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A Palynological Study of the Genus *Crotalaria* L. (Leguminosae) in Taiwan

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ABSTRACT: Pollen morphology of 18 *Crotalaria* taxa in Taiwan was studied with scanning and transmission electron microscopy. Anthers of *Crotalaria* are dimorphic, but pollen from the different types of anthers within a particular species is indistinguishable. Among the 18 species, five pollen types can be distinguished based on exine ornamentation. However, all species have the same exine stratification, and their interstitia are columellate. The overall pollen variation in *Crotalaria* species examined is small, making pollen morphology a poor criterion for identifying particular species of this genus in Taiwan.

KEY WORDS: ***Crotalaria*, Pollen morphology, Taiwan.**

INTRODUCTION

There are about 600 *Crotalaria* L. species in the tropics and subtropics, with 17 native and 3 naturalized taxa in Taiwan (Huang and Ohashi, 1977, 1993). The anthers of *Crotalaria* are dimorphic, with two different lengths (Fig 1). The ratio of long anthers to short anthers is about 3:1. Polymorphic pollen from polymorphic anthers has been observed by Erdtman (1952), Pacini and Bellani (1986), El Ghazali (1993), and Fujiki *et al.* (1997). For example, *Commelina communis* has three types of anthers (O-type, X-type and λ-type), and three different pollen types (Fujiki *et al.*, 1997). Palynological studies of *Crotalaria* by Huang (1972), Ferguson and Skvarla (1981), and Okolo and Gill (1986) did not discuss differences in pollen morphology from the two types of anthers. The present study will provide this information for *Crotalaria* species in Taiwan.

MATERIALS AND METHODS

Crotalaria flowers collected in the field and from the TAI-herbarium were fixed in 0.25% glutaraldehyde. Pollen grains in dimorphic anthers were acetolyzed (Erdtman, 1952), dehydrated in an alcohol series, dried in a Critical Point Dryer, mounted on stubs and coated with gold, and photographed with a Hitachi S-520 scanning electron microscope.

For transmission electron microscopy, anthers were prefixed in 2.5% glutaraldehyde for 24 hours, washed in 0.1 M phosphate buffer, treated with 1% OsO_4 for 2 hours, washed in 0.1 M phosphate buffer, dehydrated in an alcohol series, and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were cut with a glass knife and post-stained with uranyl acetate and lead citrate. Micrographs were taken with a Hitachi-600 microscope.

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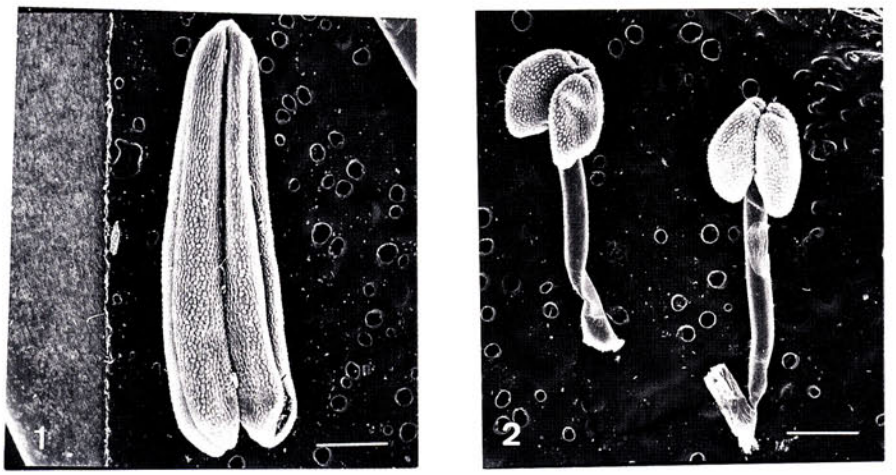


Figs. 1-2. SEM micrographs of the dimorphic anthers of *Crotalaria pallida* var. *obovata*: (1) long anther; (2) short anther. Bar = 500μm

RESULTS AND DISCUSSION

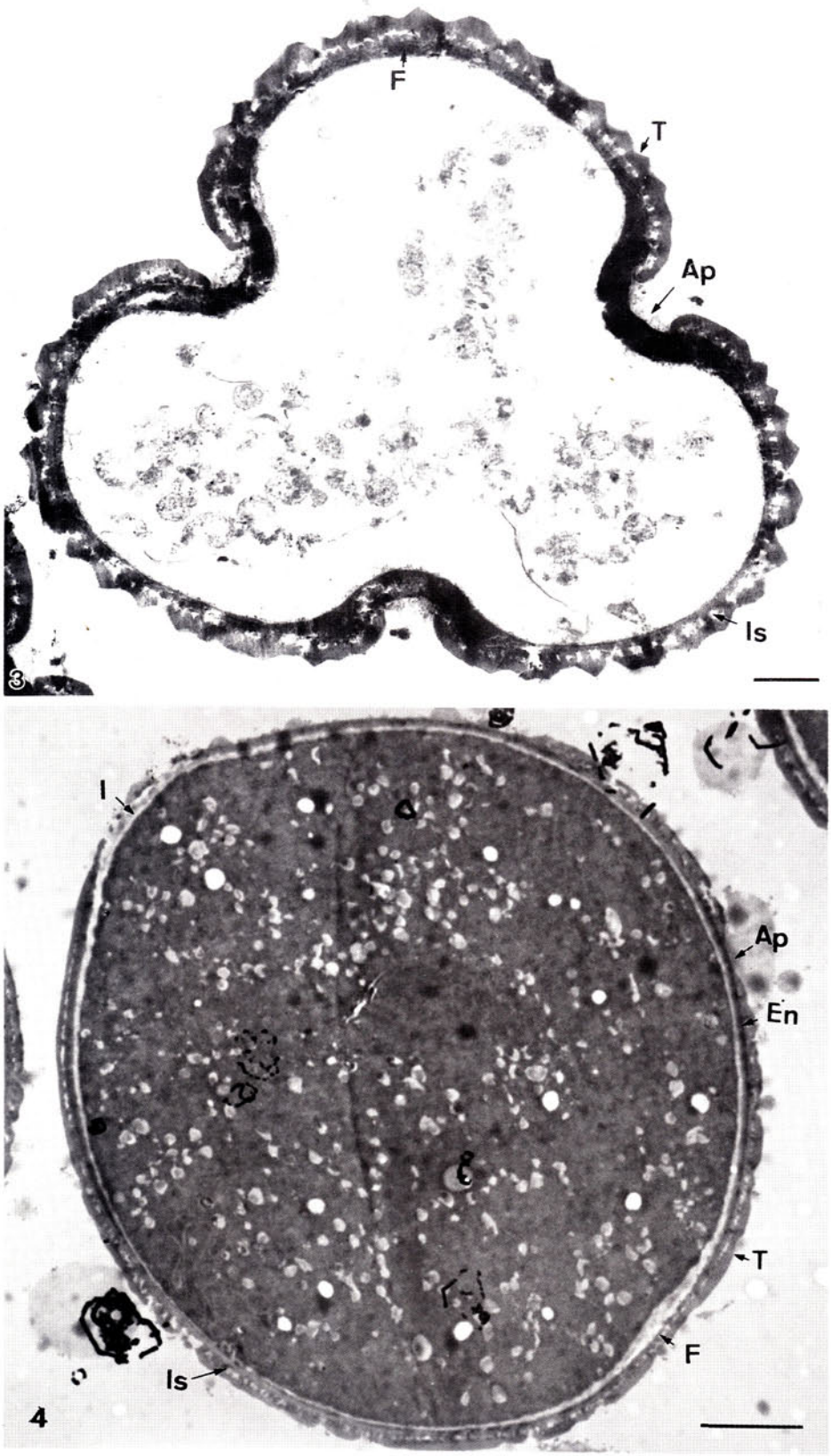
The following descriptions pertain only to Taiwan's *Crotalaria* species. Pollen is 3-colporate, prolate to subprolate, 8-13 × 17-23 μm; and amb circular, semi-angular to inter-semi-angular. The tectum is microperforate or reticulate. The ornamentation on the apocolpium area is the same as, or smoother than, on the mesocolpium area. Pollen of *Crotalaria indica* and *C. juncea* (Figs. 3-4) are representative.

Exine stratification of the various *Crotalaria* species is more or less identical. The foot layer is clear and about the same thickness as the tectum. The interstitium is columellate for all species. The endexine is thickest near the pore margin and almost absent in the apocolpium zone. The exine thins gradually near the pore region where the intine is thickest. Pollen of *C. albida*, *C. indica* and *C. linifolia* (Figs. 5-7) are representative.

