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Pollen morphology of *Cuscuta* (Convolvulaceae) in Taiwan

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Abstract. The pollen morphology of five taxa of *Cuscuta*, *C. australis*, *C. campestris*, *C. chinensis*, *C. japonica* var. *formosana* and *C. japonica* var. *japonica* (Convolvulaceae) in Taiwan was investigated by using LM, SEM and TEM. The pollen of *Cuscuta* is three to six colpate, zonocolpate or pantocolpate colpus with granules, tectum with scabrate processes, ektexine reticulate or finely reticulate, and endexine thinner than ektexine. Two distinct pollen types of *Cuscuta* spp. in Taiwan are recognized. Type 1 is small, and has colpus with granules, and ektexine finely reticulate, including *C. australis*, *C. campestris* and *C. chinensis*. Type 2 is medium in size, and has colpus with granules, scabrate processes on surface of granule and ektexine reticulate, including *C. japonica* var. *formosana* and *C. japonica* var. *japonica*. Pollen types correspond with Yuncker's subdivision of the genus *Cuscuta*, type 1 for subgenus *Grammica* and type 2 for subgenus *Monogyna*. Meanwhile, pollen characteristics are also helpful in identifying species.

Keywords: *Cuscuta*; Dodder; Pollen; Taiwan.

Introduction

Dodders (genus *Cuscuta* L.) are parasitic flowering plants of the Convolvulaceae family (Yuncker, 1932; Kuijt, 1969; Parker and Riches, 1993; Liao et al., 2000). They are, however, sometimes assigned to the Cuscutaceae family (Hadac and Chrtek, 1970; Chrtek and Osbornova, 1991). This genus is globally distributed, with most species in the tropics and subtropics, and some in the temperate regions (Beliz, 1986). The number of species varies between 100 and 200 (Yuncker, 1932; Beliz, 1986; Parker and Riches, 1993; Fang et al., 1995; Staples and Yang 1998). Liao et al. (2000) considered three species and two varieties of *Cuscuta* in Taiwan, including *C. australis*, *C. campestris*, *C. chinensis*, *C. japonica* var. *formosana* and *C. japonica* var. *japonica*.

The identification of *Cuscuta* depends mostly on the detailed characteristics of the flowers, including the shapes of perianth segments, shapes of stigma, number of styles, infra-staminal scales attached to the filaments, as well as the dehiscence of the capsule. The characteristics of the scales and fruits are often difficult to determine, even in fresh materials. Furthermore, even these characteristics are difficult to observe in a dried specimen (Parker and Riches, 1993). *Cuscuta campestris*, a North American species recently introduced in several countries of the Old World, has often been misidentified (Parker and Riches, 1993). In Taiwan, it has been misidentified as *C. australis* or *C. chinensis* (Liao et al., 2000).

Information concerning pollen morphology can be used in the analysis of fossil pollen (Moore et al., 1991; Martin, 2001), airborne pollen (Nilsson et al., 1977; Lewis and Vinay, 1983), and honey pollen (Crompton and Wojtas, 1993). It is also used as taxonomical characteristics (Hsiao and Kuoh, 1995; Perveen and Qaiser, 1998). This study describes several pollen morphological characteristics of *Cuscuta* in Taiwan. The aim is to provide more information on the fine structural characteristics, and a Key is prepared for the identification of Taiwan taxa of *Cuscuta*.

Materials and Methods

Eleven pollen samples representing five taxa of *Cuscuta* in Taiwan were examined by light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). All pollen samples were freshly collected, except that of *Cuscuta australia*, which was obtained from the herbarium of National Pingtung University of Science and Technology, Taiwan (PPI) (Table 1).

Flowers were fixed using F. A. A. for 12-24 h and then stocked in 70% alcohol. For LM and TEM, anthers were dissected, gradually dehydrated in an acetone series, slowly infiltrated/embedded in Spurr epoxy resin, and then thick- and thin-sectioned on an ultramicrotome using a diamond knife. Thick sections (500 nm) were stained with toluidine blue, and examined on a Zeiss compound microscope. Ultrathin sections (90 nm) were stained with 4% aqueous uranyl acetate and 0.4% lead citrate, and examined/imaged with a Zeiss 10C TEM at 80 kV. For SEM, anthers were critical point dried. Pollen grains were then removed from the anthers, mounted onto aluminum stubs, coated with gold palladium, and then examined/imaged using a Hitachi H2500 SEM at 15 kV.

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Table 1. Samples freshly collected for the study of pollen morphology.

Taxon	Collection site	Collector
Cuscuta australis	Heping, Taitung County[a]	C. E. Chang
C. campestris	Shenkgang, Changhua County	G. I. Liao F001
	Yuanli, Miaoli County	G. I. Liao F007
C. chinensis	Jialu, Pingtung County	G. I. Liao F002
	Wangan, Penghu County	G. I. Liao F005
C. japonica var. *formosana*	Zhangshan, Kaohsiung County	G. I. Liao F003
	Wutai, Pingtung County	G. I. Liao F006
	Lihsing, Nantou County	G. I. Liao F009
	Puli, Nantou County	G. I. Liao F010
C. japonica var. *japonica*	Shenmutsun, Nantou County	G. I. Liao F004
	Tongzihlin, Nantou County	G. I. Liao F008

[a]Material provided by the dried specimen of PPI.

