the Voyrieae, while *Voyriella* and *Leiphaimos* were placed in another tribe, the Leiphaimeae. Pollen grain characteristics were used to differentiate the two tribes. *Leiphaimos* was further subdivided into five sections. From anatomical studies, Svedelius (1902) agreed with Gilg that *Leiphaimos* and *Voyria* were not closely related. Raynal (1967), except for maintaining the type species *L. parasiticus*, found no evidence to maintain *Voyria* and *Leiphaimos* as distinct genera. Robyns (1968) treated *Voyria* in a broad sense with regard to the species occurring in Panama and, accordingly, made a transfer from *Leiphaimos* to *Voyria*.

We have found a close similarity between pollen of *Leiphaimos montanus* and *L. corymbosus*, which agrees with Jonker's (1936*b*) statement of a close relationship between the two species. The palynological similarity between *L. aphyllus* and *L. flavescens* seems to be without a gross morphological counterpart.

The pollen grains of *L. spruceanus* resemble those of *L. calycinus p.p.* and *Voyria rosea p.p.*, but the macromorphology of *L. spruceanus* differs from that of the other species (Jonker, personal communication). It is not yet possible to explain why *L. calycinus* and *Voyria rosea* show a comparable variation. Judging from a picture of *Voyria rosea* by Raynal (1967), the collections with radially symmetrical pollen grains could be regarded as *Voyria rosea*, the others as *Lei-*

phaimos calycinus. This cannot be fully confirmed until additional herbarium specimens have been studied. It should also be noted that one specimen of *L. calycinus* (*Versteeg 154*) is redetermined from *Voyria rosea* (Jonker, *in litt.*), which would be contrary to the above supposition.

L. tenellus and *L. aurantiacus* were placed together because of fusiform seeds (Miquel, 1850), and pollen morphology supports this. Jonker (1936*b*) placed *L. clavatus* in the genus *Voyria* instead of *Leiphaimos,* because of its comparatively large flowers. We have used the original name of *Voyria clavata* Splitg. in our study. Its pollen grains are similar to those of *V. caerulea* and *V. rosea,* but since we consider the pollen of *Voyria* and *Leiphaimos* to be essentially the same, with no distinct generic difference, we can only suggest a closer relationship between *V. clavata* and the above mentioned species examined of *Voyria sens. lat.*

Gilg (1895) included *Voyriella* together with *Leiphaimos* in his tribe Leiphaimeae and did not distinguish between the pollen grains of the two genera. Köhler (1905), following Gilg's taxonomic treatment, described the pollen grains of *Voyriella parviflora* as devoid of furrows and even pores (*cf.* Gilg, 1895) and with a smooth exine. Raynal (1967) noted that the pollen of *Voyriella* had short furrows with a central pore and thicker and more ornamented walls than *Voyria* and *Leiphaimos.* She gave several reasons for not placing *Voyriella* near *Voyria* and *Leiphaimos.*

We conclude from pollen morphology that *Voyriella* should better be associated with *Curtia* or *Enicostema* than with *Leiphaimos* (*cf.* Gilg, 1895). We also support the view of including *Leiphaimos* in *Voyria* (*cf.* Grisebach, 1839, 1845; Progel, 1864; Raynal, 1967; Robyns, 1968). The pollen grains of *Voyria* and *Leiphaimos* differ greatly from those of other Gentianaceae, and it is tempting to speculate whether or not the extreme simplicity of pollen in these taxa may be correlated with the saprophytic habit. The pollen grains of *Voyria* and *Leiphaimos* show too many resemblances to each other to be placed in different tribes as was done by Gilg (1895). Until a comprehensive revision is made it seems preferable to treat *Voyria* as an inclusive genus.

Literature Cited

Baillon, H. 1891. Gentianaceae - Histoire des Plantes. 10: 113–145. Paris.

Bentham, G. & J. D. Hooker. 1876. Gentianeae. *In* "Genera Plantarum." 2: 799–820. London.

Erdtman, G. 1943. An Introduction to Pollen Analysis. Waltham, Mass.

———. 1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Stockholm and Waltham, Mass.

———. 1960. The acetolysis method. A revised description. Svensk Bot. Tidskr. 54: 561–564.

———. 1966. Pollen Morphology and Plant Taxonomy. Angiosperms. (Corrected Reprint of the edition of 1952 with a new addendum.) New York and London.