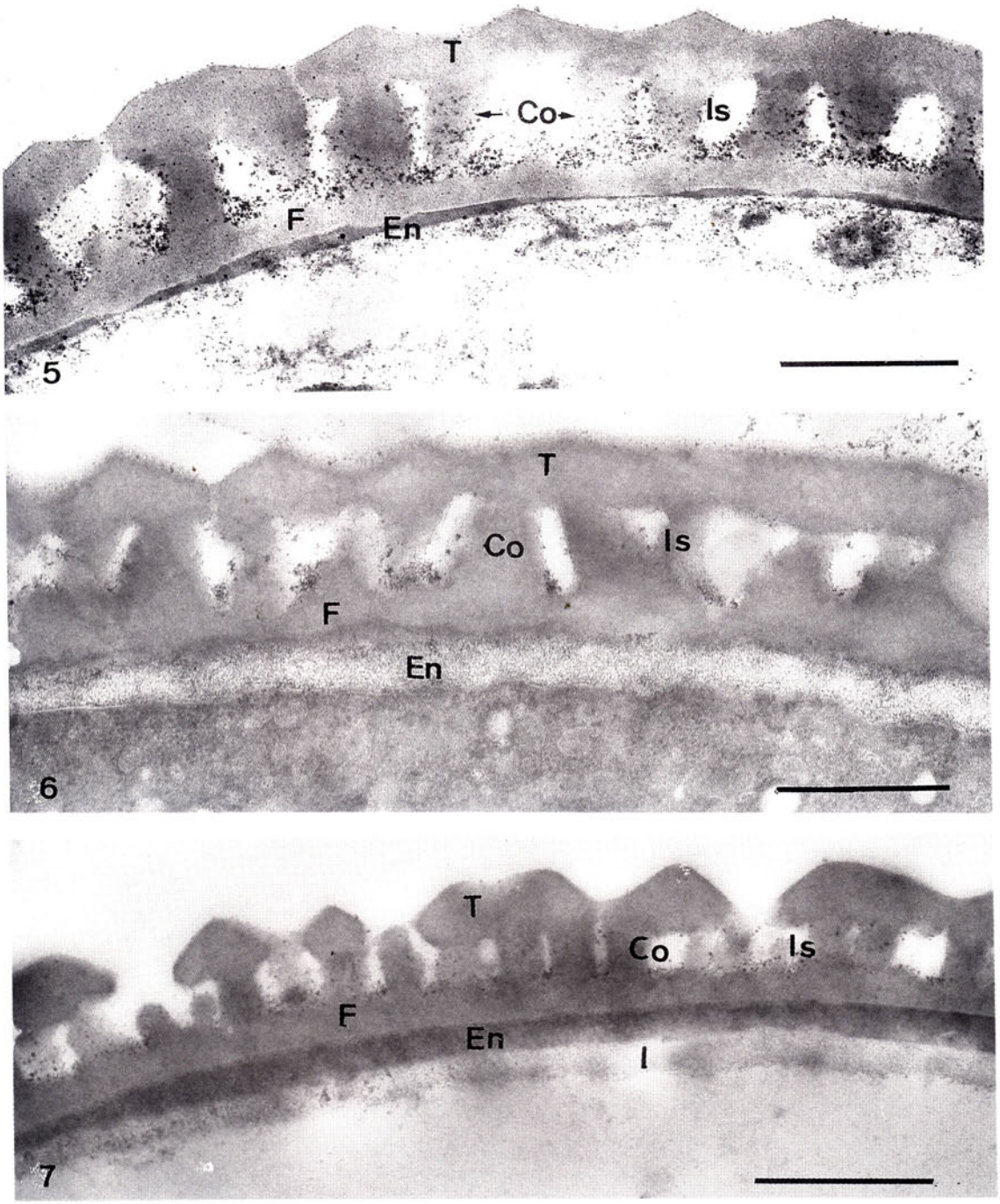
Dimorphic or polymorphic pollen from species with different types of anthers has been described in *Primula* (Erdtman, 1952), *Lagerstroemia indica* (Pacini and Bellani, 1986), *Cassia italica* (El Ghazali, 1993) and *Commelina communis* (Fujiki *et al.*, 1997). In contrast, there is not a significant difference in pollen grains from the two types of anthers within each *Crotalaria* species.

For the genus, however, five pollen types can be described (Table 1). All five pollen types have the same exine stratification, and their interstitia are columellate. A detailed summary of the pollen morphology of 18 Taiwan *Crotalaria* species is given in Table 2.

According to Erdtman (1952) and Huang (1972), the pollen shape of *Crotalaria* plants is prolate to subprolate (categories based on the P/E ratio). However, distinctions between species can be made by the specific P/E ratios of particular species. For example, pollen grains of both *C. acicularis* and *C. sessiliflora* are prolate, but the P/E ratio of the latter is larger than the former (Table 2).



Figs. 3-4. General characters of *Crotalaria juncea* L. (3) and *C. indica* L. (4). Ap: apocolpium; En: endexine; F: foot layer; I: intine; Is: interstitium; T: tectum. Bar = 2μm.



Figs. 5-7. The exine stratification of *Crotalaria albida* Heyne *ex* Roth (5); *C. incana* L. (6) and *C. linifolia* L. (7). The foot layer is clear and about the same thickness as the tectum. The interstitium is columellae for all species. Co: columellae; En: endexine; F: foot layer; I: intine; Is: interstitium; T: tectum. Bar = 1000nm

Table 1. Pollen types of Taiwan's *Crotalaria* species.

Pollen Type	Description	Crotalaria species
I	Microperforate ornamentation in the mescolpium area, but nearly smooth in the apocolpium area	*C. chinensis, C. juncea, C. pallida* var. *obovata, C. retusa, C. verrucosa, C. zanzibarica.*
II	Microperforate on both apocolpium and mesocolpium areas	*C. acicularis, C. albida, C. bialata, C. ferruginea, C. linifolia, C. micans, C. triquetra*
III	Fossulate to rugulate ornamentation on mesocolpium area, and nearly smooth on apocolpium area	*C. lanceolata*
IV	Foveolate ornamentation on mesocolpium area, but nearly smooth or with a few microperforations on apocolpium area	*C. incana, C. similis.*
V	Reticulate ornamentation on both apocolpium and mesocolpium areas	*C. elliptica, C. sessiliflora*

Ferguson and Skvarla (1981) described the variation in pollen morphology in *Crotalaria* mainly in terms of the aperture structure. Okolo and Gill (1986) described differences in pollen shape in polar view (amb type) and in the position of the aperture on the grains, using terms such as lobate, angulaperturate and planaperturate. These distinctions can be found in Taiwan's *Crotalaria* species; however, lobate grains are more abundant.

Huang (1972) observed 16 *Crotalaria* species by light microscopy, dividing them into two groups according to amb shape. One group (circular amb only) included *C. incana*, *C. juncea*, *C. similis, C. zanzibarica*, *C. linifolia*, *C. acicularis*, *C. chinensis*, *C. elliptica* and *C. verrucosa.* The other group (circular and semi-angular, or inter-semi-angular, amb) included *C. albida, C. calycina*, *C. ferruginea* and *C. sessiliflora*. We cannot compare our study to Huang (1972), because, owing to the large P/E ratio, we rarely observed the polar view.

According to Wu and Huang (1995), the evolution of the pollen grains of Taiwan *Indigofera* possibly involved reduction and multiplication or simplification. The interstitia may have undergone both reduction and multiplication from columellae to granula. Thus, the columellate interstitium may be the primitive state (Wu and Huang, 1995).

In summary, the variation of pollen features among species of *Crotalaria* in Taiwan is small, and pollen morphology is not a prominent character for distinguishing among species of this genus; at least, in Taiwan.

ACKNOWLEDGEMENTS

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Table 2. The pollen morphology of Taiwan *Crotalaria*. (The numbers of the figures are shown in the bracket under each taxa.)

Taxa	P-axil (μm)	E-axil (μm)	Average P/E ratio	Polar view	Equatorial view
C. chinensis (Figs. 26-31)	23-24	13-17	1.62	Circular	Prolate
C. juncea (Figs. 50-55)	23-25	13-14	1.75	Inter-semi-angular	Prolate
C. pallida var. *obovata* (Figs. 80-85)	18-23	13-18	1.32	Circular	Prolate to Subprolate
C. retusa (Figs. 86-91)	24-26	14-17	1.62	Semi-angular	Prolate
C. verrucosa (Figs. 122-127)	20-27	15-18	1.42	Circular to semi-angular	Prolate
C. zanzibarica (Figs.134-139)	16-18	11-13	1.43	Circular	Prolate
C. acicularis (Figs. 8-13)	19-22	13-21	1.39	Circular	Prolate
C. albida (Figs. 14-19)	12-16	8-11	1.45	Circular	Prolate
C. bialata (Figs. 20-25)	18-19	11-15	1.45	Circular	Prolate to Subprolate
C. ferruginea (Figs. 38-43)	16-22	13-19	1.21	Circular	Subprolate
C. linifolia (Figs. 62-67)	18-22	11-13	1.67	Circular	Prolate
C. micans (Figs. 74-79)	23-24	15-16	1.53	Circular	Prolate
C. triquetra (Figs. 116-121)	15-20	10-13	1.50	Circular	Prolate
C. lanceolata (Figs. 56-61)	20-21	13-16	1.41	Circular	Prolate
C. incana (Figs. 44-49)	19-23	15-19	1.22	Circular to semi-angular	Subprolate
C. similis (Figs. 104-115)	18-21	14-16	1.41	Circular to semi-angular	Prolate
C. elliptica (Figs. 32-37	20-22	13-15	1.47	Semi-angular	Prolate
C. sessiliflora (Figs. 98-103)	18-20	9-10	1.86	Circular	Prolate

Table 2. Continued.