The size of the pollen was measured by polar axis and equatorial axis when observed from the equatorial view, and 30 pollen grains were measured per sample. Studied pollen was characterized in size as small (10-25 µm) or medium (25-50 µm) according to Erdtman (1952). Pollen terminology followed Punt et al. (1994).

Results

Description of Each Taxa

Cuscuta australis R. Br. (Figure 1, Table 2)

Pollen grains 3-colpate, zonocolpate, oblate spheroidal to prolate (P/E= 0.93-1.38) in equatorial view, small in size (15.5-22.7 × 15.1-20.7 µm); circular in polar view, 15.1-20.7 µm in diameter; colpi 12.4-18.16 × 2.2-3 µm, surface with granules; exine 1.3 µm thick; tectum with scabrate processes; ektexine finely reticulate; endexine thinner than ektexine.

Cuscuta campestris Yuncker (Figure 2, Table 2)

Pollen grains 6-colpate, pantocolpate, suboblate to prolate spheroidal (P/E= 0.76-1.13) in equatorial view, small in size (17.9-22.7 × 18.3-24.7 µm); circular in polar view, 18.3-24.7 µm in diameter; colpi 10.7-15.3 × 2.7-6.7 µm, surface with granules; exine 1.8 µm thick; tectum with scabrate processes; ektexine finely reticulate; endexine thinner than ektexine.

Cuscuta chinensis Lam. (Figure 3, Table 2)

Pollen grains 3-colpate, zonocolpate, oblate to subprolate (P/E= 0.74-1.28) in equatorial view, small in size (16.7-23.5 × 17.1-26.7 µm); circular in polar view, 17.1-26.7 µm in diameter; colpi as long as polar axes, 16.7-23.5 × 5-6.7 µm, surface with granules; exine 1.9 µm thick; tectum with scabrate processes; ektexine finely reticulate; endexine thinner than ektexine.

Table 2. Summary of data on pollen morphology.

Sample	P ± std (range) (µm)	E ± std (range) (µm)	P/E ± std (range)	Aperture	Sexine
Cuscuta australis					
Heping	19.1 ± 1.60 (15.5-22.7)	16.4 ± 1.01 (15.1-20.7)	1.17 ± 0.10 (0.93-1.38)	3-colpate	Finely reticulate
C. campestris					
Shenkgang	19.9 ± 1.33 (17.9-21.9)	22.6 ± 1.39 (19.5-24.7)	0.88 ± 0.06 (0.76-0.98)	6-colpate	Finely reticulate
Yuanli	20.7 ± 1.37 (17.9-22.7)	21.2 ± 1.33 (18.3-23.1)	0.98 ± 0.06 (0.86-1.13)	6-colpate	Finely reticulate
C. chinensis					
Jialu	19.9 ± 1.74 (16.7-22.7)	22.2 ± 1.80 (17.1-25.1)	0.90 ± 0.11 (0.77-1.28)	3-colpate	Finely reticulate
Wangan	21.2 ± 1.22 (19.1-23.5)	24.1 ± 1.62 (20.7-26.7)	0.88 ± 0.06 (0.74-1.00)	3-colpate	Finely reticulate
C. japonica var. *formosana*					
Zhangshan	25.8 ± 1.96 (22.3-30.5)	28.6 ± 2.56 (22.3-32.6)	0.91 ± 0.11 (0.74-1.18)	4(3, 5)-colpate	Reticulate
Wutai	28.0 ± 2.02 (25.1-31.4)	30.9 ± 1.56 (27.8-34.6)	0.91 ± 0.05 (0.78-1.00)	4, 5-colpate	Reticulate
Lihsing	28.2 ± 1.47 (25.9-31.8)	31.3 ± 1.89 (27.4-34.2)	0.90 ± 0.07 (0.79-1.08)	4(5)-colpate	Reticulate
Puli	27.0 ± 2.12 (23.5-32.6)	28.3 ± 1.85 (25.9-33.0)	0.95 ± 0.05 (0.83-1.05)	4(3, 5)-colpate	Reticulate
C. japonica var. *japonica*					
Shenmutsun	29.2 ± 1.74 (26.5-34.6)	32.2 ± 3.09 (27.0-37.8)	0.91 ± 0.08 (0.82-1.07)	4, 5(3)-colpate	Reticulate
Tongzihlin	27.9 ± 2.48 (24.7-32.6)	30.8 ± 1.94 (27.4-34.2)	0.91 ± 0.06 (0.82-1.07)	4(3)-colpate	Reticulate

P: polar axis; E: equatorial axis; std: standard deviation. n=30.

