Seed coat micromorphology in *Irlbachia* (Gentianaceae)

by L. Cobb and P.J.M. Maas

Institute of Systematic Botany, State University of Utrecht
Transitorium 2, Heidelberglaan 2, Utrecht, the Netherlands

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ABSTRACT

The SEM was used as a taxonomic aid to the authors while writing a monograph of *Irlbachia* (Neotropical Gentianaceae). In most cases, external seed morphology was distinctive at the species level. SEM use has confirmed the authors' ideas on the synonymy of many of the species in the genus. This article describes the seed morphology of the seven species.

INTRODUCTION

The genus *Irlbachia* is a member of the *Lisianthus* group of Neotropical Gentianaceae. There are now seven species in this genus, *I. pumila, I. poeppigii, I. plantaginifolia, I. nemorosa, I. tatei, I. pratensis,* and *I. caerulescens.* These seven species range in height from 5 to 100 cm, are simple to branched; leaves are linear to subcordate; corollas are small and usually white, sometimes ranging to dark purple. *I. nemorosa* is a complex species ranging from small unbranched herbs with one flower to herbs 1 m in height with strongly branched inflorescences. The other six species are fairly distinct in their growth habits. The distribution of *Irlbachia* ranges from SE Venezuela and N. Brasil through the Guianas; *I. caerulescens* is also found throughout Brasil. Most of the species grow at low altitudes, although *I. nemorosa* ranges from 0 to 2400 m and *I. tatei* grows at altitudes above 1000 m. This great range in altitude and growth habit has caused much discussion in the naming of the species of *Irlbachia,*

SEM research conducted at:
Hugo de Vries Laboratory, University of Amsterdam under supervision of F. Bouman.

especially *I. nemorosa* (see Gentianaceae–Flora Neotropica to be publ.). In order to obtain additional arguments for the taxonomic treatment of *Irlbachia*, a scanning electron microscopic study on seed morphology was conducted on all of the species.

MATERIALS AND METHODS

Seeds for this study were obtained from specimens from the following herbaria: **BM, BR, CAY, F, GOET, INPA, K, M, MG, MO, MY, NY, RB, S, SP, U, UC, US,** and **VEN.** They were taken from capsules that were just beginning to open, thus assuring equal maturation of the seeds. The seeds were mounted on double-sided adhesive tape and then sputter-coated with a gold-palladium mixture for 2½ minutes. The specimens were studied using a Cambridge Mark 2-A scanning electron microscope. Photographs were taken at the following magnifications: X20, X200, X500, X1000, X2000, and rarely at X5000. Descriptions are based on the mature seeds–seeds at X20 and X200 magnifications and cells at X500, X1000, and X2000 magnifications. The terminology in this article is that used in Barthlott & Ehler (1977) and Radford et al. (1974).

RESULTS

The SEM research has shown that the seeds of *Irlbachia* specimens are very similar, though usually distinctive at the species level. The variation in the seeds lies in the type of cell, the size and number of papillae, and the appearance of the outer and lateral walls.

General description of the seeds:

Irlbachia Type seed

Seeds irregular in shape, spheroid to rectangular, sometimes with prominent rims, size (150–) 200 μm X 400 (–500) μm.

Testa cells circular to elliptic to puzzle piece-like.

Three types of cells:

A. dome-like with band-like thickenings in the outer wall.
B. concave cells with thin outer walls devoid of thickenings.
C. concave cells with short band-like thickenings tapering towards the center (look like collapsed domes).

Outer wall thin with or without thickenings. Lateral walls straight to sinusoidal to V-undulating. Inner walls usually pitted, rarely warty, often with papillae either scattered over the entire surface or more restricted to lateral walls.

Description on the species level:

I. pumila (Bentham) Maguire (Plate 1A–C)

Seeds irregular in shape, spheroid to rectangular, size 350–400 μm × 250 μm. Cells circular to broadly elliptic; cell surface hemispherical to dome-like. Outer wall smooth with irregularly branched, band-like thickenings continuing through lateral walls; lateral walls straight to somewhat sinusoidal; inner wall pitted.