Taxa	Wall ornamentation		Interstitium	Foot layer	Pollen type
	Mesocolpium area	Apocolpium area			
C. chinensis (Figs. 26-31)	microperforate	microperforate to nearly smooth	columellate	continuous	I
C. juncea (Figs. 50-55)	microperforate	microperforate to nearly smooth	columellate	continuous	I
C. pallida var. *obovata* (Figs. 80-85)	microperforate	microperforate to nearly smooth	columellate	continuous	I
C. retusa (Figs. 86-91)	microperforate	microperforate to nearly smooth	columellate	continuous	I
C. verrucosa (Figs. 122-127)	microperforate	microperforate to nearly smooth	columellate	continuous	I
C. zanzibarica (Figs.134-139)	microperforate	microperforate to nearly smooth	columellate	continuous	I
C. acicularis (Figs. 8-13)	microperforate	microperforate	columellate	continuous	II
C. albida (Figs. 14-19)	microperforate	microperforate	columellate	continuous	II
C. bialata (Figs. 20-25)	microperforate	microperforate	columellate	continuous	II
C. ferruginea (Figs. 38-43)	microperforate	microperforate	columellate	continuous	II
C. linifolia (Figs. 62-67)	microperforate	microperforate	columellate	continuous	II
C. micans (Figs. 74-79)	microperforate	microperforate	columellate	continuous	II
C. triquetra (Figs. 116-121)	microperforate	microperforate	columellate	continuous	II
C. lanceolata (Figs. 56-61)	Fossulate to rugulate	microperforate	columellate	continuous	III
C. incana (Figs. 44-49)	foveolate	microperforate	columellate	continuous	IV
C. similis (Figs. 104-115)	foveolate	microperforate	columellate	continuous	IV
C. elliptica (Figs. 32-37	reticulate	reticulate	columellate	continuous	V
C. sessiliflora (Figs. 98-103)	reticulate	reticulate	columellate	continuous	V

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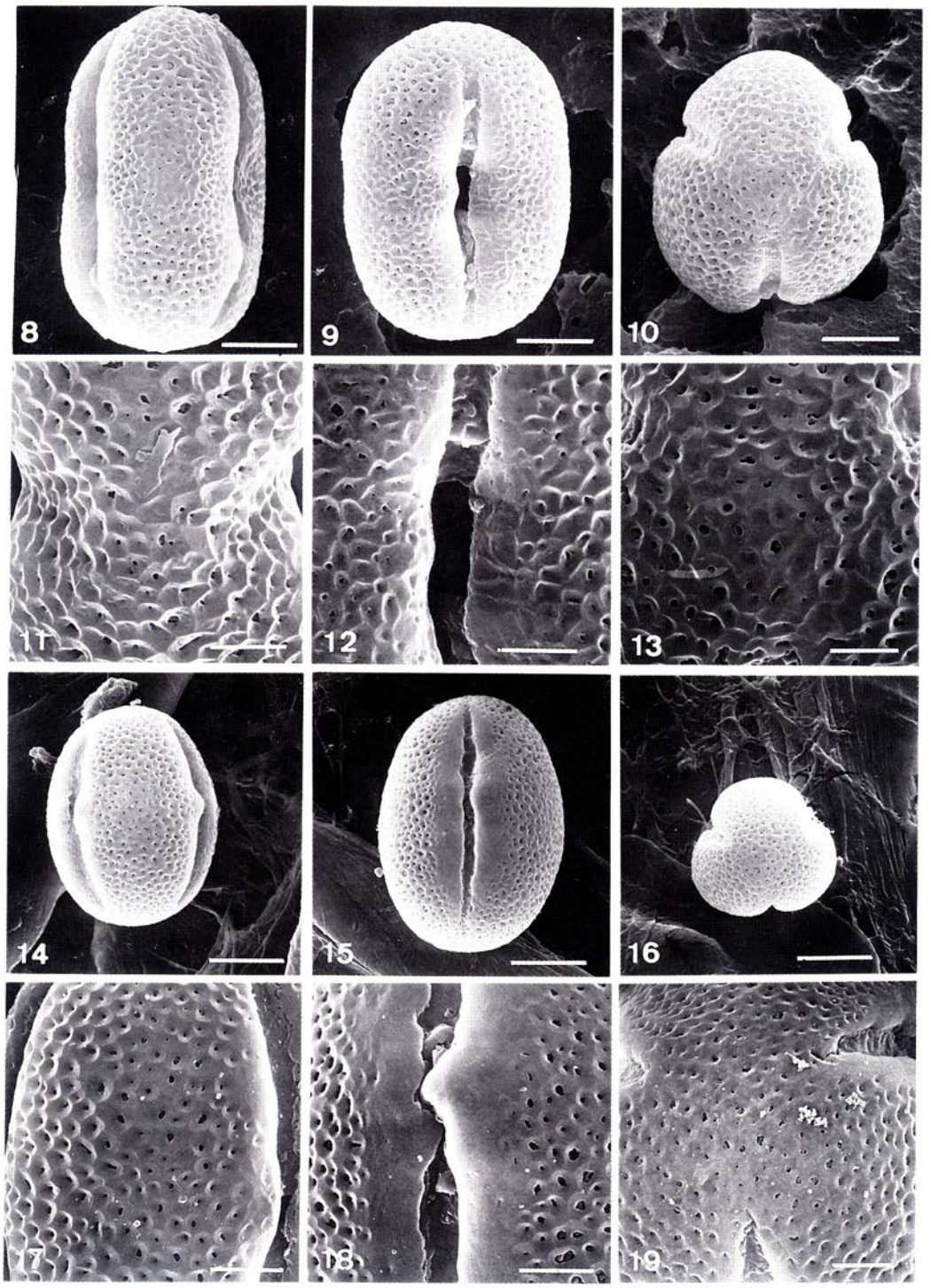
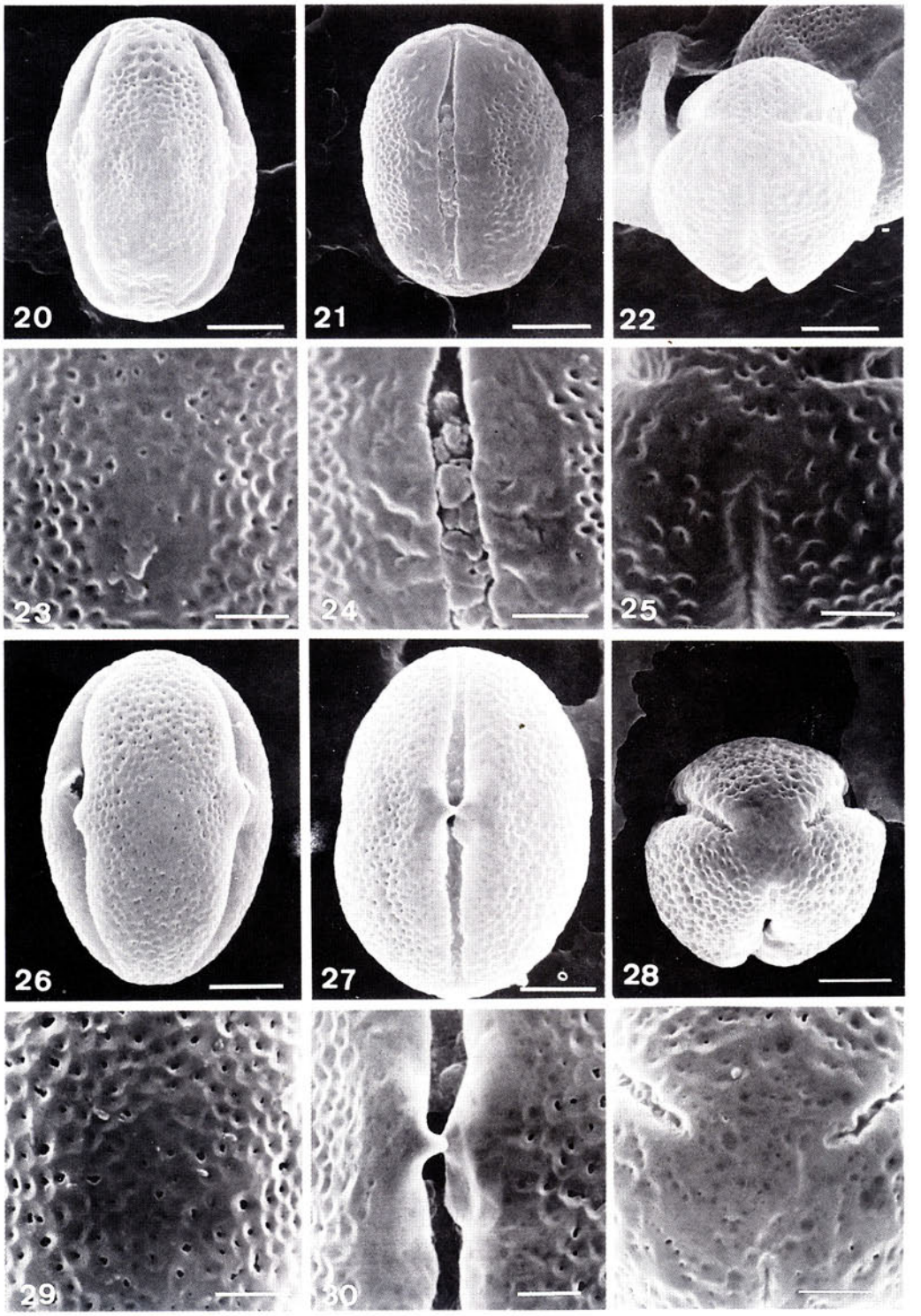
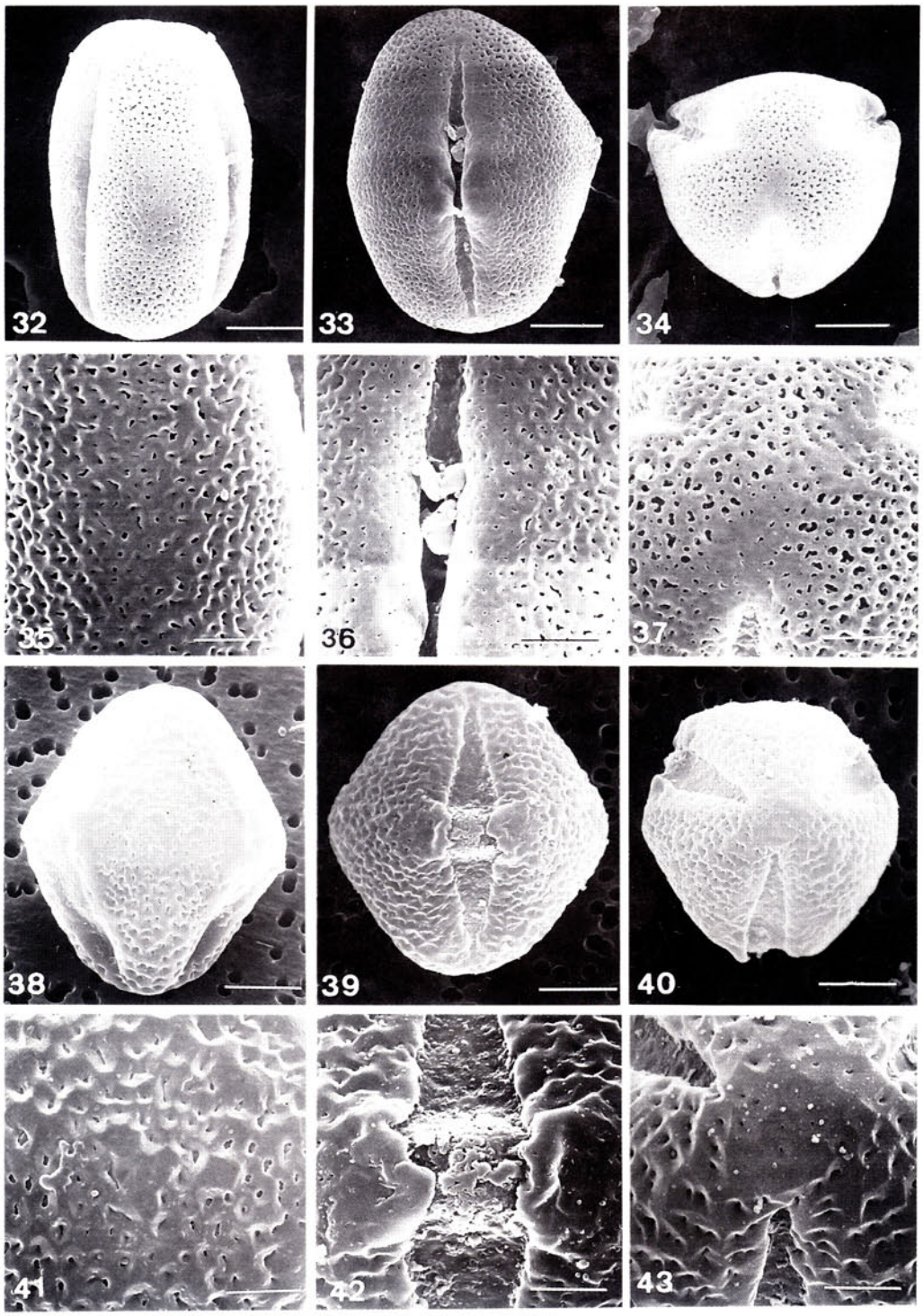