Cuscuta japonica Choisy var. **formosana** (Hay.) Yuncker (Figure 4, Table 2)

Pollen grains 4- (rarely 5- or 3-) colpate, zonocolpate, oblate to subprolate (P/E= 0.74-1.18) in equatorial view, medium in size (22.3-32.6 × 22.3-34.6 µm); inter-angular in polar view, 22.3-34.6 µm in diameter; colpi 12.5-17.3 × 4.8 µm, surface with granules, scabrate processes on surface of granule; exine 4.0 µm thick; tectum with scabrate processes; ektexine reticulate; endexine thinner than ektexine.

Notes. *Cuscuta japonica* var. *formosana* in Puli, Nantou County, produces a mixture pollen including normally zonocolpate and variously large pantocolpate grains.

Cuscuta japonica Choisy var. **japonica** (Figure 5, Table 2)

Pollen grains 4- (or 5-, rarely 3-) colpate, zonocolpate, suboblate to prolate spheroidal (P/E= 0.82-1.07) in equatorial view, medium in size (24.7-34.6 × 27.0-37.8 µm); inter-angular in polar view, 27.0-37.8 µm in diameter; colpi 15.3-20 × 3.3-5.3 µm, surface with granules, scabrate processes on surface of granule; exine 3.9 µm thick; tectum with scabrate processes; ektexine reticulate; endexine thinner than ektexine.