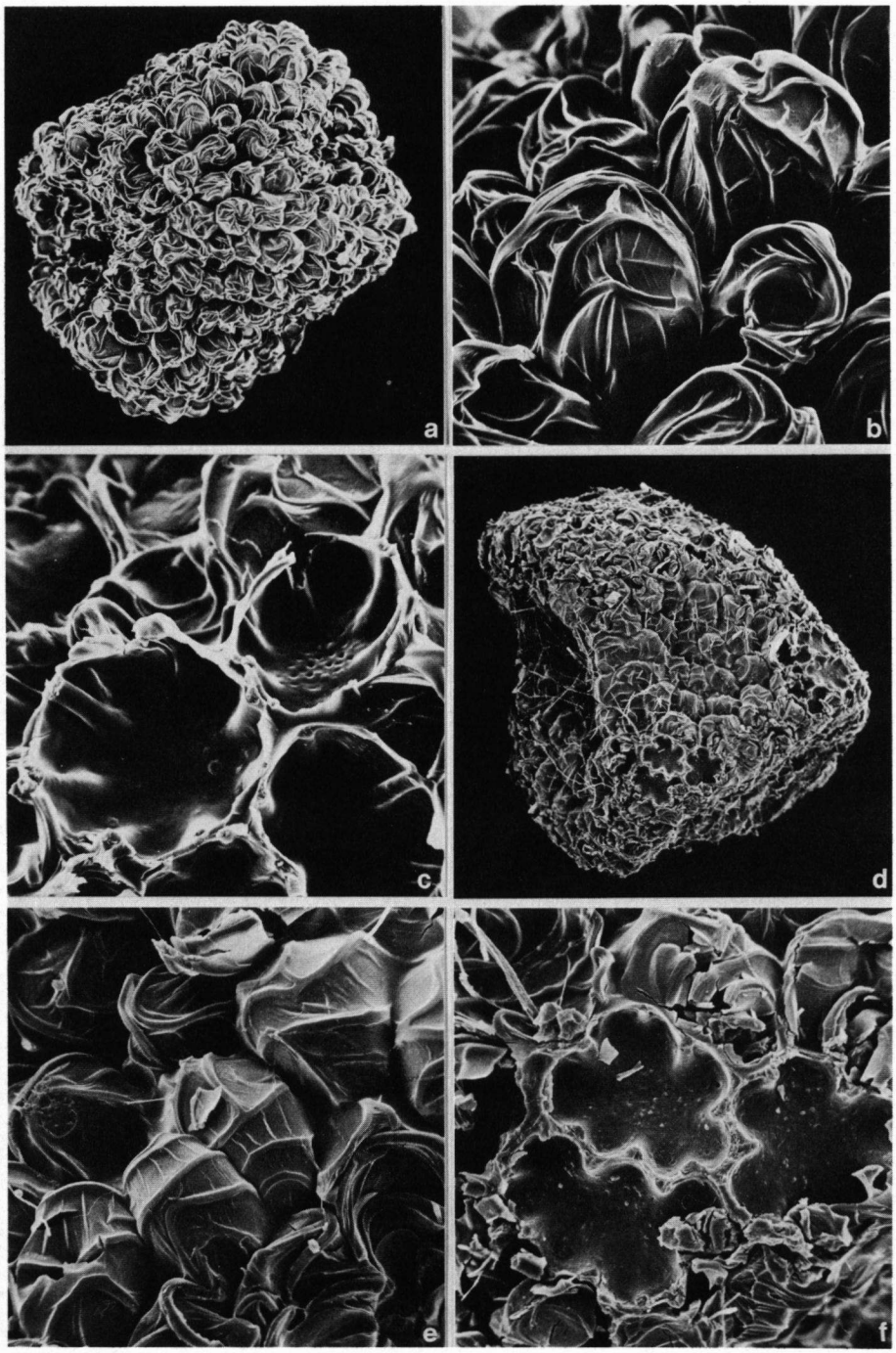

Plate 1. *I. pumila* A. seed (100×); B. detail of dome-like cells (550×); C. detail of inner wall of dome-like cells (550×).
I. poeppigii D. seed (100×); E. detail of dome-like cells (500×); F. detail of inner wall of dome-like cells (500×).

