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A Palynological Study of the Tribe Millettieae (Fabaceae) in Taiwan

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ABSTRACT: The present paper is a part of a series of palynological studies for better understanding the relationship between the genera and subfamilies of the Fabaceae (Leguminosae) in Taiwan. There are 4 genera and 12 species found as native plants of the tribe Millettieae in Taiwan. The pollen grains of these 12 native taxa were studied, using scanning electron microscopy and transmission electron microscopy to examine and photomicrograph their pollen morphology. The pollen morphology of the tribe Millettieae in Taiwan is characterized by similar exine stratification, its columellate interstitium, the discontinuous foot layer, and the differentiated exine ornamentation. The pollen type for this study is based on the features of exine ornamentation and stratification. The genus *Pongamia* is represented by a single type, the genus *Derris* by two subtypes, and the genera *Millettia* and *Tephrosia* by three subtypes respectively but they can be grouped into 4 main types. Except for the genus *Pongamia*, the palynological characteristics alone are not sufficient for identification of the genera among the tribe Millettieae in Taiwan.

KEY WORDS: Millettieae, Leguminosae (Fabaceae), Pollen Morphology, Taiwan.

INTRODUCTION

The tribe Millettieae, which is generally thought to be the intermediate group between several other tribes in Papilionoideae (Faboideae), consists of over 40 genera and 1000 species in the world (Geesink, 1984). There are 4 genera and 12 species of the tribe native in Taiwan (Huang and Ohashi, 1977, 1993). This paper is a part of a series of palynological studies about Taiwan legumes for better understanding the relationship between the genera and subfamilies (Chen and Huang, 1993; Wu and Huang, 1995; Lin and Huang, 1999) on the basis of pollen morphology.

MATERIALS AND METHODS

Flowers of 4 genera and 12 species of Millettieae were mostly collected in the field in Taiwan (Table 1). Some were obtained from the TAI-herbarium specimens. All collections were fixed in 0.25% glutaraldehyde. Pollen grains were acetolyzed (Erdtman, 1952), dehydrated in an ethanol series, and dried in a Critical Point Dryer, placed on specimen stubs, and sputter coated with gold. The examinations and photomicrographs were made by a Hitachi S-520 scanning electron microscope.

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Table 1. Data of pollen materials used.

Taxa	Collection site	Collectors and collection numbers	Types of microscope used SEM	TEM
Derris laxiflora Benth.	Pingtung: Fenkang	*T. C. Huang 14080-1*	+	+
	Taichung: Kukuan	*T. C. Huang 14782*	+	+
Derris oblonga Benth.	Taitung: Lanyu	*T. C. Huang 9243*	+	
	Taitung: Lanyu	*Y. C. Hsu s.n.*, 1993	+	+
Derris trifoliata Lour.	Taitung: Lanyu	*T. C. Huang 5333*	+	+
Millettia nitida Benth.	Taichung: Kukuan	*T. C. Huang 14780*	+	+
	Taichung: Kukuan	*T. C. Huang 14781*	+	+
	Taichung: Kukuan	*T. C. Huang 15324*		+
Millettia pachycarpa Benth.	Nantou: Luantashan	*K. C. Wang s.n.*, 1992	+	+
	Hsinchu: Ching-chuan	*K. C. Wang s.n.*, 1992	+	+
	Ilan: Shuanshi	*Y. C. Hsu.s.n.*,1992	+	+
Millettia pulchra var. *microphylla* Dunn.	Pingtung: Chiaroshui	*T. C. Huang 14664*	+	+
	Pingtung: Chiaroshui	*T. C. Huang 15565-7*	+	+
Millettia reticulata Benth.	Pingtung: Wutai	*T. C. Huang 15256*	+	+
	Taoyuan: Taoyuan	*M. J. Wu 1369*	+	+
Pongamia pinnata (L.) Pierre ex Merr.	Taipei: NTU compass	*Y. C. Hsu s.n.*, 1991	+	+
		Y. C. Hsu s.n., 1993	+	+
Tephrosia candida (Roxb.) D. C.	Pingtung: Neipu	*S. Z. Yang 24727-8*	+	+
	Nantou: Puli	*T. C. Huang 13147*	+	+
Tephrosia noctiflora Boj.	Taichung: Lunchin	*T. C. Huang 15273*	+	+
	Taichung: Lunchin	*T. C. Huang 15279*	+	+
	Taichung: Lunchin	*T. C. Huang 15284*		+
	Chanhua: Lukan	*T. C. Huang 15293*	+	+
Tephrosia obovata Merr.	Pingtung: Ho-pi	*T. C. Huang 14686*		+
	Pingtung: Maopitou	*T. C. Huang 15554*	+	
	Pingtung: Chiaroshui	*T. C. Huang 15582*	+	+
Tephrosia purpurea Pers.	Kouhsiung: Taiyangku	*T. C. Huang 14493**	+	+
	Kouhsiung: Yuehshihchieh	*T. C. Huang 15339**	+	+
	Tainan: Anping	*S. F. Huang 3142***	+	+
	Taitung	*S. Sasaki s.n.****	+	

*The white flower; ** The red flower; *** The red flower classified as *T. ionphlebia*

The preparation of materials for transmission electron microscopy follows that of Spurr (1969). The pollen grains were prefixed in 2.5% glutaraldehyde for 24 hours, washed in 0.1M phosphate buffer, postfixed in 1% osmium tetraoxide for 1-2 hours, rinsed in 0.1 M phosphate buffer for 30 minutes, dehydrated in a graded ethanol series, transferred to 100% acetone, and embedded in Spurr's resin. Ultra-thin sections were cut with a glass knife about 60-75 nm. The sections were pre-stained with 5% uranyl acetate and post-stained with lead citrate, then examined and photographed with a Hitachi 600 microscope.

The specimens used in this study were deposited in the TAI-herbarium of the Department of Botany, National Taiwan University.

RESULTS AND DISCUSSION

Pollen grains of Taiwan's Millettieae are characterized by 3-colporate, prolate to oblate-spheroidal, 18-29 x 17-30 μm, and amb circular to semi-angular. The pollen wall stratification can be shown in Figure 1: ectexine has a uniform thickness; endexine is thinner in the polar area and becomes thicker toward the ora, and in the ora area it becomes a membrane; intine in the polar area is also thinner, but towards the ora it becomes thicker and becomes thickest in the ora area. The interstitium is columellate for all taxa. The foot layer is discontinuous, and from obscure to clearly distinct. The tectum is foveolate, fossulate, reticulate to verrucate. The ornamentation on the apocolpium area is the same as, or smoother than, on the mesocolpium area (Table 2). The terminology used in this paper for exine ornamentation follows that of Thomson and Pflug (1953) except one term "verrucate" is adopted from Couper and Grebe (1961).

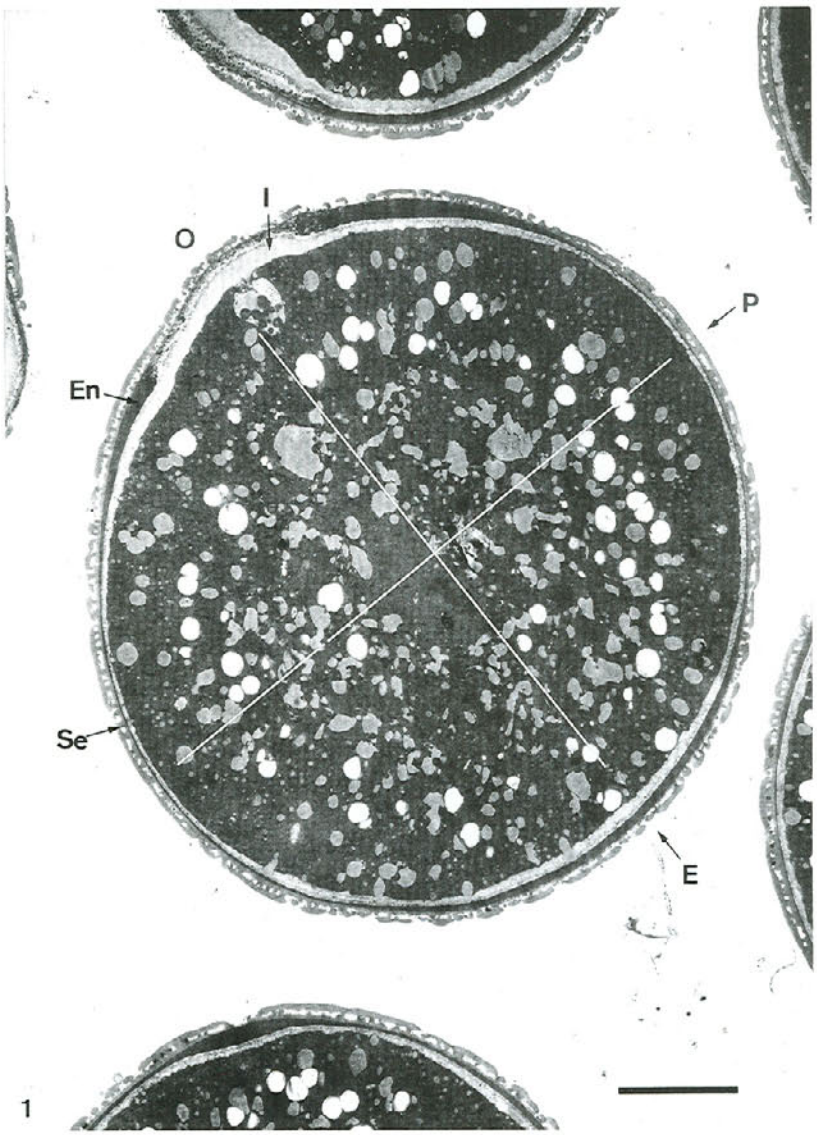


Fig. 1. General characters of *Tephrosia noctiflora*. E: equatorial area, P: polar area, O: ora, En: endexine, I: intine, Se: sexine. Bar=5 μm. (TEM, *Huang 15284*)