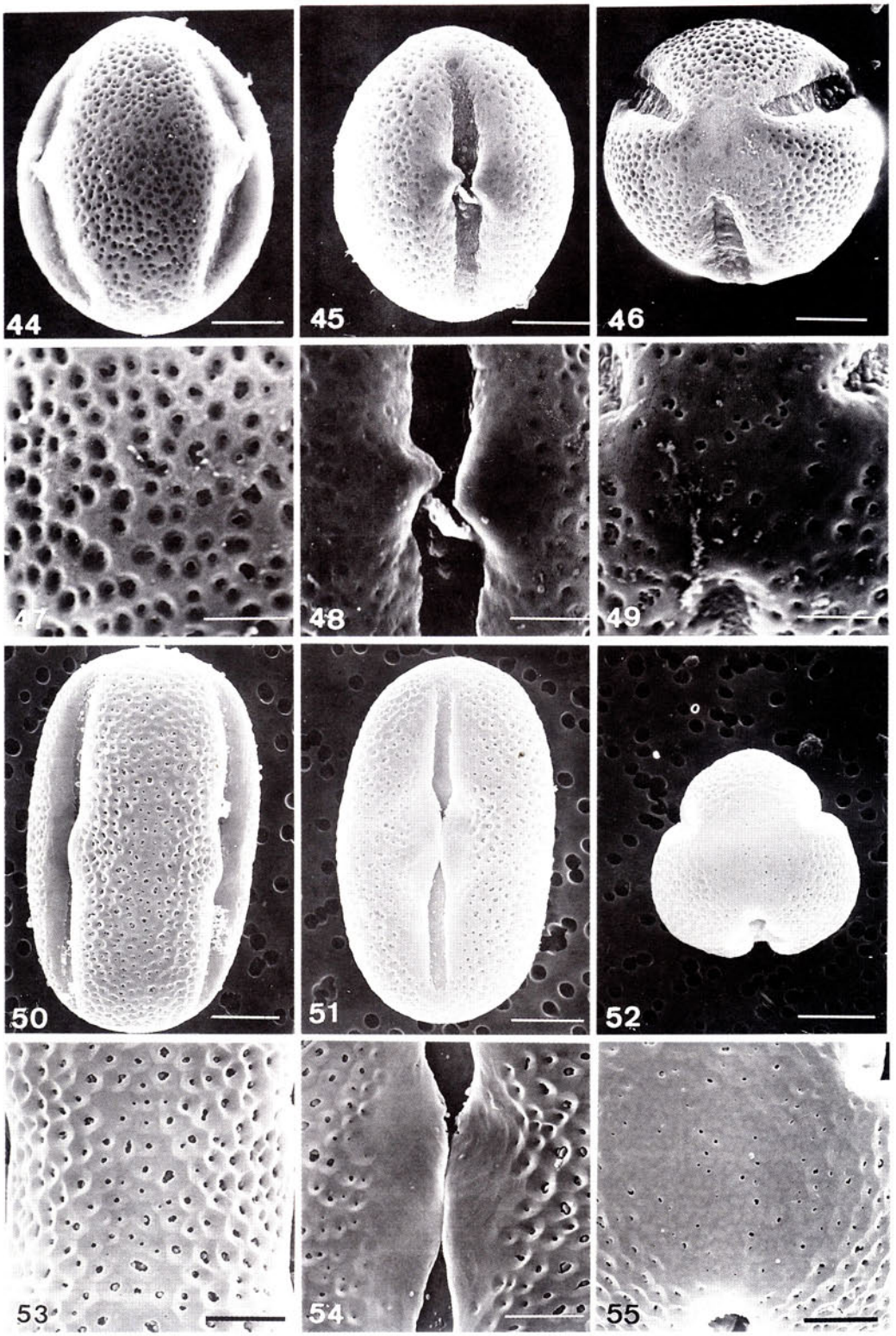
Figs. 8-19. Pollen grains of *Crotalaria acicularis* Buch. –Ham. *ex* Benth. (8-13) and *C. albida* Heyne *ex* Roth (14-19). 8-9 and 14-15, equational view; 10 and 16, polar view; 11 and 17 mesocolpium areas, showing microperforate ornamentation; 12 and 18, colpus membrane; 13 and 19, apocolpium areas, showing microperforate ornamentation; 8-10 and 14-16, bar = 5μm; 11-13 and 17-19, bar = 1.5μm.