Faegri, K. 1956. Recent trends in palynology. Bot. Rev. (Lancaster) 22: 639–664.

Figdor, W. 1896. Ueber *Cotylanthera* Bl., ein Beitrag zur Kenntnis tropischer Saprophyten. Ann. Jard. Bot. Buitenzorg 14: 213–240.

Gilg, E. 1895. Gentianaceae. *In* A. Engler & K. Prantl, "Die natürlichen Pflanzenfamilien." 4(2): 50–108. Leipzig.

Gillett, J. M. 1959. A revision of *Bartonia* and *Obolaria* (Gentianaceae). Rhodora 61: 43–62.

Gray, A. 1848. Chloris Boreali-Americana. Decade I. Mem. Amer. Acad. Arts. 3: 1–56.

———. 1868. Characters of a new genus consisting of two species of parasitic Gentianeae. Jour. Linn. Soc. Bot. 11: 22–23.

Grisebach, A. H. R. 1839. Genera et Species Gentianearum. Stuttgart and Tübingen.

———. 1845. Gentianaceae. *In* A. P. de Candolle, "Prodromus Systematis Naturalis Regni Vegetabilis." 9: 38–141.

Holm, T. 1897. *Obolaria virginica* L.: A morphological and anatomical study. Ann. Bot. (London) 11: 369–383.

Huynh, K. L. 1968. Étude de l'arrangement du pollen dans la tetrade chez les Angiospermes sur la base de données cytologiques. Bull. Soc. Bot. Suisse. 78: 151–191.

Iversen, J. & J. Troels-Smith. 1950. Pollenmorfologiske Definitioner og Typer. Danmarks Geol. Unders. 4rk. 3, 8.

Johow, F. 1889. Die chlorophyllfreien Humuspflanzen nach ihren biologischen und anatomisch-entwickelungsgeschichtlichen Verhältnissen. Jahrb. Wiss. Bot. 20: 475–525.

Jonker, F. P. 1936*a*. Gentianaceae. *In* A. Pulle, "Flora of Suriname." 4: 400–427.

———. 1936*b*. Ueber einige Gentianaceae aus Suriname. Recueil Trav. Bot. Neérl. 33: 250–253.

Knoblauch, E. 1894. Beiträge zur Kenntnis der Gentianaceae. Bot. Centralbl. 60: 321–334.

Köhler, A. 1905. Der systematische Wert der Pollenbeschaffenheit bei den Gentianaceen (Inaugural Dissertation). Mitt. Bot. Mus. Univ. Zürich 25: 1–72.

Miquel, F. A. G. 1849. Voyriae species quasdam Surinamenses recenset. Tijdschr. Wis.-Natuurk. Wetensch. Eerste Kl. Kon. Ned. Inst. Wetensch. 2: 122–125.

———. 1850. Stirpes Surinamenses selectae. Natuurk. Verh. Holl. Maatsch. Wetensch. Haarlem. 7: 146–151.

Nilsson, S. 1964. On the pollen morphology in *Lomatogonium* A. Br. Grana Palynol. 5: 298–329.

———. 1967. Pollen morphological studies in the Gentianaceae-Gentianinae. Grana Palynol. 7: 46–145.

Oehler, E. 1927. Entwicklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentianaceen. Planta 3: 671–733.

Progel, A. 1865. Gentianaceae. *In* C. F. P. von Martius, "Flora Brasiliensis." 6(1): 197–248. München, Wien, and Leipzig.

Raynal, A. 1967. Etude critique des genres *Voyria* et *Leiphaimos* (Gentianaceae) et révision des *Voyria* d'Afrique. Adansonia, Ser. 2. 7: 53–71.

Robyns, A. 1968. Notes on some American species of *Voyria* (Gentianaceae). Ann. Missouri Bot. Gard. 55: 398–400.

Skvarla, J. J. 1966. Techniques of pollen and spore electron microscopy. Part 1. Staining, dehydration and embedding. Oklahoma Geol. Notes 26: 179–186.

Smith, Francis. 1968. "Dyads" in Proteaceae. Grana Palynol. 8: 86–87.

Straka, H. 1964. Palynologia madagassica et mascarenica. 1. Vorwort, 2. Einleitung. Pollen & Spores 6(1): 239–288.

Svedelius, N. 1902. Zur Kenntnis der saprophytischen Gentianaceen. Bih. Kongl. Svenska Vetensk.-Akad. Handl. 28(3): 1–16.