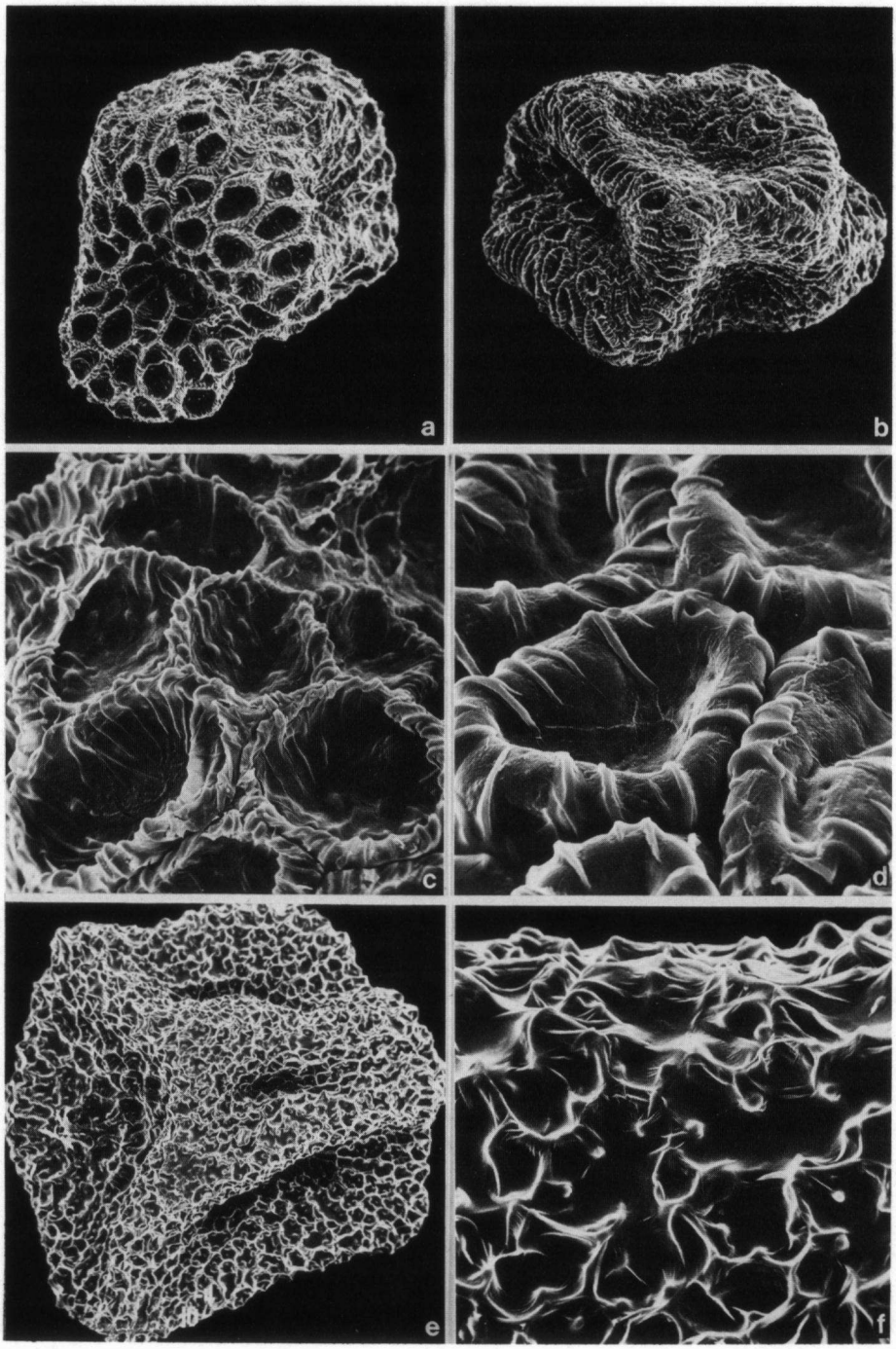

Plate 2. *I. plantaginifolia* A. seed (100×); B. seed (110×); C. detail of concave cells (500×); D detail of concave cells (1100×).
I. tatei E. seed (120×); F. detail of concave cells (500×).

I. poeppigii (Grisebach) Cobb & Maas comb. nov. (Plate 1D–F)

Basionym: *Pagaea poeppigii* Grisebach in de Candolle, Prodr. 9: 71. 1845. Type. Poeppig 2854 (holotype, **GOET**; isotypes, **F, G, MO**) Amazonas, Brasil.

Seeds irregular in shape, from pyramidal to rectangular with ridges and slightly sunken areas, size 350 μm × 200–275 μm. Cells circular to elliptic; cell surface hemispherical to domelike. Outer wall smooth with few band-like thickenings, sometimes branched and continuing through lateral walls; lateral walls sinusoidal; inner wall pitted.

I. plantaginifolia Maguire (Plate 2A–D)

Seeds irregular in shape, from rectangular to spheroid, ridges prominent with obvious sunken areas, size 350 μm × 200–250 μm. Cells usually elliptic, sometimes circular; cell surface concave. Outer wall with short band-like thickenings arising from lateral walls and tapering towards center (looks somewhat like a "collapsed" dome of *I. pumila);* lateral walls thick, straight, with sunken anticlinal boundaries; inner wall scattered with small papillae, sometimes pits outlined by outer wall.

I. nemorosa (Willdenow) Merrill (Plate 3A–F)