Table 2. The pollen morphology of Taiwan Millettieae species (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
Derris laxiflora (Figs. 2-9)	21-24	20-22	1.04	Circular	Oblate-spheroidal to subprolate
Derris oblonga (Figs. 10-16)	20-23	19-20	1.08	Circular to semi-angular	Suboblate to subprolate
Derris trifoliata (Figs. 17-23)	20-23	20-27.5	1.13	Circular to semi-angular	Prolate-spheroidal to subprolate
Millettia nitida (Figs. 24-30)	28-29	27-30	1.09	Circular to semi-angular	Oblate-spheroidal to subprolate
Millettia pachycarpa (Figs. 31-37)	21-25	19-24	1.13	Semi-angular	Spheroidal to prolate
Millettia pulchra var. *microphylla* (Figs. 38-44)	19-22	17-22	1.13	Semi-angular	Oblate-spheroidal to subprolate
Millettia reticulata (Figs. 45-51)	19-22	17-20	1.13	Circular	Oblate-spheroidal to prolate
Pongamia pinnata (Figs. 52-58)	18-22	18-20	1.08	Circular to semi-angular	Oblate-spheroidal to subprolate
Tephrosia candida (Figs. 59-66)	24-28	20-25	1.18	Circular	Prolate -spheroidal to prolate
Tephrosia noctiflora (Figs. 67-74)	29-30	21-23	1.38	Circular	Prolate -spheroidal to prolate
Tephrosia obovata (Figs. 75-81)	23-24	18-20	1.33	Circular	Subprolate to prolate
Tephrosia purpurea (Figs. 82-88)	19-20	17-19	1.20	Semi-angular	Oblate-spheroidal to prolate

The exine stratification is similar throughout this tribe, but the exine ornamentation varies greatly among genera within the tribe (Table 3). Thus, for various species in the same genus sometimes the specific difference is greater than their generic difference; on the other hand, in different genera it is possible to find similar exine ornamentation. Based on exine ornamentation the distinction between different genera is difficult, excepting the genus *Pongamia* which has a distinct "verrucate" wall ornamentation. There can be one to several subtypes within each genus, *i.e.*, two subtypes for *Derris*, three subtypes for *Millettia*, only one pollen type for *Pongamia* and three subtypes for *Tephrosia* (Table 4). But all these subtypes also can be grouped into four main pollen types for the Taiwan Millettieae species.

Pollen variations for *Derris oblonga* and *Millettia pachycarpa* have been observed as follows. The exine ornamentation displays a continuous variation (Figs. 10-16) in a single

Table 2. Pollen wall ornamentation and stratification of Taiwan Millettieae species.

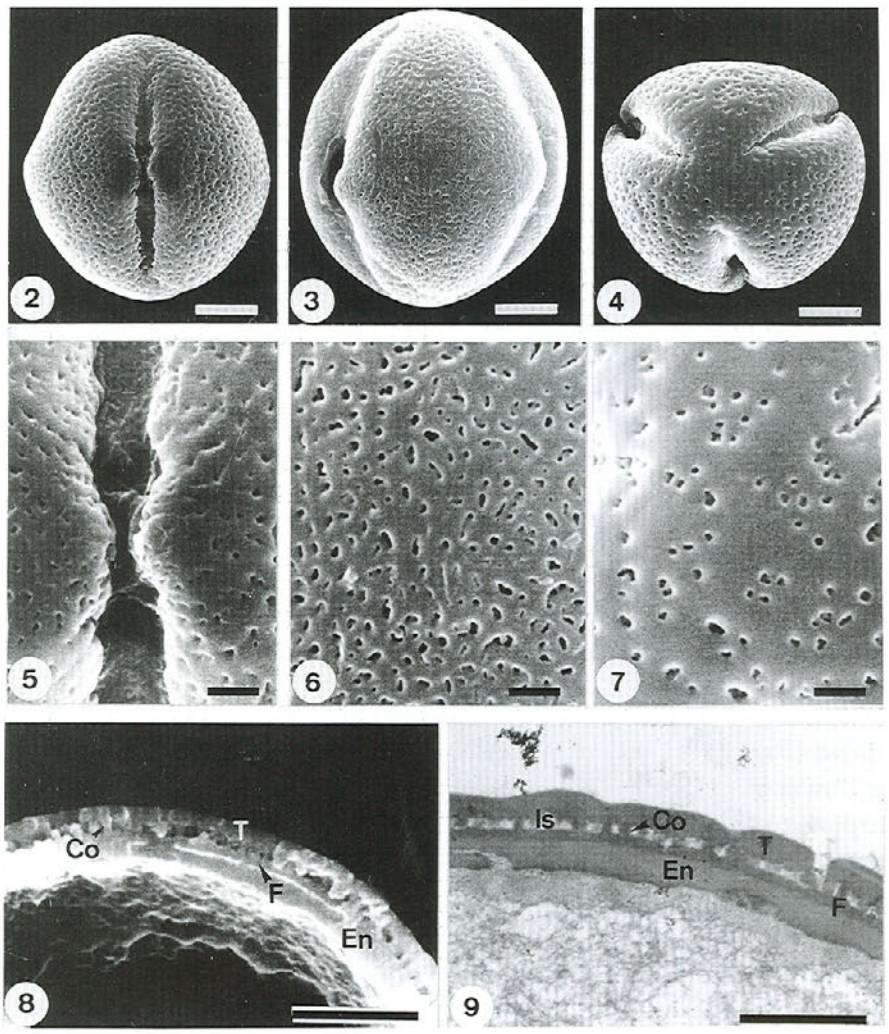
Taxa	Wall ornamentation		Interstitium	Foot layer		Pollen type
	Mesocolpium area	Apocolpium area				
Derris laxiflora (Figs. 2-9)	Foveolate (Fossulate)	Foveolate (Fossulate)	Collumellate	Distinct,	discontinuous	D1
Derris oblonga (Figs. 10-16)	Fossulate	Fossulate	Collumellate	Distinct,	discontinuous	D2
Derris trifoliata (Figs. 17-23)	Foveolate (Fossulate)	Foveolate (Fossulate)	Collumellate	Distinct,	discontinuous	D1
Millettia nitida (Figs. 24-30)	Reticulate	Foveolate	Collumellate	Obscure,	discontinuous	M3
Millettia pachycarpa (Figs. 31-37)	Foveolate	Foveolate	Collumellate	Distinct,	discontinuous	M1
Millettia pulchra var. *microphylla* (Figs. 38-44)	Fossulate	Fossulate	Densely collumellate	Distinct,	discontinuous	M2
Millettia reticulata (Figs. 45-51)	Reticulate	Foveolate	Collumellate	Obscure,	discontinuous	M3
Pongamia pinnata (Figs. 52-58)	Verrucate with Foveolate	Verrucate with Foveolate	Collumellate	Distinct,	discontinuous	P
Tephrosia candida (Figs. 59-66)	Fossulate	Foveolate (Fossulate)	Collumellate	Distinct,	discontinuous	T1
Tephrosia noctiflora (Figs. 67-74)	Reticulate	Foveolate	Collumellate	Distinct,	discontinuous	T2
Tephrosia obovata (Figs. 75-81)	Foveolate (fossulate)	Foveolate (Fossulate)	Collumellate	Distinct,	discontinuous	T3
Tephrosia purpurea (Figs. 82-88)	Foveolate (fossulate)	Foveolate	Collumellate	Distinct,	discontinuous	T3

flower of *Derris oblonga*. There is different exine ornamentation in two specimens of *Millettia pachycarpa* (Figs. 31-37), and it has been determined that the difference is due to maturation of the flower bud.