Key to the Taxa of Cuscuta Pollen in Taiwan

1. Grains small, 10-25 µm; ektexine finely reticulate 2
   2. Grain pantocolpate .... *C. campestris*

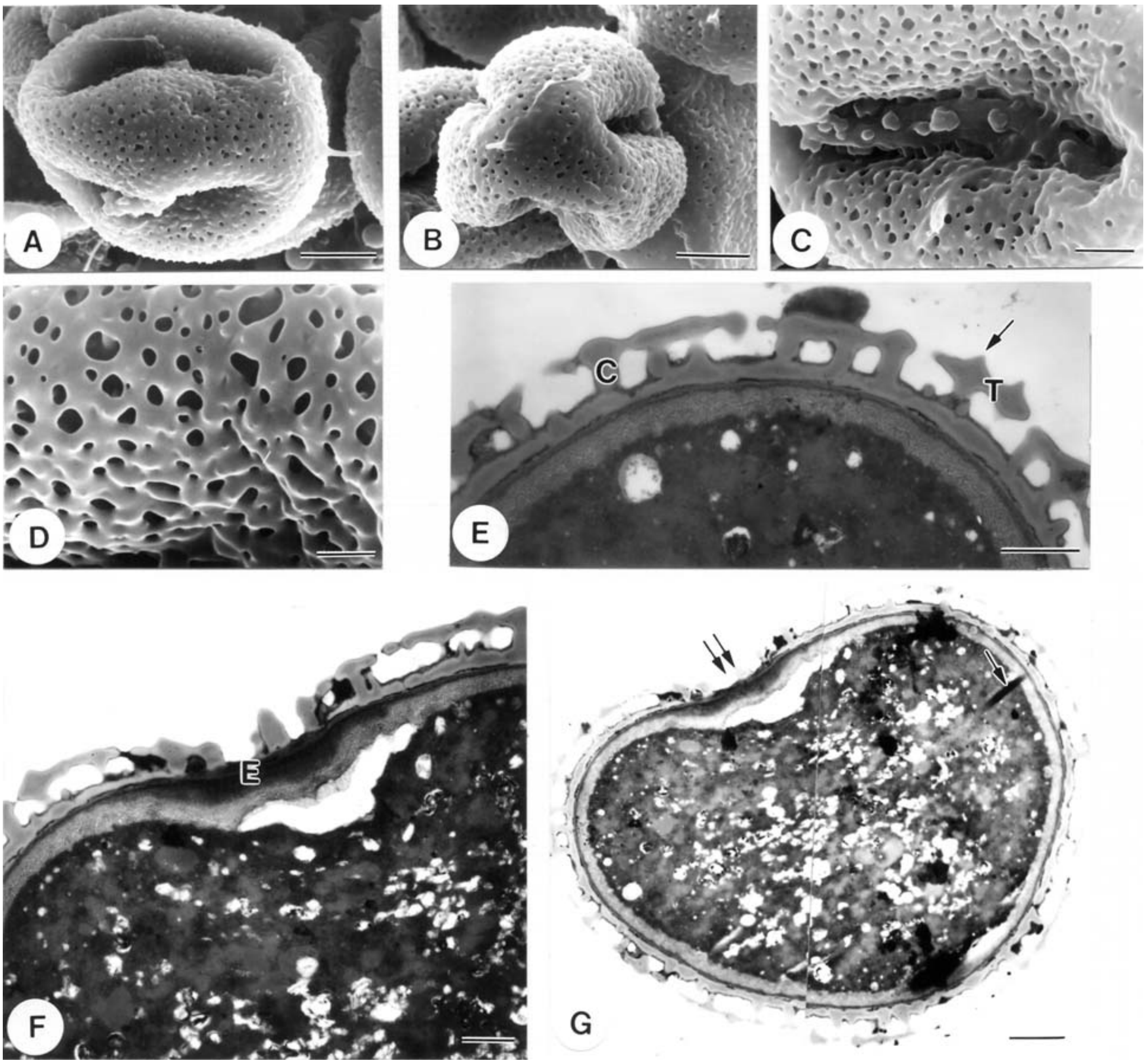

Figure 1. Micrographs of *Cuscuta australis* pollen. A-D: SEM; E-G: TEM. A, equatorial view; B, polar view; C, colpus membrane with granules; D, surface pattern at the mesocolpium; E, transverse section through equatorial plane at mesocolpium showing prominent columellae (C), arrow indicates scabrate process on tectum (T); F, section showing the thickening of the endexine (E) and the absence of the tectum at apertural region; G, oblique section through plane between apocolpium and mesocolpium, only one colpus (double arrow) is visible. Note that some areas show slight shrinkage when mounted on the large slide (arrow). Bars: A, B = 5 µm; C, G = 2 µm; D-F = 1 µm.

2. Grain zonocolpate .... *C. australis*, *C. chinensis*

1. Grains medium, 25-50 µm; ektexine reticulate .... *C. japonica* var. *formosana*, *C. japonica* var. *japonica*

Discussion

Sengupta has an important work on the pollen morphology of Convolvulaceae (Sengupta, 1972). His work distinguished four main pollen types in Convolvulaceae, based on the number and distribution of apertures mainly using light microscopy. The pollen types are tricolpate, penta-hexa-colpate, dodecacolpate, and pantoporate, respectively. When dealing with the genus *Cuscuta*, Sengupta pointed out that the pollen grains of 19 species are tricolpate and scrobiculate, the pollen grains of *C. monogyna* are tricolpate and reticulate, and the pollen grains of *C. reflexa* are penta-hexa-colpate and reticulate. Meanwhile he also postulated that *Cuscuta* pollen evolved from tricolpate to penta-hexa-colpate with a diagram. The five taxa of *Cuscuta* we studied were not examined in Sengupta's work. The pollen of *Cuscuta* in Taiwan is three to six colpate, zonocolpate or pantocolpate, colpus with granules, tectum with scabrate processes, ektexine reticulate or finely reticulate, and endexine thinner than ektexine, hence we distinguished two main pollen types of *Cuscuta* spp. in Taiwan, based on the pollen size and fine structure of pollen wall mainly using electron microscopy. Type 1