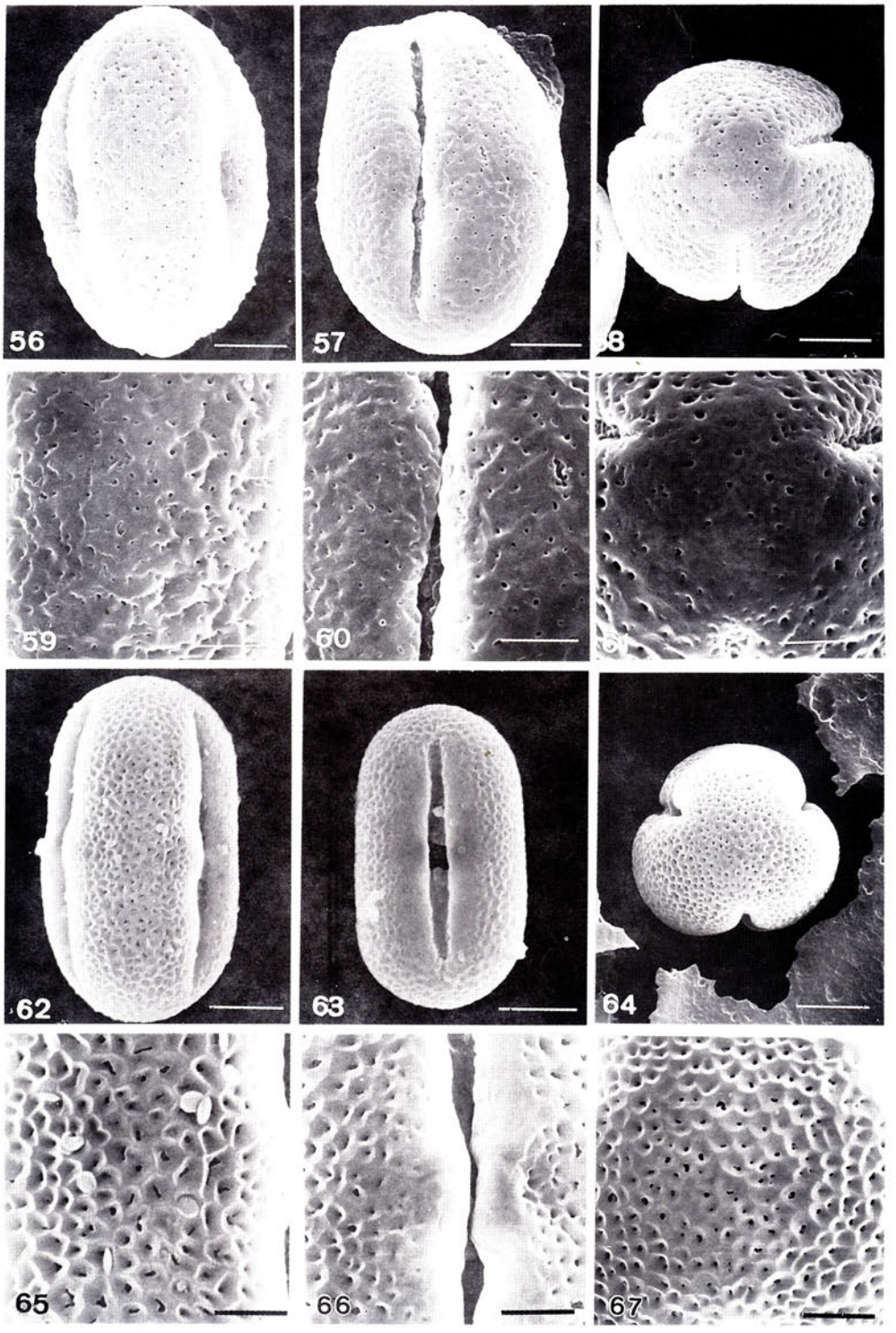
Figs. 20-31. Pollen grains of *Crotalaria bialata* Schrank (20-25) and *C. chinensis* L. (26-31). 20-21 and 26-27, equational view; 22 and 28, polar view; 23 and 29, mesocolpium areas, showing microperforate ornamentation; 24 and 30, colpus membrane; 25 and 31, apocolpium areas, showing microperforate ornamentation; 20-22 and 26-28, bar = 5μm; 23-25 and 29-31, bar = 1.5μm.



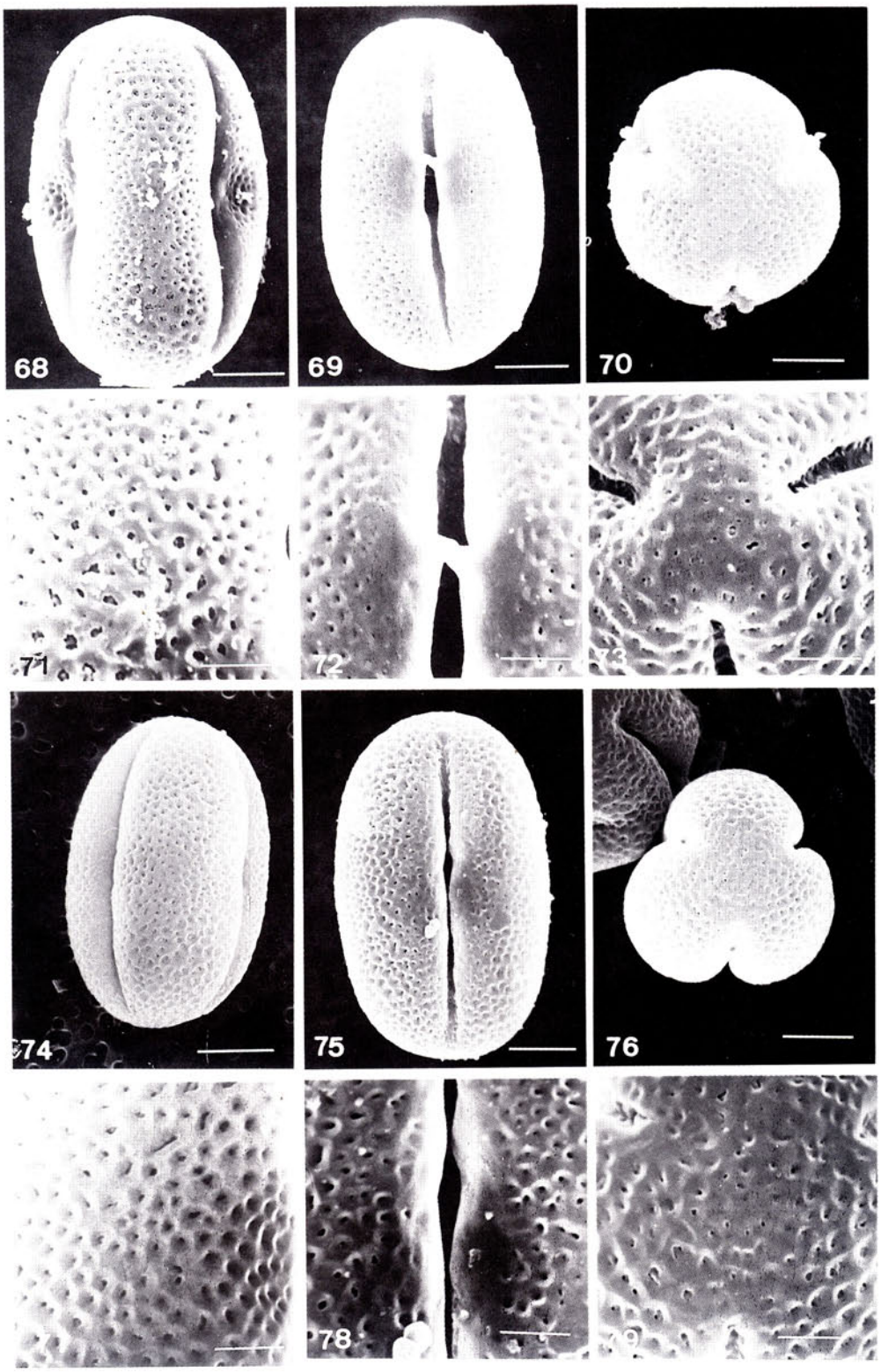
Figs. 32-43. Pollen grains of *Crotalaria elliptica* Roxb. (32-37) and *C. ferruginea* Grah. *ex* Benth (38-43). 32-33 and 38-39, equational view; 34 and 40, polar view; 35, mesocolpium area, showing reticulate ornamentation; 41, mesocolpium area, showing microperforate ornamentation; 36 and 42, colpus membrane; 37, apocolpium area, showing reticulate ornamentation; 43, apocolpium area, showing microperforate ornamentation; 32-34 and 38-40, bar = 5μm; 35-37 and 41-43, bar = 1.5μm.