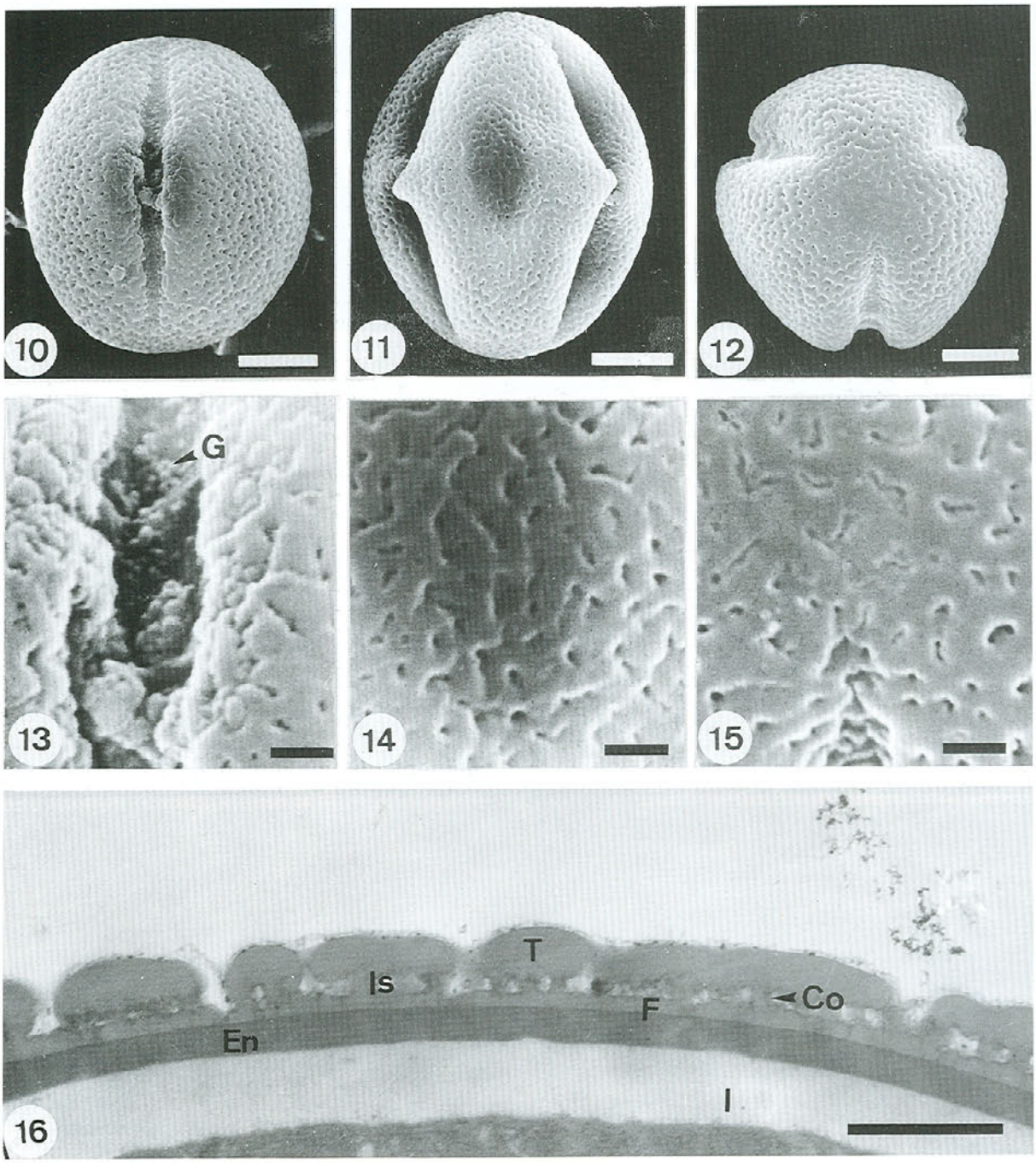
Tephrosia purpurea has two kinds of flower colors, white and red; some plants are wholly glabrous and have purple veins and short inflorescences. Because of these prominent contrasting charecteristics, in the past, the species was treated as two different taxa (Hayata, 1920; Hosokawa, 1932). In this research we have studied the general morphology of specimens from Tayangku and Yuehshihchieh, the red-flowered specimen from Anping, and the specimen classified as *T. ionphlebia* (Table 1). The pollen features from these specimens were compared. Their pollen morphology was extremely uniform (Figs. 82-88), thus supporting the findings in Ohashi *et al.* (1984) to group these three taxa together as a conspecific species. Except for the genus *Pongamia*, the palynological characteristics alone are not sufficient for identification of the genera among the tribe Millettieae in Taiwan.

Table 4. Pollen types based on ornamentation of Taiwan's Millettieae species.

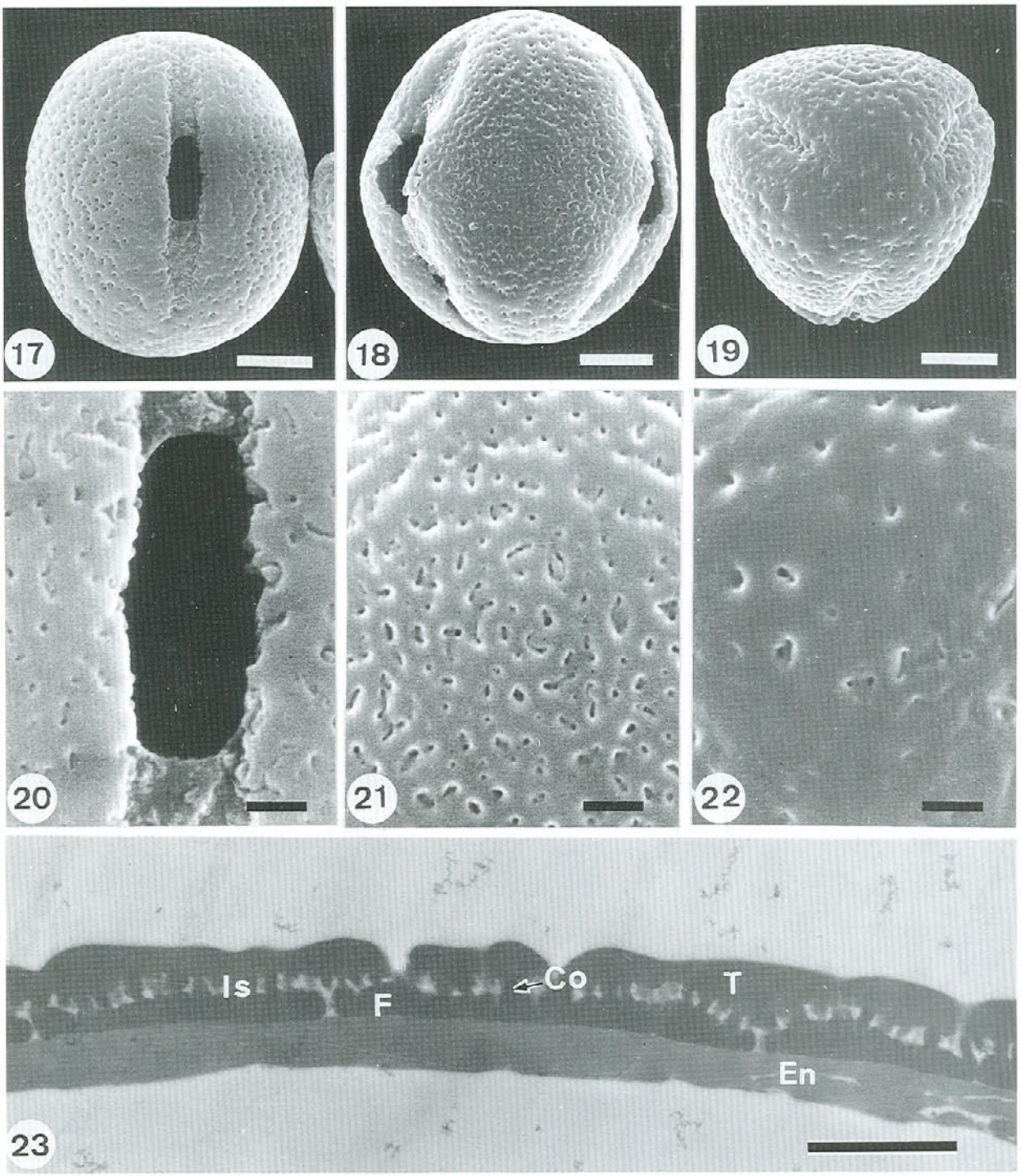
	Pollen Type	Description	Taxa
I	D1, M1, T3	Foveolate (to fossulate) on both mescolpium and apocolpium areas	*Derris laxiflora* *Derris trifoliata* *Millettia pachycarpa* *Tephrosia obovata* *Tephrosia purpurea*
II	D2, M2, T1	Fossulate on both mescolpium and apocolpium areas	*Derris oblonga* *Millettia pulchra var. microphylla* *Tephrosia candida*
III	P	Verrucate with foveolate on both mesocolpium and apocolpium areas	*Pongamia pinnata*
IV	M3, T2	Reticulate on mesocolpium area and foveolate on apocolpium area	*Millettia nitida* *Milletia reticulata,* *Tephrosia noctiflora*