Seeds irregular in shape with prominent ridges (sometimes almost winged), often with deeply sunken areas, size 225–400 μm × 300–500 μm. Two types of cell surface, hemispherical to dome-like and concave. Hemispherical to domelike cells circular to broadly elliptic. Outer wall smooth with branched band-like thickenings (very similar to *I. pumila*); lateral walls straight to sinusoidal; inner wall papillae often with warty surface scattered or more restricted to lateral wall. Warty surface seen only when "dome" is removed. Concave cells circular to ovate-elliptic to somewhat polygonal. Outer wall thin; lateral walls straight to slightly undulating, not always the same height; inner wall with few to many papillae along lateral walls (papillae outlined by outer wall), occasionally pitted. Two specimens, Steyermark et al. 113399 (**VEN**) and Moore et al. 9663 (**UC**), also have sunken anticlinal boundaries.

Some of the seeds in this species have just the concave cells, while others have both concave and dome-like cells. There is a tendency that seeds from high altitude plants have only concave cells with lateral walls and papillae less pronounced, while those from lowland plants often have both concave and domelike cells.

Papillae attachment, seen in side view, varies from the lateral wall to the inner wall (Figure 1). Papillae shape is conical with either a rounded, V-shaped, or 3-pronged apex (Figure 2).

I. tatei (Gleason) Maguire (Plate 2E–F)

Seeds irregular in shape and size; usually with deeply sunken areas; ridges prominent, size (175–) 250–350 μm × 350–400 μm. Cells not uniform in size and shape, often puzzle piece-like; cell surface concave. Outer wall usually smooth, sometimes with "stretch marks," rarely with very short, tapering