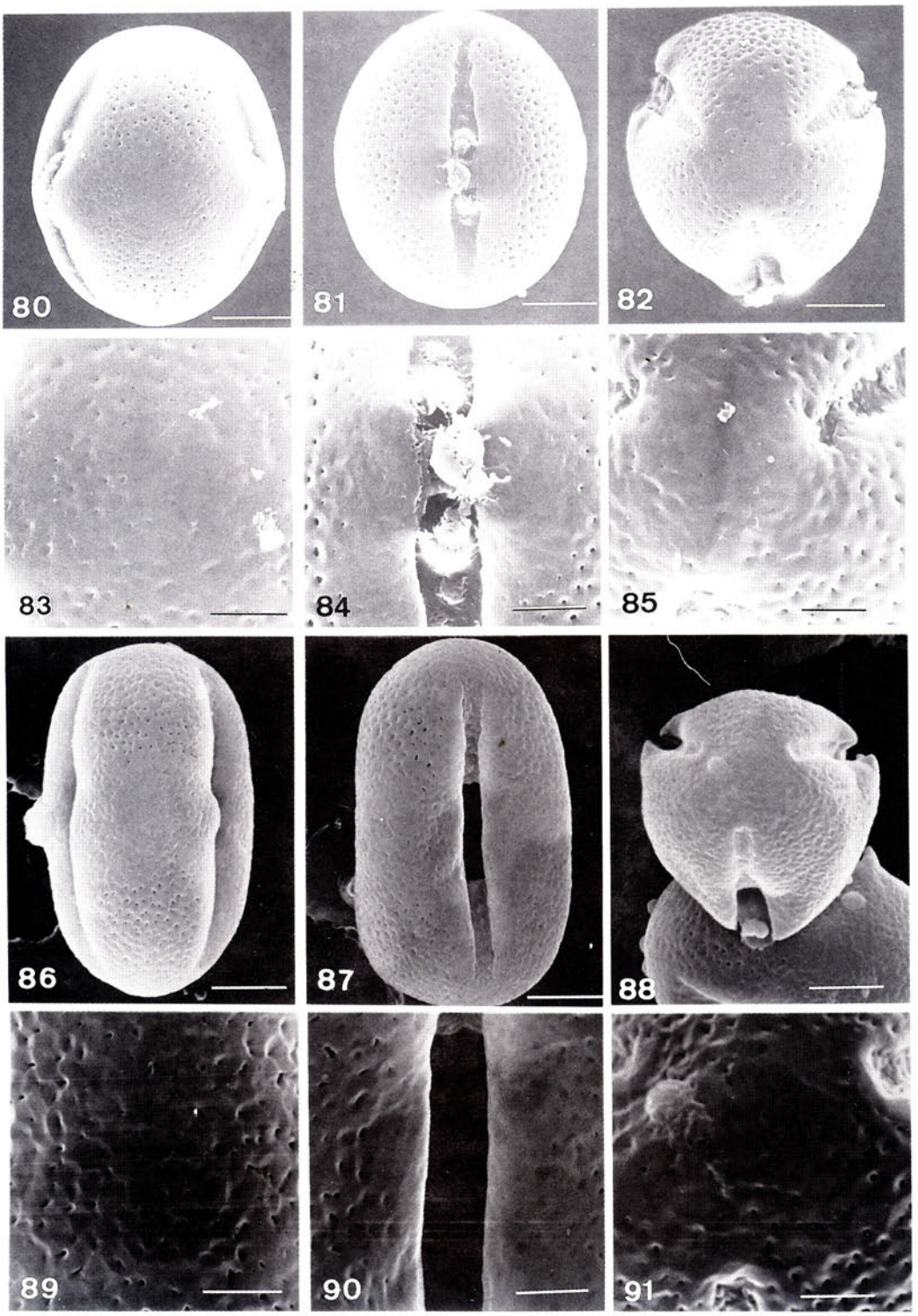
Figs. 44-55. Pollen grains of *Crotalaria incana* L. (44-49) and *C. juncea* L. (50-55). 44-45 and 50-51, equational view; 46 and 52, polar view; 47, mesocolpium area, showing faveolate ornamentation; 53, mesocolpium area, showing microperforate ornamentation; 48 and 54, colpus membrane; 49 and 55, apocolpium areas, showing microperforate ornamentation; 44-46 and 50-52, bar = 5μm; 47-49 and 53-55, bar = 1.5μm.



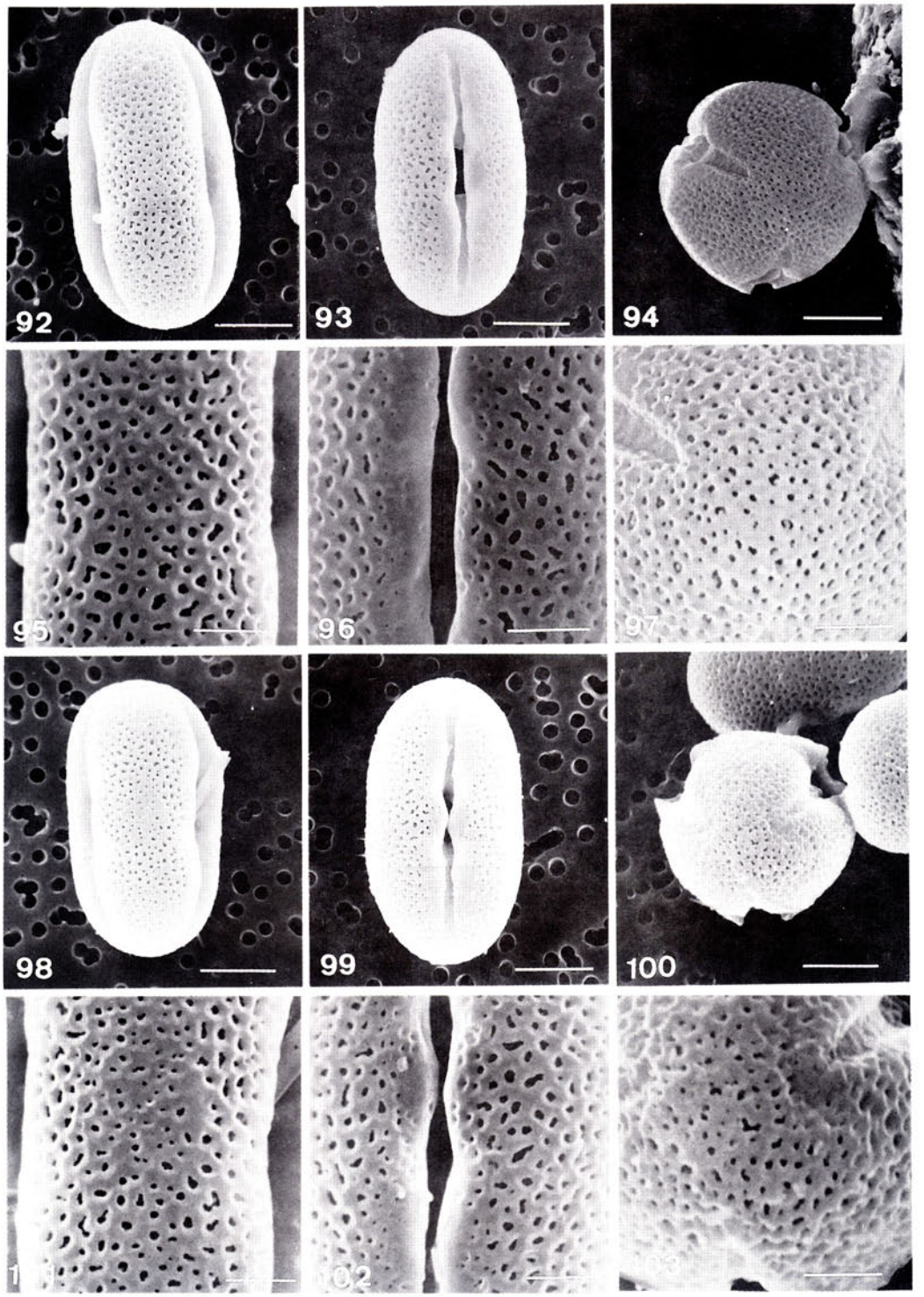
Figs. 56-67. Pollen grains of *Crotalaria lanceolata* E. Mey. (56-61) and *C. linifolia* L. (62-67). 56-57 and 62-63, equational view; 58 and 64, polar view; 59, mesocolpium area, showing fossulate to rugulate ornamentation; 65, mesocolpium area, showing microperforate ornamentation; 60 and 66, colpus membrane; 61 and 67, apocolpium areas, showing microperforate ornamentation; 56-58 and 62-64, bar = 5μm; 59-61 and 65-67, bar = 1.5μm.