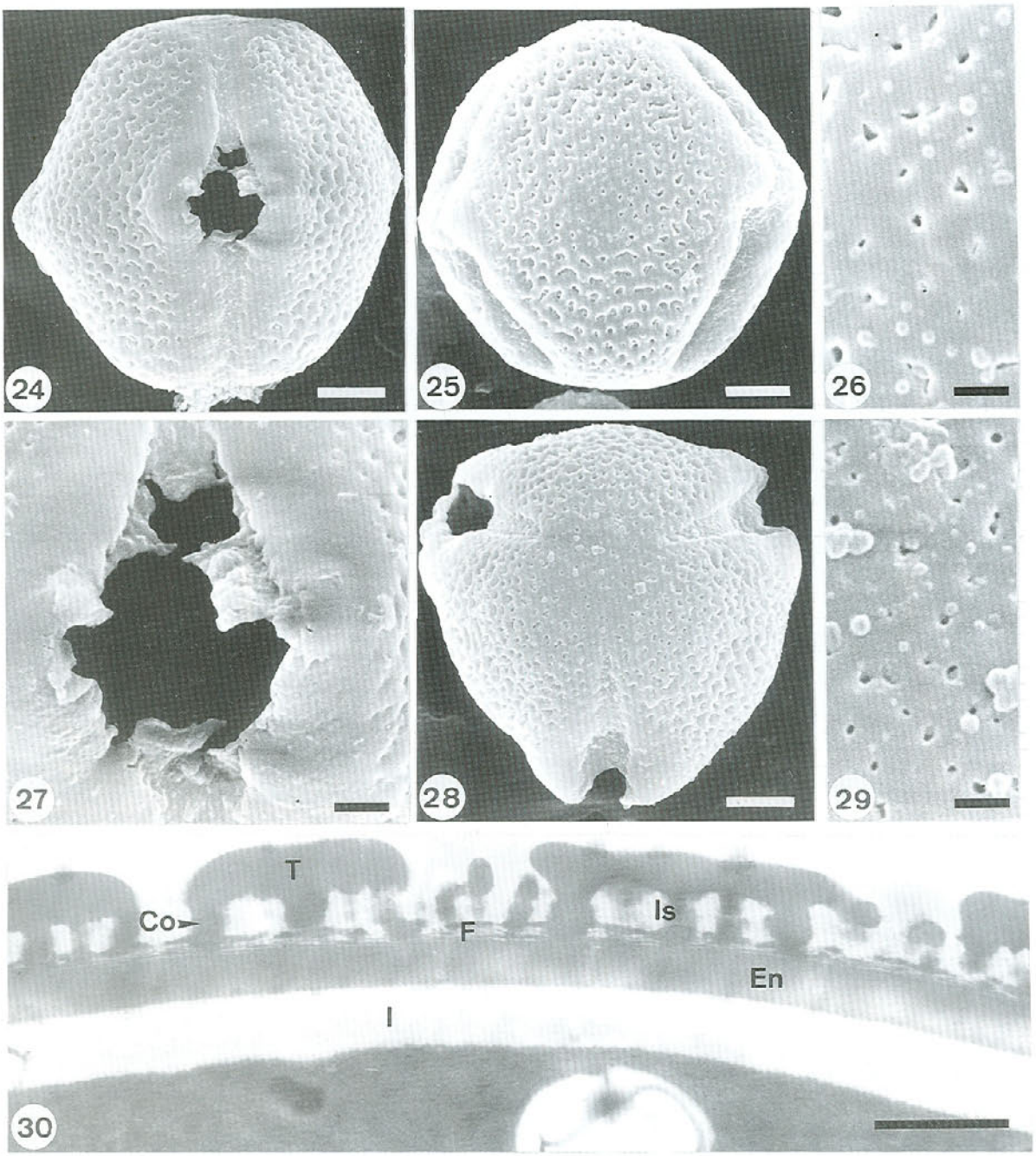
Figs. 2-9. *Derris laxiflora*. 2-3. Equatorial view, oblate-spheroidal to subprolate; 4. Polar view, circular; 5. Colpus membrane; 6. Mesocolpium area foveolate (to fossulate); 7. Apocolpium area foveolate (to fossulate); 8-9. Interstitium collumellate; foot layer distinct and discontinuous. Figs. 2-7: SEM, *T.-C. Huang 14081*; Figs. 8-9: TEM, *T.-C. Huang 14780*. 2-4, Bar=5 μm; 5-9, Bar=1 μm.



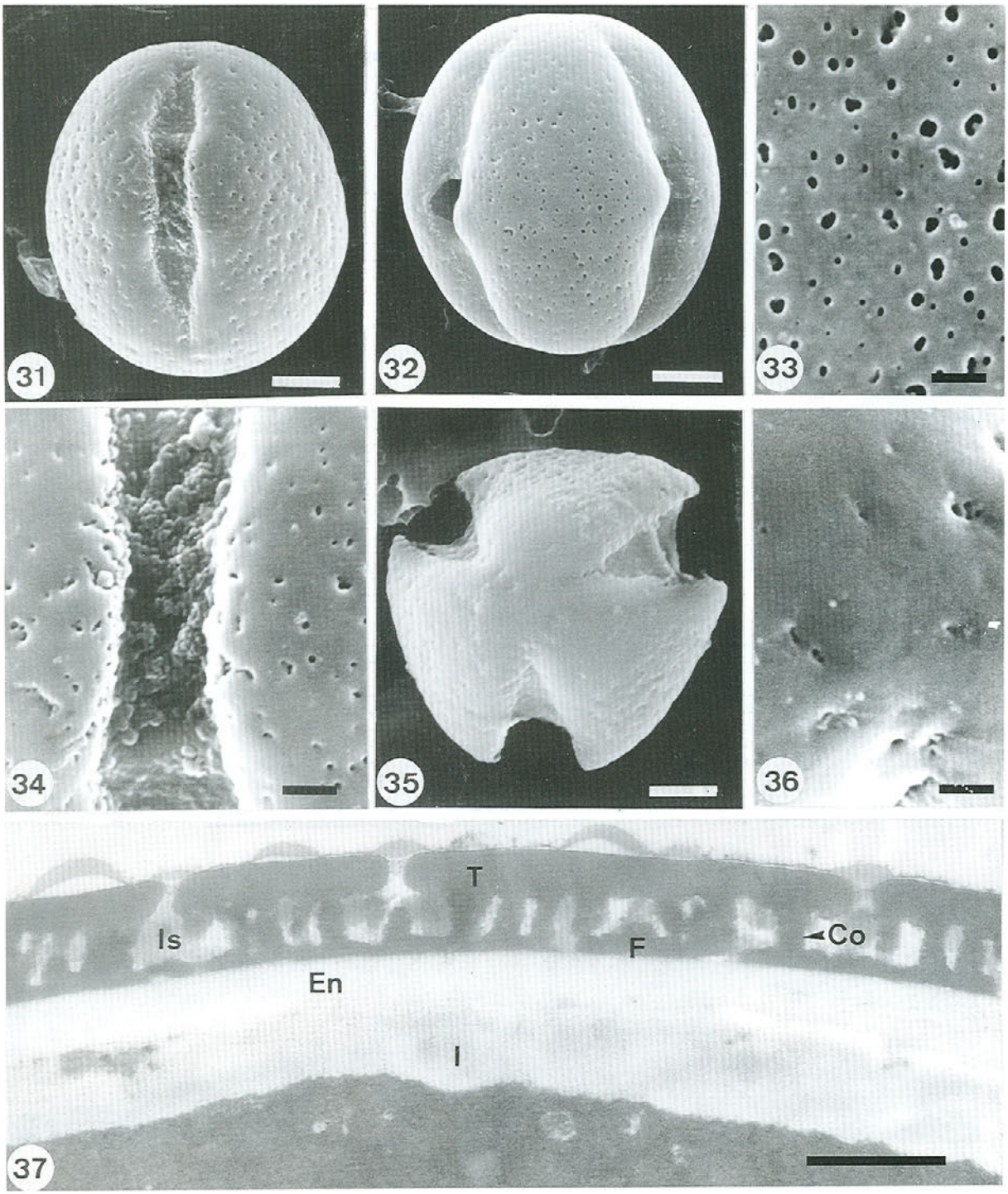
Figs. 10-16. *Derris oblonga*. 10-11. Equatorial view, suboblate to subprolate; 12. Polar view, circular to semi-angular; 13. Colpus membrane; 14. Mesocolpium area fossulate; 15. Apocolpium area fossulate; 16. Interstitium collumellate; foot layer distinct and discontinuous. Figs. 10-15: SEM, *Hsu s.n.* 1993. TEM, Fig. 16: *Hsu s.n.* 1993. 10-12, Bar=5 μm; 13-16, Bar=1 μm.