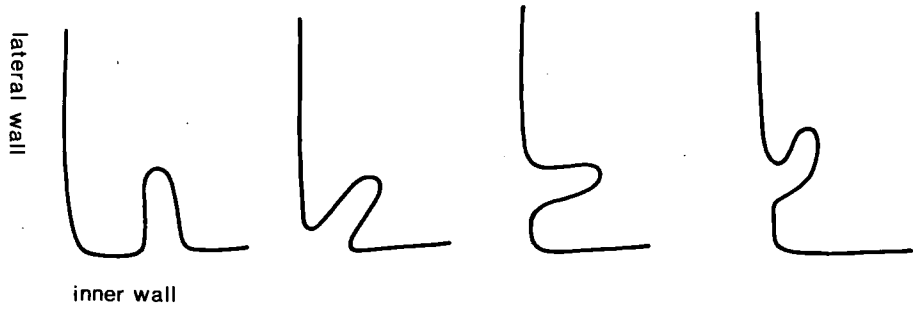

Fig. 1. Papillae attachment on the inner/lateral walls of *Irlbachia nemorosa.*

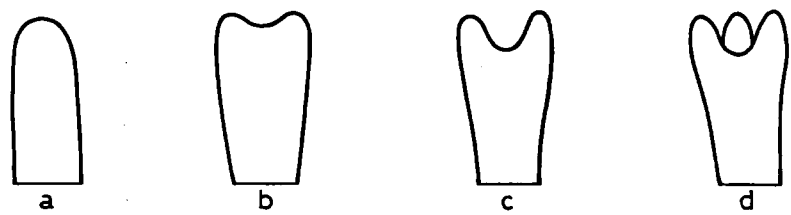

Fig. 2. Shape of papillae of *Irlbachia nemorosa:* conical with a. rounded apex, b. & c. V-shaped apex, and d. 3-pronged apex.

bands; lateral walls irregular to V-undulating; inner wall with papillae near the lateral walls, pits sometimes outlined by outer wall.

I. pratensis (Humboldt, Bonpland, & Kunth) Cobb & Maas comb. nov. (Plate 4A–B)

Basionym: *Lisianthus pratensis* H.B.K., Nov. Gen. Sp. 3: 180. 1818. Type. A collection of Humboldt & Bonpland (holotype, **P** photo seen) Esmeraldas, Amazonas, Venezuela, 21–23 May 1800.

Seeds very irregular in shape, size (250–) 350–400 μm × 400–475 μm. Cells ovate-elliptic to polygonal, shallow; cell surface concave. Outer wall with few, thin band-like thickenings continuing over the entire surface or stopping abruptly; lateral walls low, thick, irregularly undulating with papillae on or near the top; inner wall smooth.

I. caerulescens (Aublet) Grisebach (Plate 4C–F)

Seeds often angular to almost cubic, size (150–) 250–350 μm × (200–) 300–400 μm. Cells puzzle piece-like, shallow, often difficult to distinguish between the separate cells; cell surface concave. Outer wall thin often with "stretch marks" between thickened angles of lateral walls; lateral walls thin, low, sinusoidal to V-undulating with papillae confined to angles; inner wall warty and sometimes pitted.