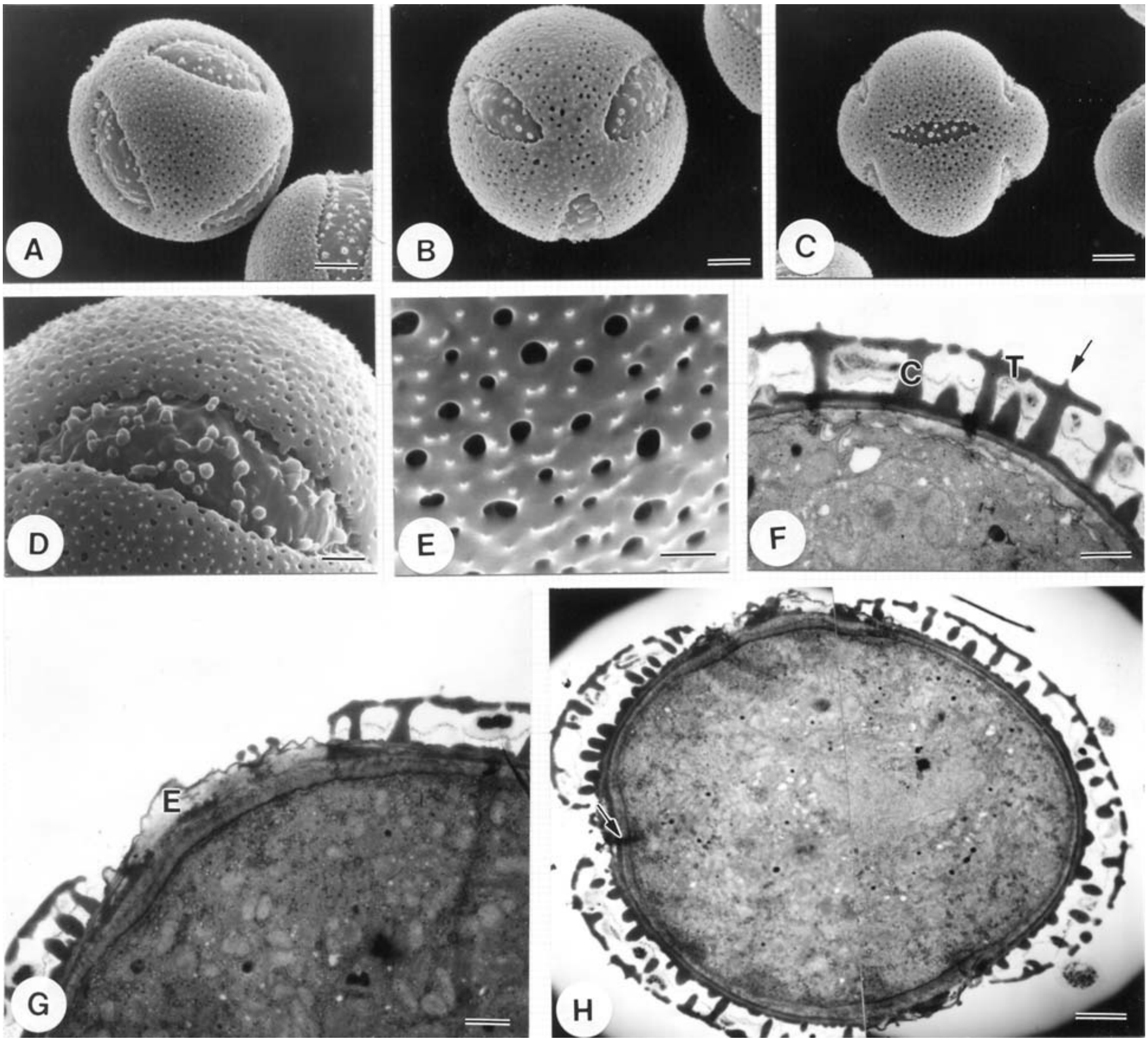

Figure 2. Micrographs of *Cuscuta campestris* pollen. A-E: SEM; F-H: TEM. A, equatorial view; B, polar view; C, subequatorial view; D, colpus membrane with granules; E, surface pattern at the mesocolpium; F, transverse section through equatorial plane at mesocolpium showing prominent columellae (C), arrow indicates scabrate process on tectum (T), note the columellae is longer than that in Figure 1- E; G, section showing the thickening of the endexine (E) and the absence of the tectum at the apertural region; H, transvers section of pollen grain at the subpolar region, three colpi are visible. Note that some areas show slight shrinkage when mounted on the large slide (arrow). Bars: A-C = 5 µm; D, H = 2 µm; E-G = 1 µm.

is small, and has a colpus with granules, and ektexine finely reticulate, including *C. australis*, *C. campestris* and *C. chinensis*. Type 2 is medium in size, and has a colpus with granules, scabrate processes on surface of granule and ektexine reticulate, including *C. japonica* var. *formosana* and *C. japonica* var. *japonica*.

Yuncker (1932) divided the genus *Cuscuta* into three subgenera, subgenus Grammica, subgenus Cuscuta, and subgenus Monogyna, respectively, with these based on the arrangement of the styles and the shapes of the stigmas. In this preliminary study, we find that the pollen types we proposed correspond with Yuncker's subdivision of the genus *Cuscuta*, type 1 for subgenus *Grammica* and type 2 for subgenus *Monogyna*. The relation between the fine structure of the pollen wall and the subdivision of the genus *Cuscuta* are worth further study with more taxa.

Huang (1972) studied the pollen morphology of two species of *Cuscuta* in Taiwan using light microscopy. He de-