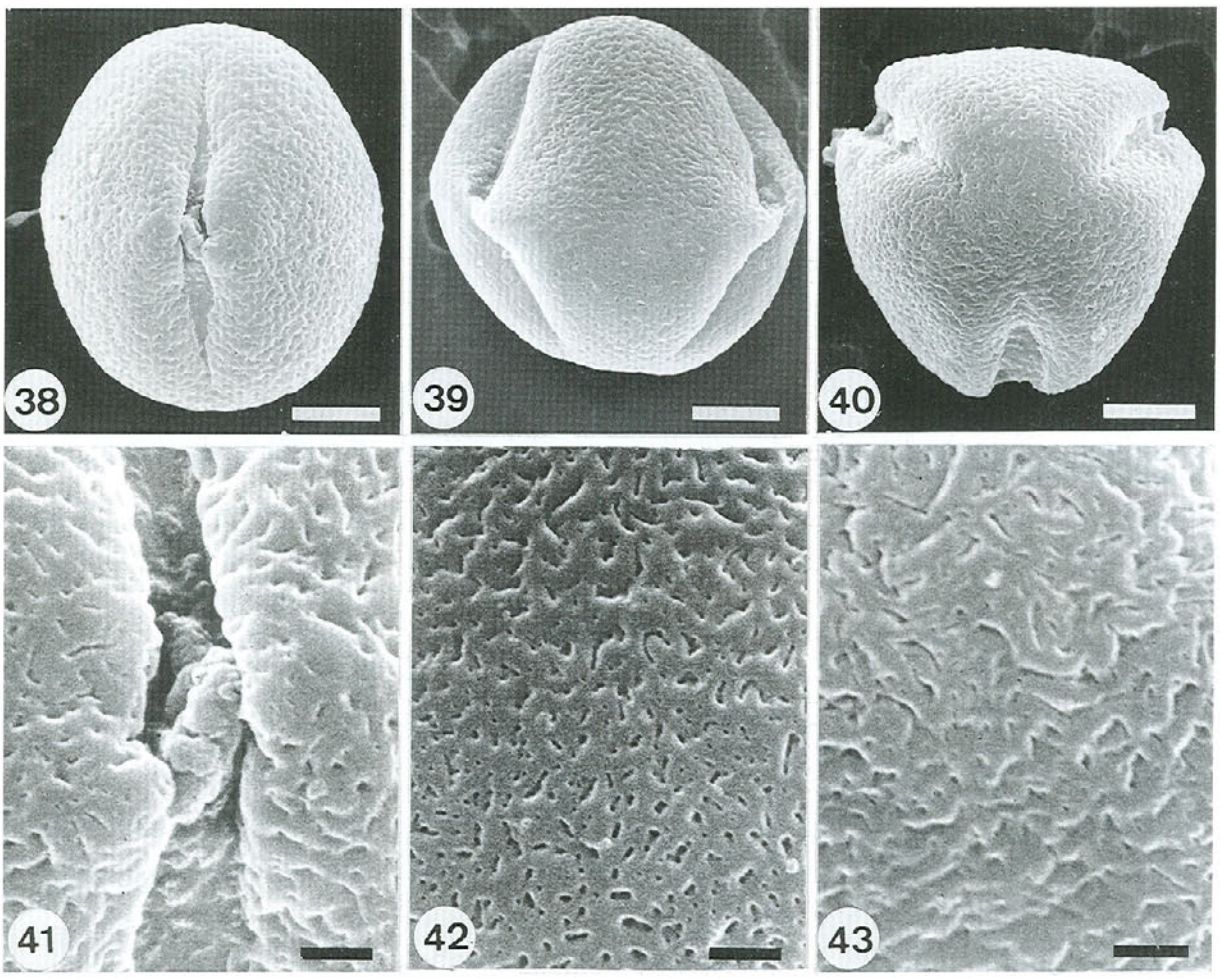
Figs. 17-23. *Derris trifoliata*. 17-18. Equatorial view, prolate-spheroidal to subporlate; 19. Polar view, circular to semi-angular; 20. Colpus membrane; 21. Mesocolpium area foveolate (to fossulate); 22. Apocolpium area foveolate (to fossulate); 23. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 17-22: SEM, T.-C. *Huang 5333*; Fig. 23: TEM, *T.-C. Huang 5333*. 17-19, Bar=5 μm; 20-23, Bar=1 μm.

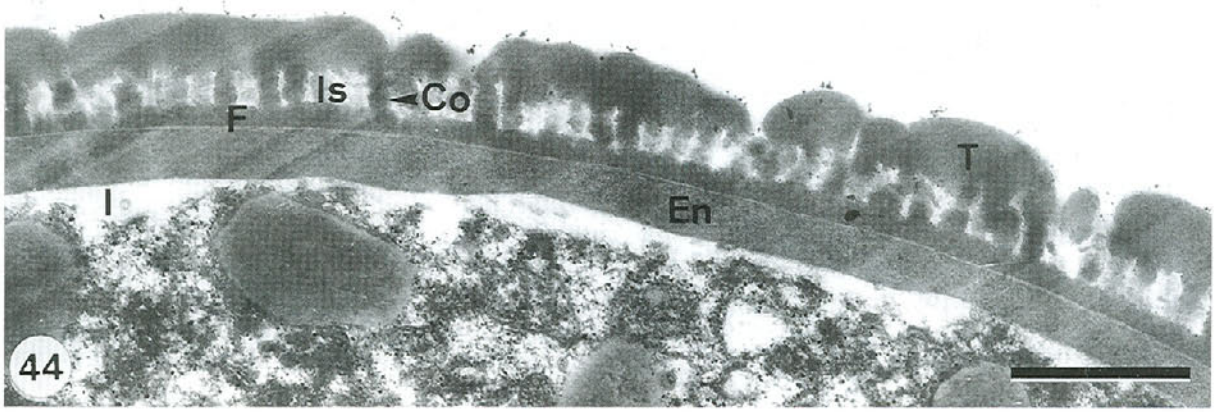


Figs. 24-30. *Millettia nitida*. 24-25. Equatorial view, oblate-spheroidal to subprolate; 26. Mesocolpium area reticulate; 27. Colpus membrane; 28. Polar view, circular to semi-angular; 29. Apocolpium area foveolate; 30. Interstitium collumellate, foot layer obscure and discontinuous. Figs. 24-29: SEM, *T.-C. Huang 14780*; Fig. 30: TEM, *T.-C. Huang 15324*. 24-25, 28, Bar=5 μm; 26-27, 29-30, Bar=1 μm.