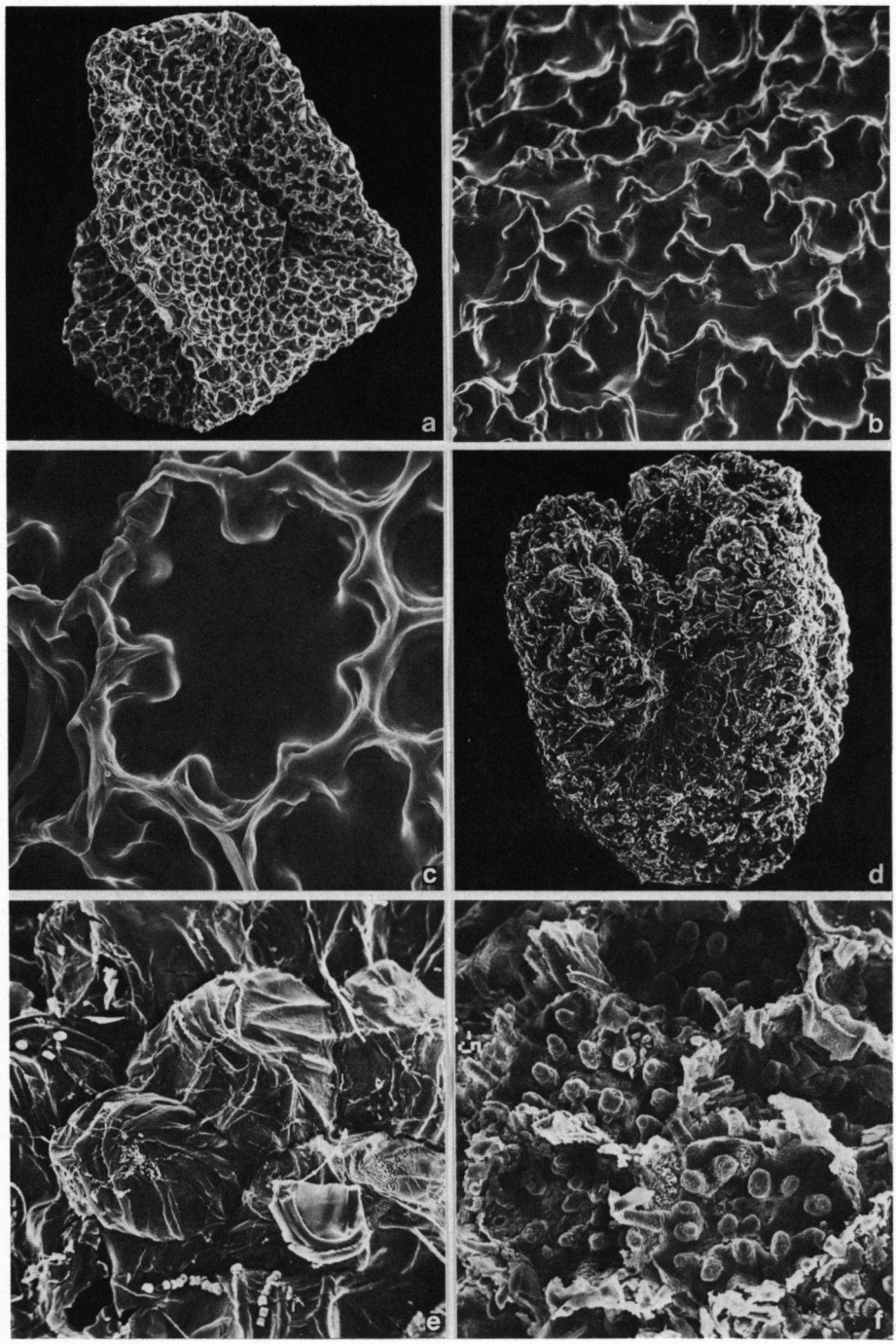

Plate 3. *I. nemorosa* A. seed with concave cells (110×); B. detail of concave cells (510×); C. detail of concave cell (1100×); D. seed with both concave and dome-like cells (110×); E. detail of dome-like cells (550×); F. detail of inner wall with papillae of dome-like cells (500×).

