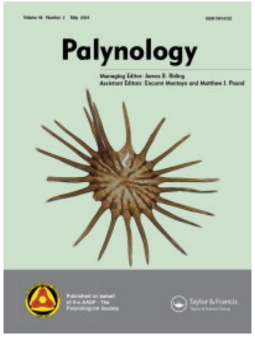

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ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tpal20

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To cite this article: Chien-Ti Chao, Hsy-Yu Tzeng & Yen-Hsueh Tseng (29 Apr 2024): Pollen morphology of Taiwanese Asparagaceae species: insights from scanning electron microscopy, Palynology, DOI: 10.1080/01916122.2024.2337676

To link to this article: https://doi.org/10.1080/01916122.2024.2337676

PALYNOLOGY
https://doi.org/10.1080/01916122.2024.2337676

Pollen morphology of Taiwanese Asparagaceae species: insights from scanning electron microscopy

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ABSTRACT
This study employed scanning electron microscopy to investigate the pollen morphology of Taiwanese Asparagaceae species, revealing that their pollen morphology closely aligns with that of other monocotyledonous plant taxa such as Asparagales, Commelinales, and Bromeliales. Despite the overall similarity, variations in pollen morphology across different genera were noted, highlighting its potential utility in classification. Unlike the wide diversity observed in flower morphology, Asparagaceae pollen morphology appears conservative, rendering it less effective as a taxonomic marker at the species level. The study also delved into the effects of pollenkitt and harmomegathy on pollen morphology, discovering that pollenkitt partially or completely coats the pollen surface, potentially complicating the identification of exine sculpture. Furthermore, the harmomegathic effect was observed to influence aperture dynamics. Consequently, observing both hydrated and dried pollen is advisable, as it can more accurately depict the pollen's aperture morphology and offer additional insights for pollen grain analysis.

KEYWORDS
pollen morphology; Asparagaceae; scanning electron microscopy; pollenkitt; harmomegathy

1. Introduction

Seed plants produce pollen grains from cones or flowers, which act as carriers of male gametes (Shivanna and Tandon 2014; Halbritter et al. 2018). These pollen grains are transported to the surface of the stigma and produce a pollen tube that carries sperm cells into the ovules of the ovary, completing the fertilization process (Shivanna and Tandon 2014; Halbritter et al. 2018). The surface of the pollen is typically hard and composed of sporopollenin, the hardest biotic material known to date (Mackenzie et al. 2015). Pollen morphology varies among plant taxa and exhibits high conservation, often serving as taxonomic evidence or applied in phylogeny reconstruction (Scotland 1993; Vezey et al. 1994; Ghavami et al. 2009; Ferrauto and Pavone 2016; Klimko et al. 2018).

As a large family of monocotyledons, Asparagaceae consists of 118 genera and approximately 3200 species, distributed worldwide (excluding Antarctica) (Christenhusz et al. 2017). Seven subfamilies have been identified in Asparagaceae: Agavoideae, Aphyllanthoideae, Asparagoideae, Brodiaeoideae, Convallarioideae, Lomandroideae, and Scilloideae (APG 2016; Tanaka and Nguyen 2023). Pollen morphology within this family has been extensively studied. Previous research conducted by Radulescu (1973a, 1973b) and Schulze (1980a, 1980b, 1982a, 1982b, 1983) used light microscopy across several subfamilies, while scanning electron microscopy (SEM) has been widely used since the mid-1960s with the ability to provide highly detailed surface images (Halbritter et al. 2018). Recent research has adopted this approach to study a variety of groups, e.g. Asphodeloideae (Liliaceae s.l.) (Diaz Lifante et al. 1990), Ruscaceae (Rudall and Campbell 1999), *Muscari* Mill. (Pehlivan and Özler 2003), *Deacaena* Vand. and *Chrysodracon* P.L. Lu & Morden (Klimko et al. 2018), *Hyacinthella* Schur (Acar Şahīn and Eroğlu 2022), *Beschorneria* Kunth and *Asimina* Adans. (Hesse et al. 2009), and *Asparagus* L. (Ozler and Pehlivan 1970). In addition, pollen morphology is often a fundamental element of new taxon descriptions, such as those by Böhnert and Lobin (2017), Eroğlu et al. (2019), and Yildirim and Sefali (2020), besides the aforementioned references.

In Taiwan, Huang (1972) conducted the earliest pollen morphology study of Asparagaceae using light microscopy. Subsequently, several master's theses focused on the revision of Ophiopogoneae (Sang 1995), *Aspidistra* Ker Gawl. (Wang 2004), and Polygonateae (Chao 2010) reported on pollen morphology. However, many species have not been observed using any of the aforementioned methods. Additionally, variations in pretreatment protocols and microscopes can affect pollen morphology and image resolution. Therefore, the objective of this study is to comprehensively observe the pollen morphology of Asparagaceae of Taiwan using SEM and assess the significance of pollen morphology in the systematic classification of this family.