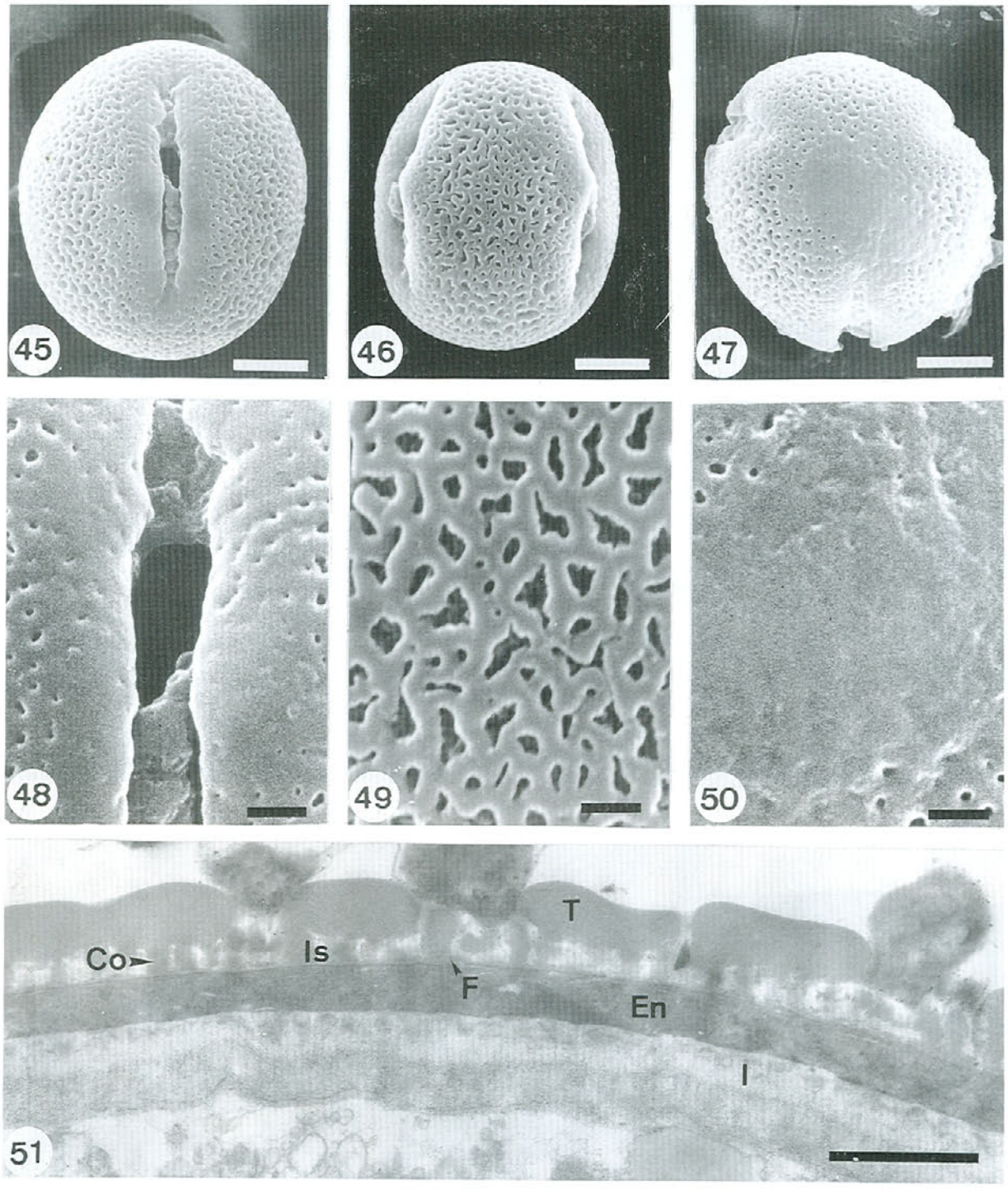


Figs. 31-37. *Millettia pachycarpa*. 31-32. Equatorial view, spheroidal to prolate; 33. Mesocolpium area foveolate; 34. Colpus membrane; 35. Polar view, semi-angular; 36. Apocolpium area foveolate; 37. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 31-36: SEM, *Wang s. n.* 1992 in Ching-chuan; Fig. 37: TEM, *Wang s. n.* 1992 in Ching-chuan. 31-32, 45, Bar=5 μm; 33-34, 36-37, Bar=1 μm.

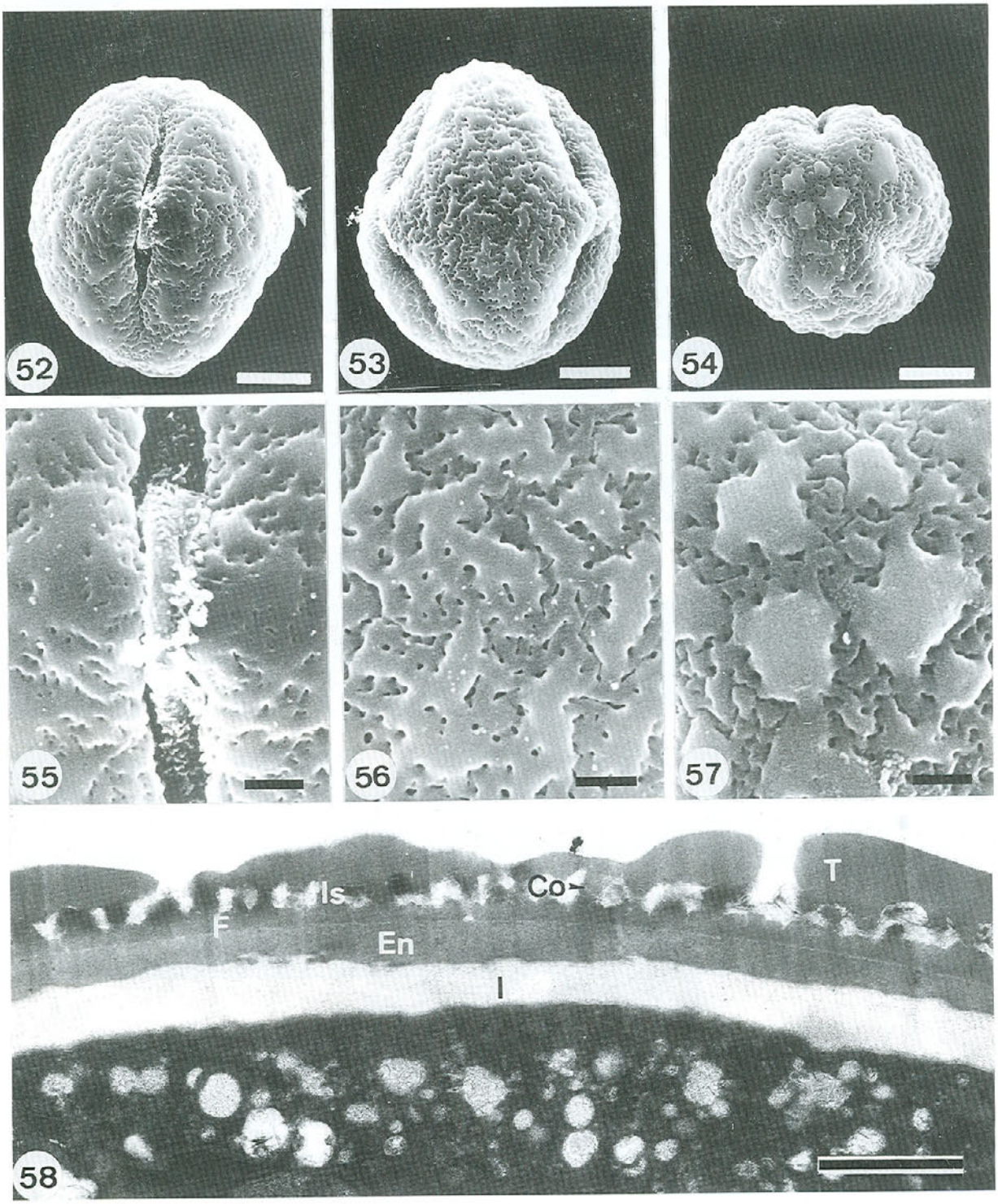




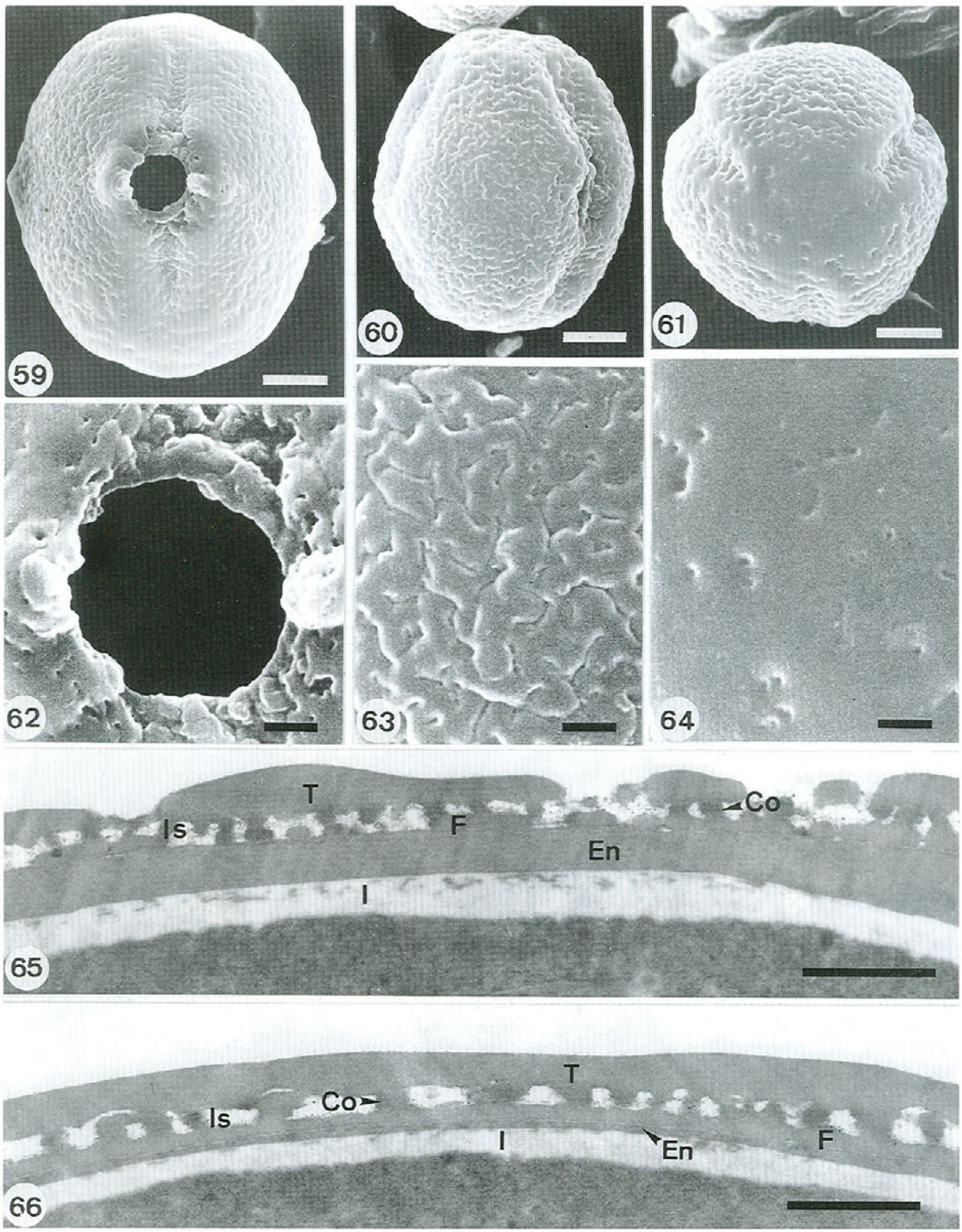
Figs. 38-44. *Millettia pulchra* var. *microphylla*. 38-39. Equatorial view, oblate-spheroidal to subprolate; 40. Polar view, semi-angular; 41. Colpus membrane; 42. Mesocolpium area fossulate; 43. Apocolpium area fossulate; 44. Interstitium densely collumellate, foot layer distinct and discontinuous. Figs. 38-43: SEM, *T.-C. Huang 15565*; Fig. 44: TEM, *T.-C. Huang 15567*. 38-40, Bar=5 μm; 41-44, Bar=1 μm.