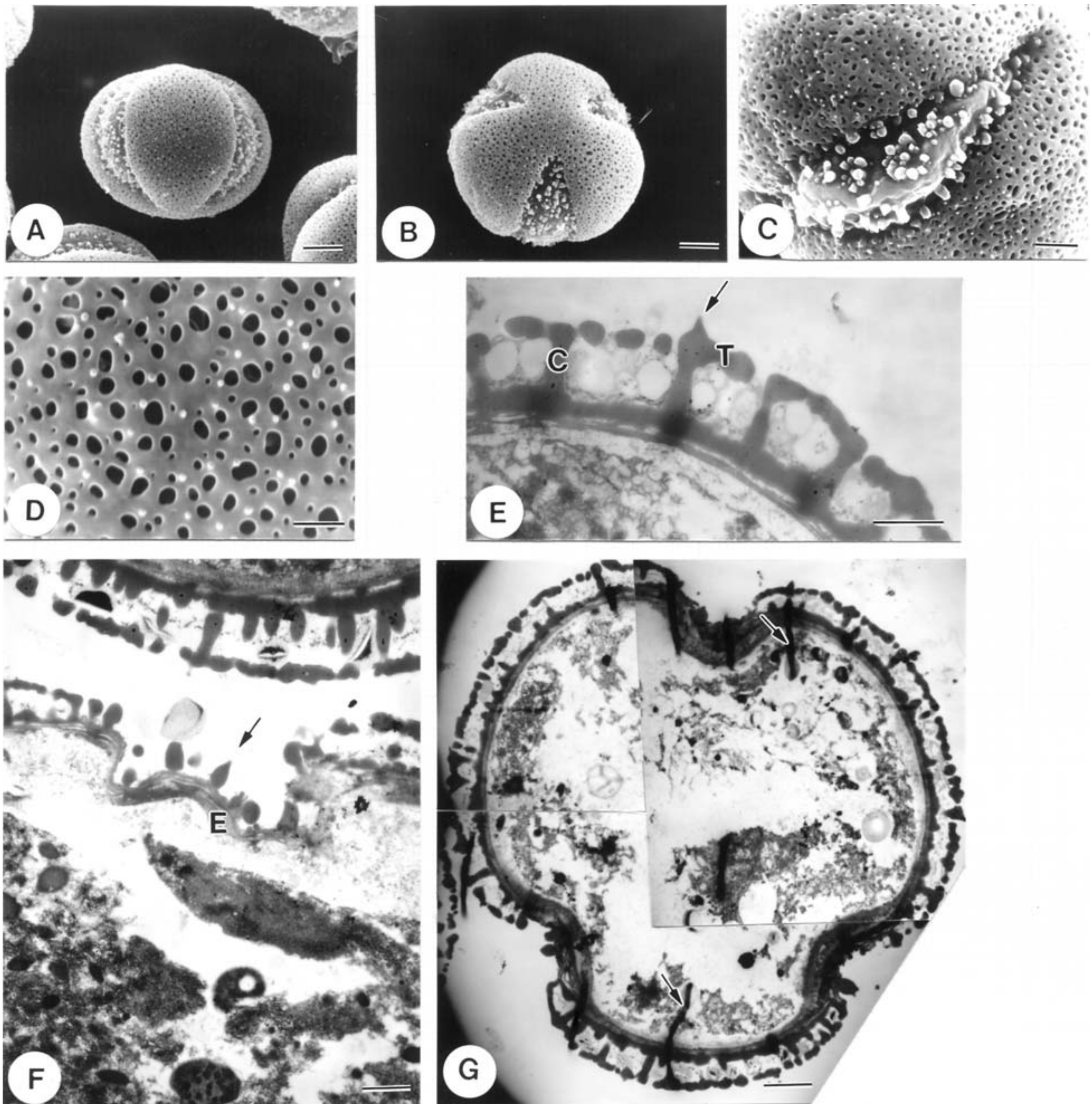

Figure 3. Micrographs of *Cuscuta chinensis* pollen. A-D: SEM; E-G: TEM. A, equatorial view; B, polar view; C, colpus membrane with granules; D, surface pattern at the mesocolpium; E, transverse section through equatorial plane at mesocolpium showing prominent columellae (C), arrow indicates scabrate process on tectum (T), note the columellae is longer than that in Figure 1- E; F, section showing the thickening of the endexine (E) and the absence of the tectum at the apertural region (arrow); G, transverse section of pollen grain at the equatorial region, three colpi are visible. Note that some areas show slight shrinkage when mounted on the large slide (arrow). Bars: A, B = 5 µm; C, G = 2 µm; D-F = 1 µm.

scribed the pollen of *C. chinensis* as tectum psilate, and *C. japonica* var. *formosana* as sexine rugulate. Wang et al. (1995) investigated the pollen morphology of *C. chinensis* from China using light microscopy, and showed that the nexine was as thick as the sexine. However, the observations in our work showed that the tectum of the pollen of *C. chinensis* has scabrate processes, and endexine is thinner than ektexine. Moreover, the pollen sculpture of *C. japonica* var. *formosana* has ektexine reticulate. The difference between these results is probably due to the use of different microscopies.

Cuscuta campestris is a dodder common worldwide (Parker and Riches, 1993). It was firstly reported in Taiwan by Liao et al. in 2000. In Taiwan, the first collected *Cuscuta campestris* was misidentified as *C. chinensis* in 1964. Then, another specimen was misidentified as *C. australis* in 1971.