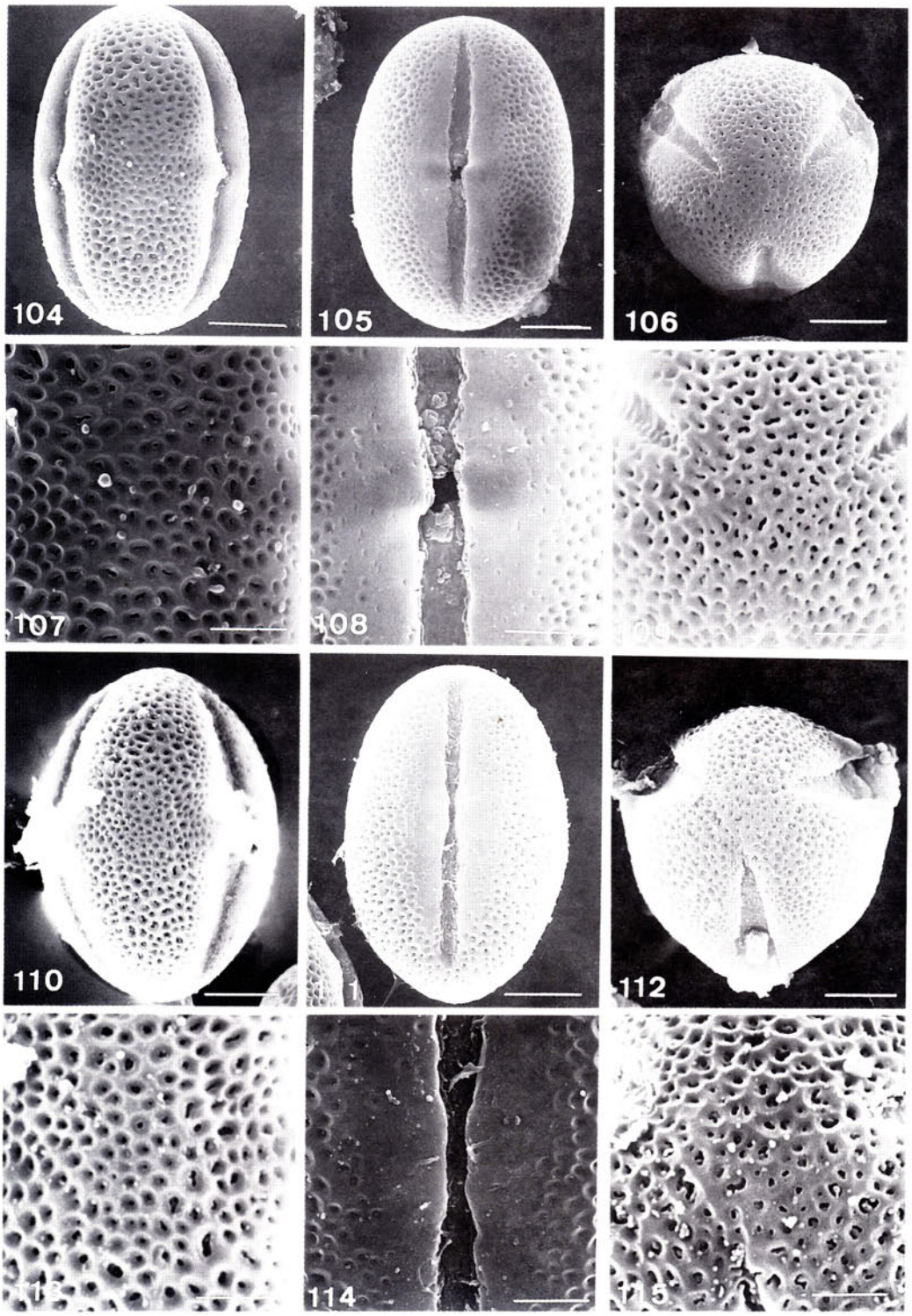
Figs. 68-79. Pollen grains of the long anthers (68-73) and the short anthers (74-79) of *Crotalaria micans* Link. 68-69 and 74-75, equational view; 70 and 76, polar view; 71 and 77, mesocolpium areas, showing microperforate ornamentation; 72 and 78, colpus membrane; 73 and 79, apocolpium areas, showing microperforate ornamentation; 68-70 and 74-76, bar = 5μm; 71-73 and 77-79, bar = 1.5μm.



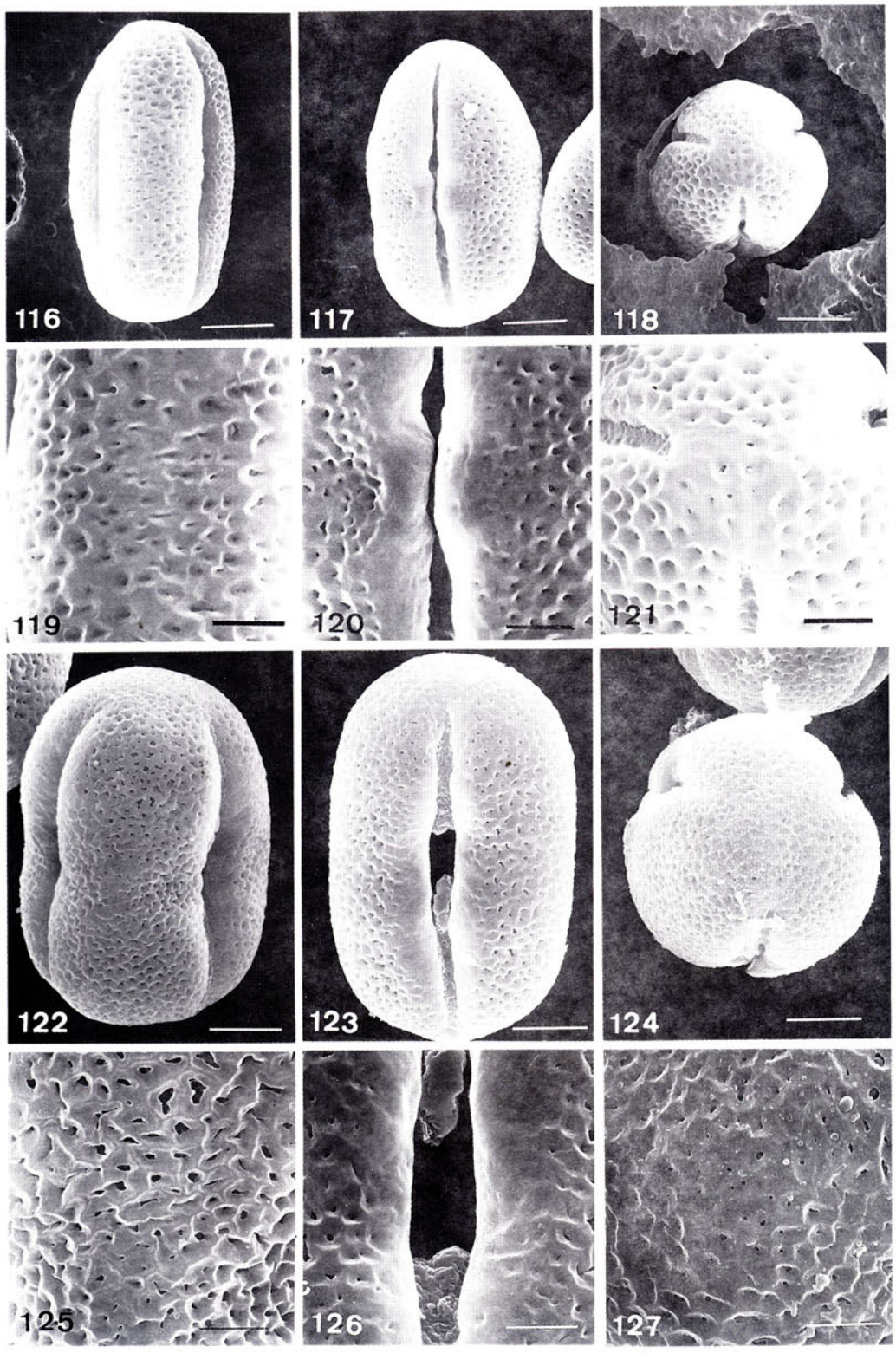
Figs. 80-91. Pollen grains of *Crotalaria pallida* Ait. var. *obovata* (G. Don) Polhill (80-85) and *C. retusa* L. (86-91). 80-81 and 86-87, equational view; 82 and 88, polar view; 83 and 89, mesocolpium areas, showing microperforate ornamentation; 84 and 90, colpus membrane; 85 and 91, apocolpium areas, showing microperforate ornamentation; 80-82 and 86-88, bar = 5μm; 83-85 and 89-91, bar = 1.5μm.