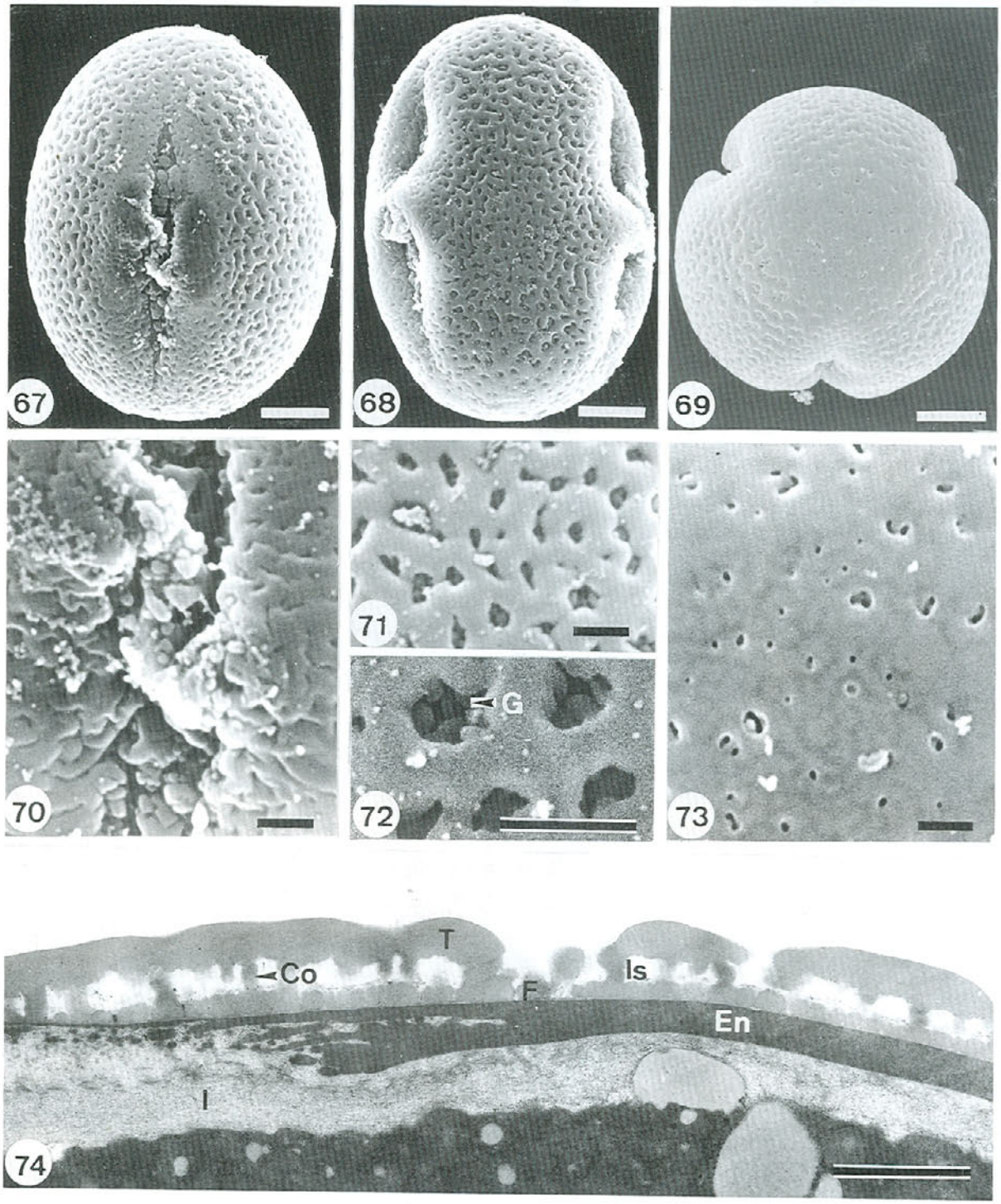
Figs. 45-51. *Millettia reticulata*. 45-46. Equatorial view, oblate-spheroidal to prolate; 47. Polar view, circular; 48. Colpus membrane; 49. Mesocolpium area reticulate; 50. Apocolpium area foveolate; 51. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 46-50: SEM, *T.-C. Huang 15256*; Fig. 51: TEM, *Wu 1369*. 45-47, Bar=5 μm; 48-51, Bar=1 μm.



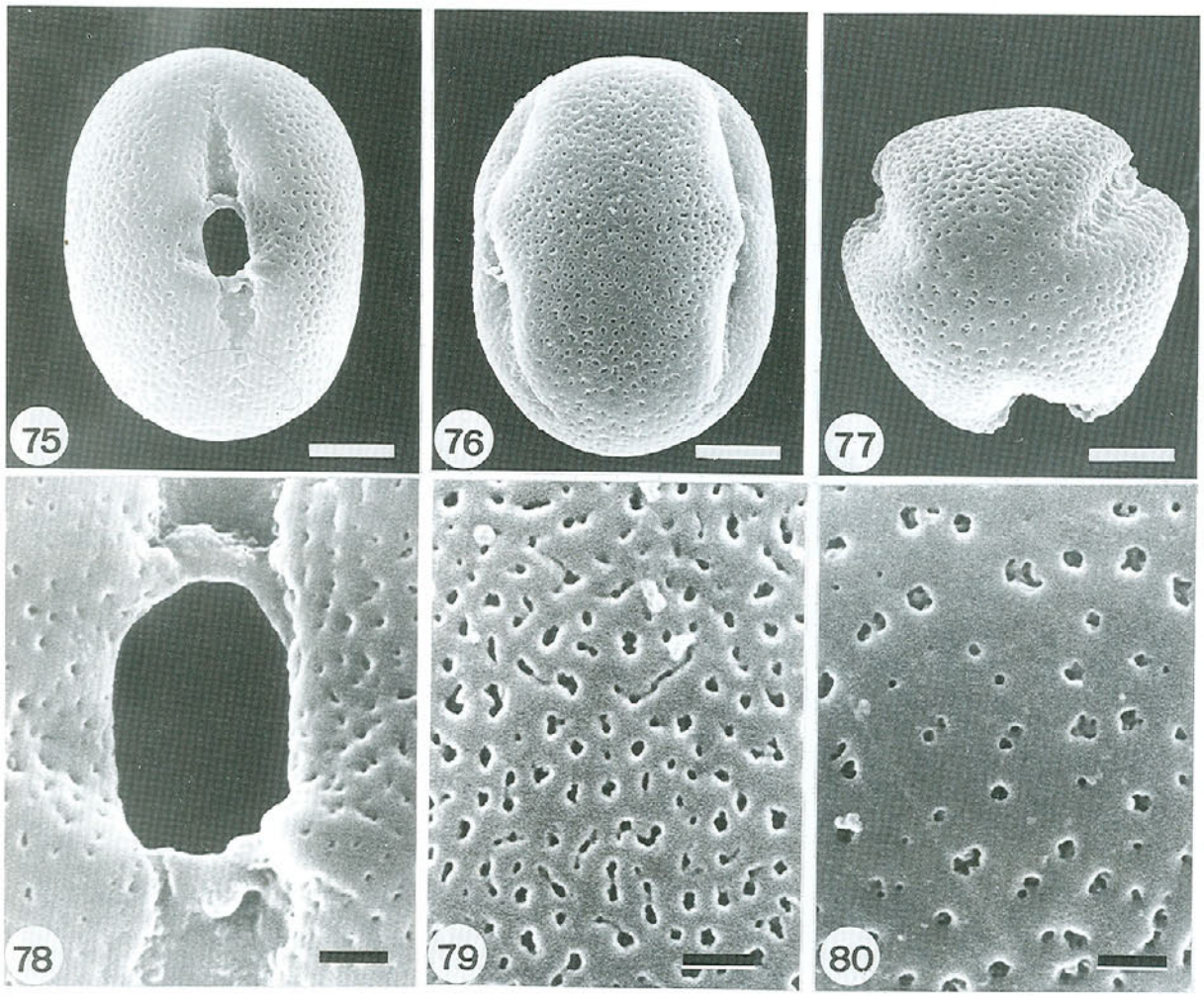
Figs. 52-58. *Pogamia pinnata*. 52-53. Equatorial view, oblate-spheroidal to subprolate; 54. Polar view, circular to semi-angular; 55. Colpus membrane; 56. Mesocolpium area verrucate with foveolate; 57. Apocolpium area verrucate with foveolate; 58. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 52-57: SEM, *Hsu s. n.* 1993; Fig. 58: TEM, *Hsu s. n.* 1993. 52-54, Bar=5 μm; 55-58, Bar=1 μm.