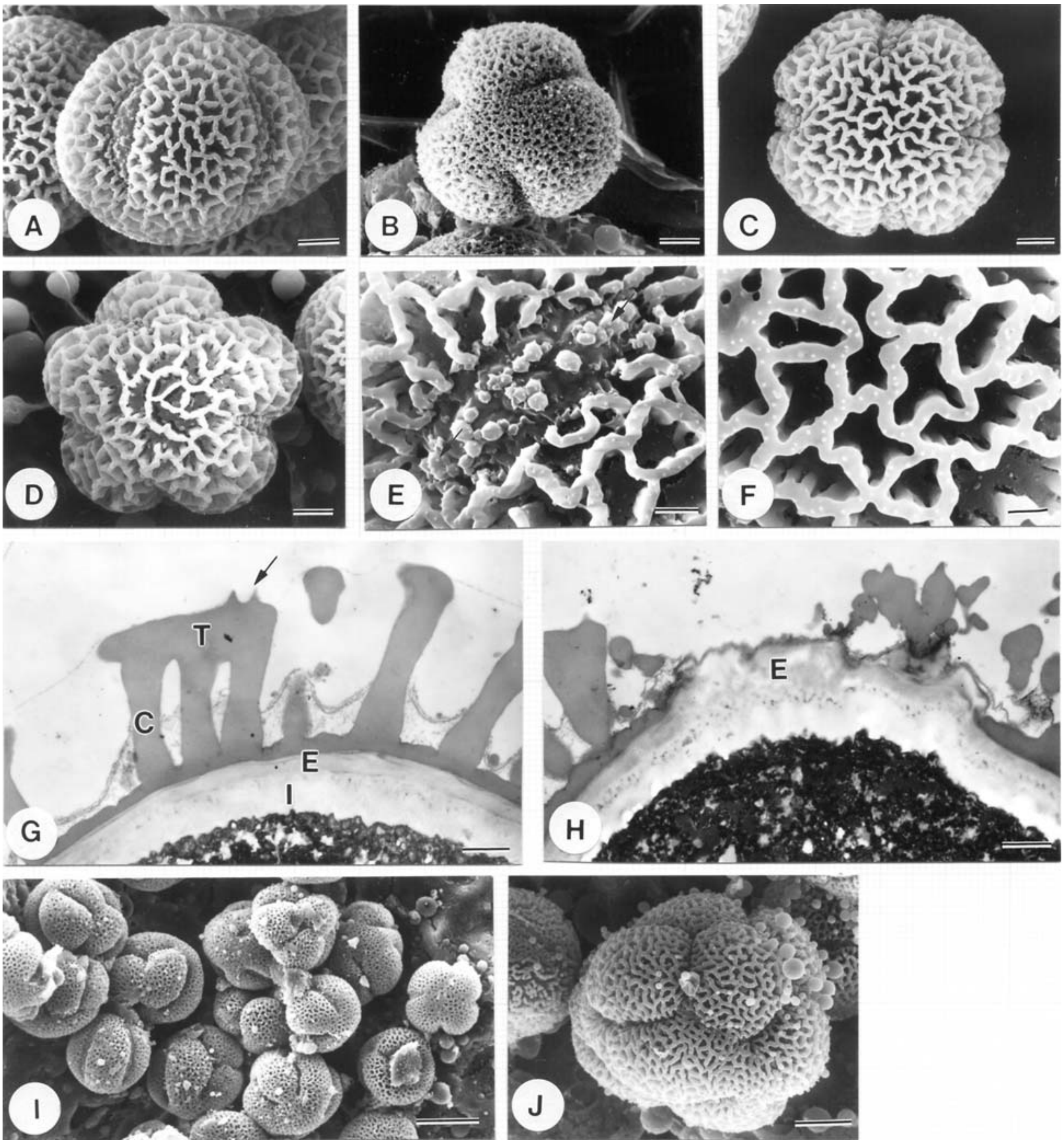

Figure 4. Micrographs of *Cuscuta japonica* var. *formosana* pollen. A-F, I & J: SEM; G & H: TEM. A, equatorial view; B-D, polar view; E, colpus membrane with granules, note the scabrate processes (arrows) on surface of granule; F, surface pattern at the mesocolpium; G, transverse section through equatorial plane at mesocolpium showing prominent columellae (C), endexine (E) and intine (I), arrow indicates scabrate on tectum (T); H, section showing the thickening of the endexine (E) and the absence of the tectum at the apertural region; I-J, showing variously large pantocolpate grains and normally zonocolpate grains. Bars: A-D = 5 µm; E, F = 2 µm; G, H = 1 µm; I = 20 µm; J = 10 µm.