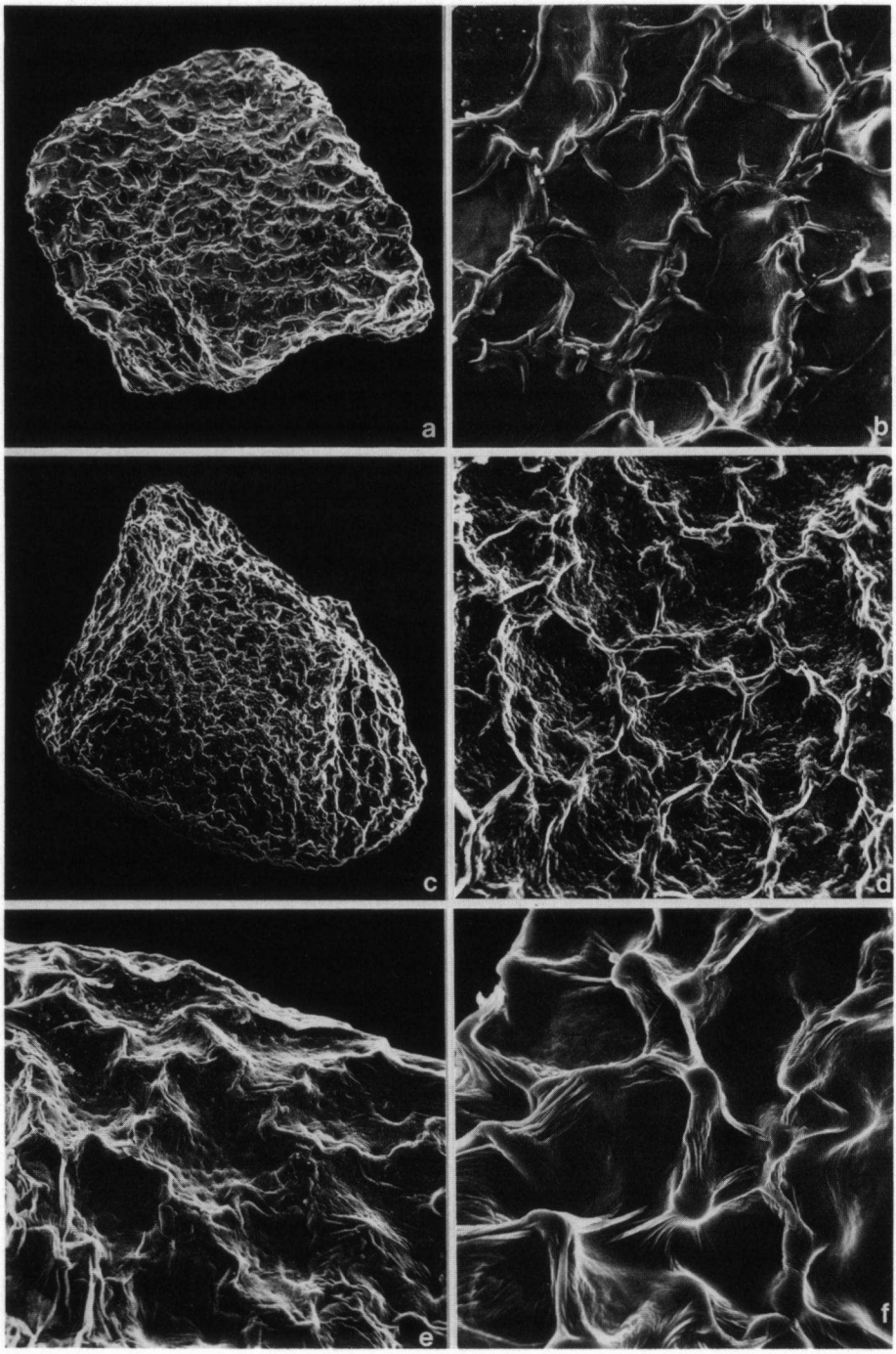

Plate 4. *I. pratensis* A. seed (110×); B. detail of concave cells (580×).
I. caerulescens C. seed (120×); D. detail of concave cells (500×); E. detail of concave cells (550×); F. detail of concave cells (1000×).

DISCUSSION

This seed morphological study supports the authors' views on the classification within the genus *Irlbachia,* especially the species *I. nemorosa.* The other *Irlbachia* species are quite distinctive in their macromorphological and micromorphological features, while *I. nemorosa* is very variable. In the monograph on *Irlbachia* that will be published in Flora Neotropica, twelve species have been brought to the level of synonymy under the species name *I. nemorosa.* This was not done because they were identical, but because the characteristics flowed so smoothly from one species to the next that no separations could be made. If one sees specimens of two extremes of the continua of characteristics, however, it is understandable that he will put them in separate species. With the evidence found in the SEM study and careful examination of specimens from the entire distribution range showing continua in the characteristics, we propose that *I. nemorosa* be considered one species, a species in the process of change.