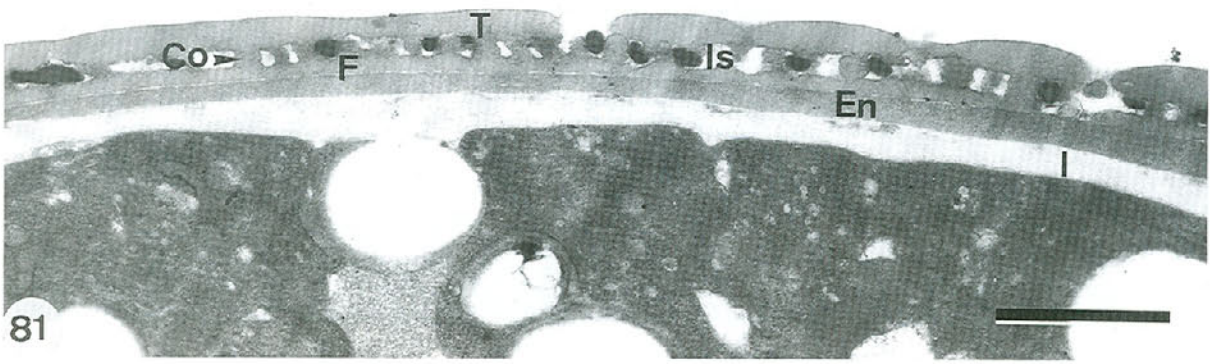


Figs. 59-66. *Tephrosia candida*. 59-60. Equatorial view, prolate -spheroidal to prolate; 61. Polar view, circular; 62. Colpus membrane; 63. Mesocolpium area fossulate; 64. Apocolpium area foveolate (to fossulate); 65-66. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 59-64: SEM, *S.-Z. Yang 24728*; Figs. 65-66: TEM, *S.-Z. Yang 24728*. 59-61, Bar=5 μm; 62-66, Bar=1 μm.

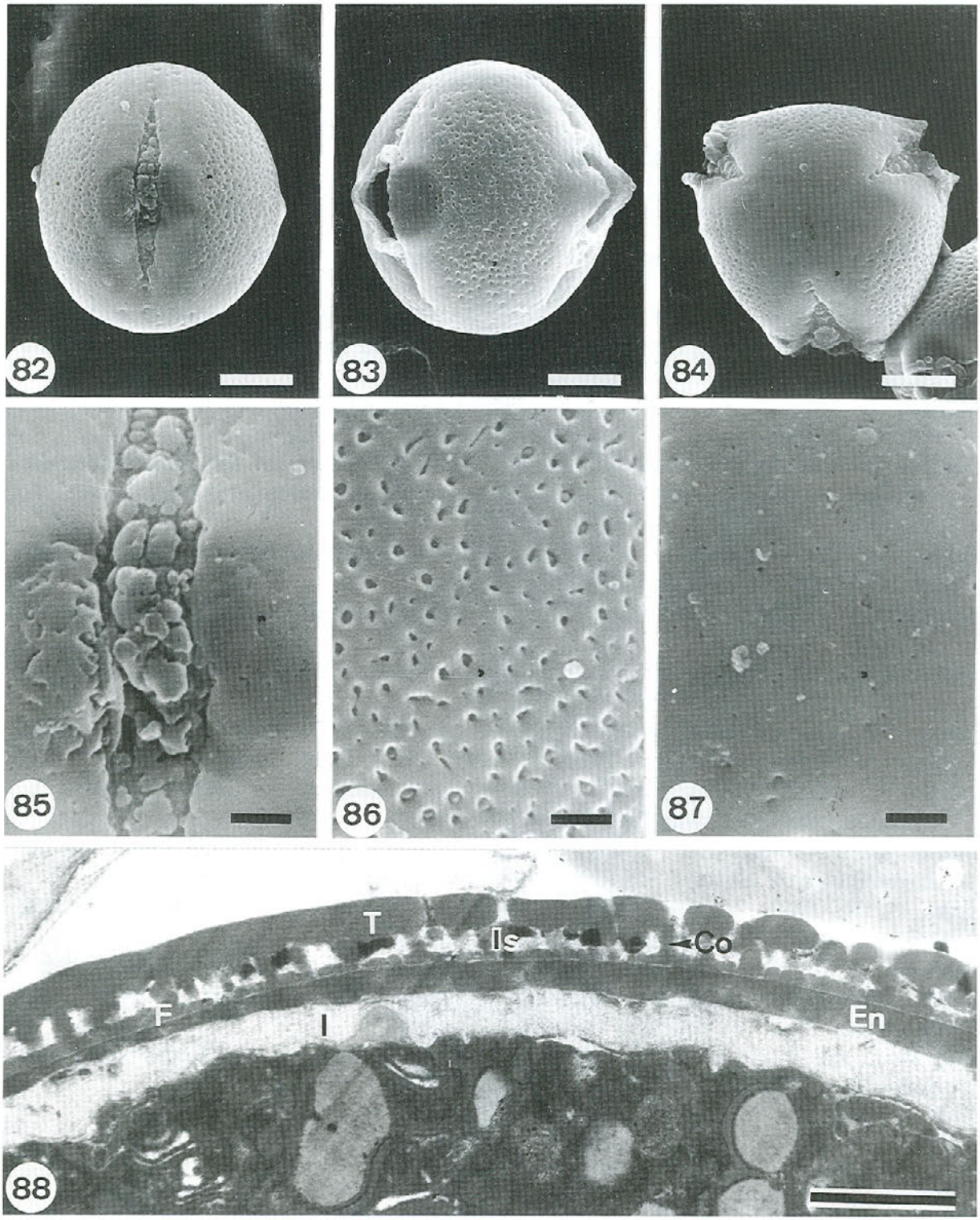


Figs. 67-74. *Tephrosia noctiflora*. 67-68. Equatorial view, prolate-spheroidal to prolate; 69. Polar view, circular; 70. Colpus membrane; 71-72. Mesocolpium area reticulate; 73. Apocolpium area foveolate; 74. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 67-73: SEM, *T.-C. Huang 15293* ; Fig. 74: TEM, *T.-C. Huang 15284*. 67-69, Bar=5 μm; 70-74, Bar=1 μm.





Figs. 75-81. *Tephorsia obovata*. 75-76. Equatorial view, subprolate to prolate; 77. Polar view, circular; 78. Colpus membrane; 79. Mesocolpium area foveolate (to fossulate); 80. Apocolpium area foveolate (to fossulate); 81. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 75-80: SEM, *T.-C. Huang 14686*; Fig. 81: TEM, *T.-C. Huang 15582*. 75-77, Bar=5 μm; 78-81, Bar=1 μm.



Figs. 82-88. *Tephrosia purpurea*. 82-83. Equatorial view, oblate-spheroidal to prolate; 84. Polar view, semi-angular; 85. Colpus membrane; 86. Mesocolpium area foveolate (to fossulate); 87. Apocolpium area foveolate; 88. Interstitium collumellate, foot layer distinct and discontinuous. Figs. 82-87: SEM, *T.-C. Huang 14493*; Fig. 88: TEM, *T.-C. Huang 15339*. 82-84, Bar=5 μm; 85-88, Bar=1 μm.

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台灣魚藤族植物花粉之研究

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摘　　要

本文為台灣豆科植物花粉系列研究之一，藉由花粉形態特徵進一步探討台灣豆科植物屬及亞科間之關係。以台灣魚藤族(Millettieae)植物為主，利用掃瞄式及穿透式電子顯微鏡觀察野外現生的本族植物花粉形態及內部層次，共四屬十二種。根據觀察的結果，本族花粉的外壁層次一致，蓋基間層為圓柱狀，基層為連續至不連續狀，並具有分化的外壁紋飾。個別屬之類型差異則以花粉外壁紋飾及層次為依據，水黃皮屬(*Pongamia*)為單一類型、魚藤屬(*Derris*)可分為兩亞型、雞血藤屬(*Millettia*)及灰毛豆屬(*Tephrosia*)則各為三個亞型；經整合後，本族花粉形態則可分為四個花粉類型。由於屬間花粉之差異並不明顯，因此，除了水黃皮屬具有較明顯之特徵外，台灣魚藤族花粉特徵並不適合單獨利用為屬間分類之依據。

關鍵詞：魚藤族、豆科、花粉形態、台灣。

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