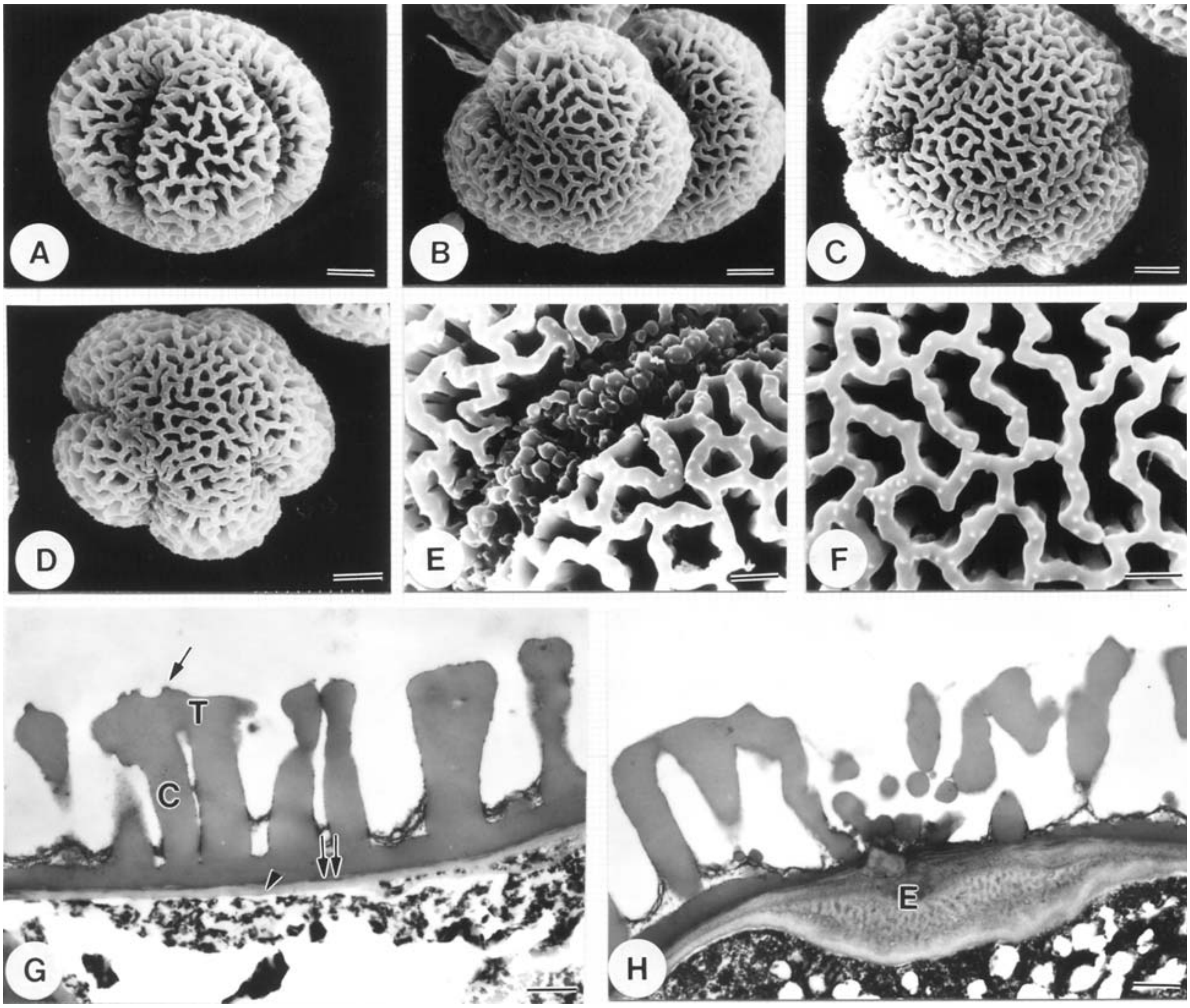

Figure 5. Micrographs of *Cuscuta japonica* var. *japonica* pollen. A-F: SEM; G & H: TEM. A, equatorial view; B-D, polar view; E, colpus membrane with granules, note the scabrate processes on surface of granule; F, surface pattern at the mesocolpium; G, transverse section through equatorial plane at mesocolpium showing prominent columellae (C), arrow indicates scabrate on tectum (T). Note both endexine (double arrow) and intine (arrowhead) are thinner than those in Figure 4- G; H, section showing the thickening of the endexine (E) and the absence of the tectum at the apertural region. Scale bars: A-D = 5 µm; E, F = 2 µm; G, H = 1 µm.

Thereafter, almost all collected species were misidentified as *C. australis*. These misidentifications followed from similarities in gross morphology. Actually, the characteristics of pollen of *C. campestris* can be easily distinguished from those of the pollen of other species in *Cuscuta*, since the pollen of *C. campestris* is pantocolpate and the other pollen are zonocolpate.

Although the gross morphology and characteristics of the pollen of *Cuscuta japonica* var. *formosana* and *C. japonica* var. *japonica* are very similar, the tongue-shaped stigma lobes and shorter corolla, and thicker endexine and intine of *C. japonica* var. *formosana* distinguish it from *C. japonica* var. *japonica*.

Moore et al. (1991) stated that the pantocolpate condition of some species, including *Cuscuta*, that are normally trizonocolpate, is thought to be associated with meiotic irregularities or increasing ploidy levels, and sometimes with hybridization. In this work, *Cuscuta japonica* var. *formosana* in Puli, Nantou County, also produces a mixture of pollen, including normally zonocolpate grains and variously large pantocolpate grains. Although the cause of the dimorphism of C. *japonica* var. *formosana* is not yet clear, it is not a problem when using pollen characteristics in the key for identification of *Cuscuta* spp. in Taiwan.

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台灣菟絲子屬植物之花粉形態

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本文以光學顯微鏡、掃描式電子顯微鏡及穿透式電子顯微鏡觀察比較分佈於台灣的旋花科菟絲子屬（*Cuscuta*）植物的花粉形態，包括：菟絲子、平原菟絲子、中國菟絲子、台灣菟絲子及日本菟絲子。菟絲子屬的花粉具有 3-6 溝，溝分佈在赤道面或散佈表面，溝具顆粒，覆蓋層具瘤狀突起，外壁外層雕紋是網紋或細網紋，外壁內層比外壁外層薄。台灣的菟絲子屬可分為兩個花粉型：第一型是小粒，溝具顆粒，外壁外層雕紋細網狀，包括菟絲子、平原菟絲子及中國菟絲子；第二型是中間粒，溝具顆粒，顆粒的表面有瘤狀突起，外壁外層雕紋網狀，包括台灣菟絲子及日本菟絲子。花粉的分型和 Yuncker所分亞屬有一致性，第一型為 *Grammica* 亞屬而第二型為 *Monogyna* 亞屬，同時花粉的特徵也有助於物種的鑑定。

關鍵詞：菟絲子屬；菟絲子；花粉；台灣。