The external morphological characteristics of the seeds overlap in the *Irlbachia* species; for example, the approximate size of the seeds and the cells, the papillae, the pitted surface of the inner walls, the band-like thickenings on the outer walls, and the dome-like and "collapsed" dome-like cells in many of the species. The order of the species in the article shows the overlap in the characteristics:

dome-like cells – *I. pumila, I. poeppigii, I. nemorosa*
papillae on inner/lateral walls –*I. plantaginifolia, I. nemorosa, I. tatei, I. pratensis, I. caerulescens*
pitted inner wall –*I. pumila, I. poeppigii, I. plantaginifolia, I. nemorosa, I. tatei, I. caerulescens*
band-like thickenings on outer wall – *I. pumila, I. poeppigii, I. nemorosa, I. plantaginifolia, I. tatei, I. pratensis*
concave cells – *I. nemorosa, I. plantaginifolia, I. tatei, I. pratensis, I. caerulescens.*

This overlap in characteristics indicates a very close relation between the species of *Irlbachia.*

Note: Taxonomic treatments of three very closely related genera, *Calolisianthus, Chelonanthus,* and *Helia,* and a treatment at the genus level of this group of Gentians are being conducted at this time. The results of these treatments may show that some or all of the species must be transferred to one genus. This being the case, *Irlbachia* (the oldest genus) may contain more than the seven species listed in this article (see Gentianaceae, Flora Neotropica – to be publ.).

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SPECIMENS SEEN

I. pumila

Level, J.S. L-53 (S); Maguire, B. 34502 (S); Schultes, R.E. et F. Lopez 9902 (US); Spruce, R. 2950 (BM).

I. poeppigii

Ducke, A. 22386 (RB); Holt, E.G. et E.R. Blake 566 (US); Lisbôa, P. 793 (INPA); Nascimento, O.C. 391 (INPA); Pires, J.M. et al. 13962 (U); Poeppig, E.F. 2854 (GOET); Schomburgk, R. 989 (K).

I. plantaginifolia

Bunting, G.S. 3991 (U); Maguire, B. et al. 42593 (VEN); Steyermark, J.A. 102825 (US); Williams, Ll. 14202 (F).

I. nemorosa

Badillo, V. 6144 (MY); Bunting, G.S. 4129 (MY); Cardona, F. 2079, 2694, 2958, 2978 (US), 2986 (MO); Maas, P.J.M. et al. 4240, 5369, 5370 (U); Maguire, B. 27324 (S), 36396 (US); Martius, C.F.P. von s.n. (M); Moore, H.E. 9663 (UC), 9755 (US); Phelps, K.D. 517 (US); Prance, G.T. 3721 (U, US), 4866, 18042 (U); Ravelo, O. 15 (MY); Schomburgk, R. 147 (BM); Schultes, R.E. 5863, 8721 (US); Schultes, R.E. et I. Cabrera 16296 (GH), 16523 (US); Silva, N.T. et M.R. Santos 4704 (U); Spruce, R. 2524, 3055 (BR); Steward, W.C. Pr20262 (U); Steyermark, J.A. 227, 345 (F), 732 (US), 57929 (F), 59087, 59393 (US), 59507 (F), 97961 (G), 113399 (VEN); Tate, G.H.H. 1360 (US); Trujillo, B. 13567 (MY); Ule, E. 8731 (MG); Williams, Ll. 13937 (US); Wurdack, J.J. et L.S. Adderley 42926 (US).

I. tatei

Cardona, F. 376 (US); Maguire, B. 29556, 29625 (VEN); Steyermark, J.A. 58167, (US), 58297, 108877 (F); Tillett, S.S. 751–106 (U).

I. pratensis

Huber, O. 2665 (U); Maguire, B. 34694 (US), 37628 (S); Pires, J.M. et P. Leite 14803 (U); Spruce, R. 2005 (GOET); Steyermark, J.A. 112805 (VEN); Wurdack, J.J. et L.S. Adderley 42949 (US).

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