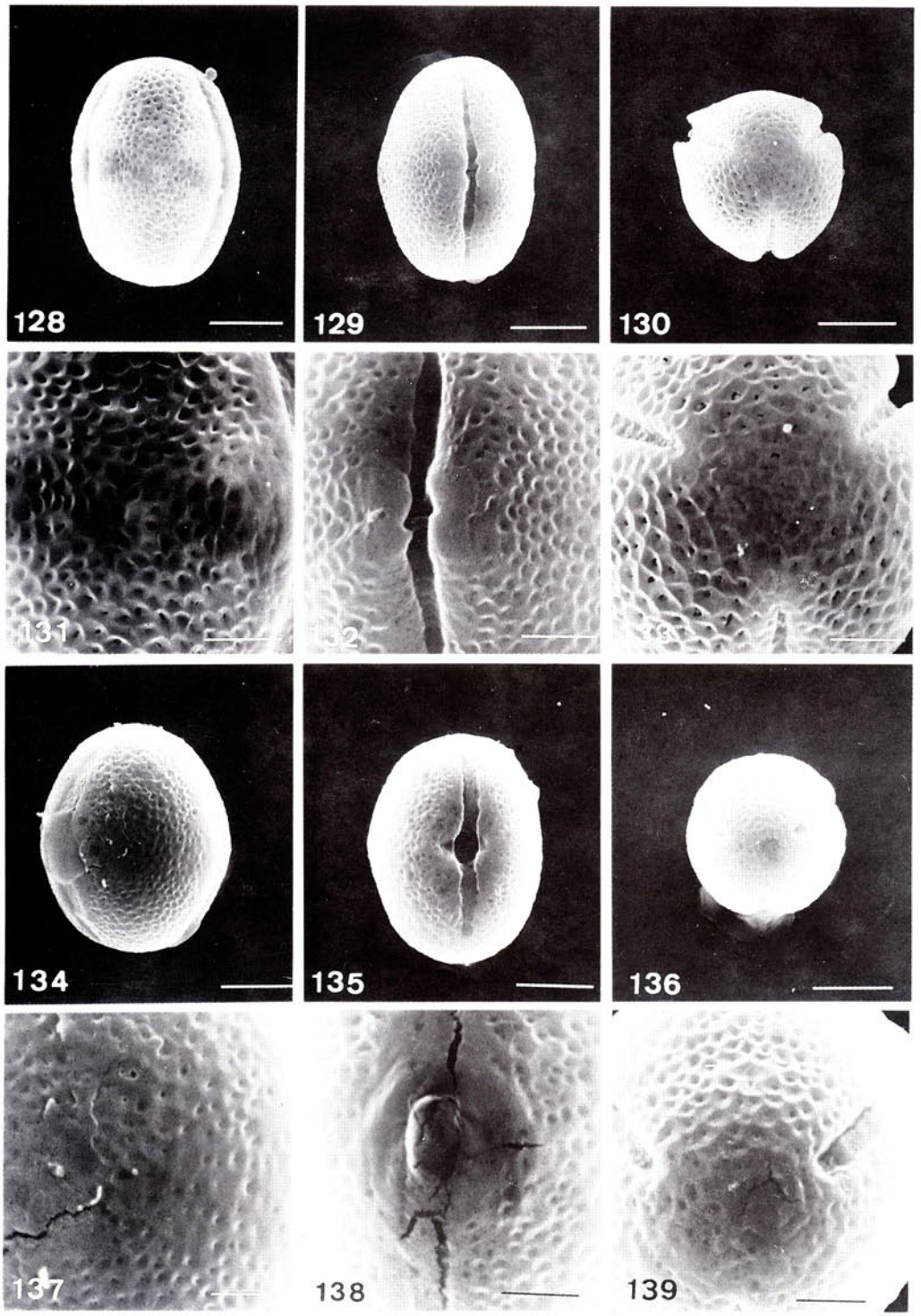
Figs. 92-103. Pollen grains of the long anthers (92-97) and the short anthers (98-103) of *Crotalaria sessiliflora* L. 92-93 and 98-99, equational view; 94 and 100, polar view; 95 and 101, mesocolpium areas, showing reticulate ornamentation; 96 and 102, colpus membrane; 97 and 103, apocolpium areas, showing reticulate ornamentation; 92-94 and 98-100, bar = 5μm; 95-97 and 101-103, bar = 1.5μm.



Figs. 104-115. Pollen grains of the long anthers (104-109) and the short anthers (110-115) of *Crotalaria similis* Hemsl. 104-105 and 110-111, equational view; 106 and 112, polar view; 107 and 113, mesocolpium areas, showing fovelate ornamentation; 108 and 114, colpus membrane; 109 and 115, apocolpium areas, showing microperforate ornamentation; 104-106 and 110-112, bar = 5μm; 107-109 and 113-115, bar = 1.5μm.



Figs. 116-127. Pollen grains of *Crotalaria triquetra* Dalzell (116-121) and *C. verrucsa* L. (122-127). 116-117 and 122-123, equational view; 118 and 124, polar view; 119 and 125, mesocolpium areas, showing microperforate ornamentation; 120 and 126, colpus membrane; 121 and 127, apocolpium areas, showing microperforate ornamentation; 116-118 and 122-124, bar = 5μm; 119-121 and 125-127, bar = 1.5μm.



Figs. 128-139. Pollen grains of the long anthers (128-133) and the short anthers (134-139) of *Crotalaria zanzibarica* Benth. 128-129 and 134-135, equational view; 130 and 136, polar view; 131 and 137, mesocolpium areas, showing microperforate ornamentation; 132 and 138, colpus membrane; 133 and 139, apocolpium areas, showing microperforate ornamentation; 128-130 and 134-136, bar = 5μm; 131-133 and 137-139, bar = 1.5μm.

臺灣產野百合屬植物之花粉型態研究

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摘　　要

臺灣產野百合屬植物花粉皆為三溝孔粒，無論從光學顯微鏡或掃描式電子顯微鏡來看，不僅同一種植物之兩型花藥所含花粉形態相近，種間之花粉形態亦無太大差異，然而，依據外壁紋飾，仍可區分為下列幾個類型。第 I 類型：溝間區之外壁紋飾為細穿孔紋，愈接近溝緣，孔徑漸小，至溝緣處則平滑無紋；極區紋飾為稀疏細穿孔紋，近於平滑；蓋基間層顯著柱狀，基層顯著，如黃野百合。第 II 類型：溝間區之外壁紋飾為細穿孔狀，愈接近溝緣，孔徑漸小，至溝緣處則平滑無紋；極區紋飾與溝間區相同；蓋基間層顯著柱狀，基層顯著，如圓葉野百合。第 III 類型：溝間區之外壁紋飾為溝紋至散條紋，極區紋飾為稀疏細穿孔紋；蓋基間層柱狀，與蓋頂層相等厚度或較薄；基層顯著，如披針葉豬屎豆。第 IV 類型：溝間區之外壁紋飾為圓孔狀，愈接近溝緣，孔徑漸小，至溝緣處則平滑無紋；極區紋飾為細穿孔紋；蓋基間層柱狀，基層顯著，如恆春野百合。第 V 類型：溝間區之外壁紋飾為網狀，愈接近溝緣，網眼漸小，在溝緣附近，並有少許細穿孔紋；極區紋飾與溝間區相同；蓋基間層柱狀，基層顯著，如雙子野百合。

關鍵詞：野百合屬，花粉型態，臺灣。

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