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Published online 29 Apr 2024

2. Materials and methods

2.1. Pollen material

During field surveys, 13 genera and 22 species were collected, with three individuals collected from each location. Mature and indehiscent anthers were preserved in a solution of 70% ethanol (EtOH) and DMP (2-2, Dimethoxypropane) solution. Voucher specimens were then deposited in the herbarium of Chung Hsing University (TCF) for future reference (Table 1).

2.2. Pretreatment and observation

Acetolysis has been regarded as a standard method of pretreatment of pollen for several decades, and is widely applied in the seed plants. This method was proposed by Erdtman (1952); the matter inside and on the surface of pollen could be removed after acetolysis, and only exine was left. However, the pollen of taxa with thin or few exine elements, such as *Beilschmiedia* Nees (Lauraceae) (Hesse and Waha 1989), or *Guatteria* Ruiz & Pav. (Annonaceae) (Morawetz and Waha 1985), is very fragile and would be destroyed by such a method. Therefore, pollen pretreatment using the DMP method is better for these species and thus is adopted here (Halbritter 1998). In order to capture more pollen characters, both hydrated and dry pollen were observed in this study. For the hydrated pollen, mature anthers were packaged using filter paper and immersed in DMP for at least 30 min at room temperature. Subsequently, the anthers were transferred into acetone for 30 min and then dried with a critical point dryer (Quoram E3100). Dried pollen grains were taped onto the stubs with copper tape, then coated with gold using a sputter coater (QUORUM SC7620). Dry pollen grains were directly taped onto the stubs and coated. The prepared pollen was observed under a scanning electronic microscope (HITACHI S-3400N).

2.2.1. Data analysis

Descriptive terminology of pollen morphology followed Halbritter et al. (2018); analysis of aperture types followed Halbritter and Hesse (1993). Cluster analysis was conducted to categorize samples based on a mixed dataset containing variables of different types. Gower's distance metric, ideal for handling mixed data types, was calculated to assess the similarity between samples. Following this, hierarchical clustering was performed using Ward's linkage method, which minimizes the total within-cluster variance, thus ensuring the formation of homogeneous clusters. All analyses were executed in R statistical software (R Core Team, 2023), employing the 'cluster' package for the computation of Gower distances and hierarchical clustering.

3. Results

The pollen of Taiwanese Asparagaceae was monad, suboblate to oblate spheroidal, small to medium in size, inaperture, monosulcate or ulcus. In addition, the shape of dried pollen grains could differ from that of hydrated pollen grains due to the closure of the aperture. Descriptions of the pollen morphology of the studied taxa are listed in Table 2.

In this study, the equatorial axis length (E) measurements revealed *Dracaena angustifolia* (Medik.) Roxb. had the longest axis at 49.35 ± 0.89 μm (Plate 4), whereas *Asparagus cochinchinensis* (Lour.) Merr. was the shortest at 16.84 ± 1.01 μm (Plate 1). Similarly, the polar axis length (P) varied, with *Disporopsis pernyi* (Hua) Diels displaying the longest axis of 38.09 ± 1.58 μm (Plate 8, Figures 1–5), and *A. cochinchinensis* the shortest at 16.55 ± 0.99 μm (Plate 1). The P/E ratio ranged from a high of 0.99 in *Aspidistra mushaensis* Hayata and *Thysanotus chinensis* Benth. to a low of 0.76 in *Heteropolygonatum altelobatum* (Hayata) Y.H. Tseng, H.Y. Tzeng, C.T. Chao (Plate 8, Figures 6–10). Pollen grains exhibited two primary shapes – suboblate and oblate spheroidal – with species such as *Heteropolygonatum altelobatum*, *Liriope*

Table 1. Collection information for the materials of this study.

Subfamily	Taxon	Location	Coll. no.
Asparagoideae	*Asparagus cochinchinensis* (Lour.) Merr.	Yunlin County: Kuken	Chao 2191
Convallarioideae	*Aspidistra attenuata* Hayata	Pingtung County: Chenlishan	Chao 3935
Convallarieae	*A. daibuensis* Hayata	Pingtung County: County road no. 199	Chao 3903
	A. mushaensis Hayata	Taichung City: Takeng	Chao 3630
	Rohdea fargesii (Baill.) Y.F.Deng	Taichung City: Wuling farm	Chao 2108
Dracaeneae	*Dracaena angustifolia* Roxb.	Taitung County: Lanyu	Chao 4223
Ophiopogoneae	*Liriope graminifolia* (L.) Baker	Pingtung County: Tahan logging trail	Chao 4046
	L. muscari (Decne.) L.H. Bailey	Taitung County: Lanyu	Chao 3223
	L. spicata Lour.	New Taipei City: Pienfutung park	Chao 4401
	Ophiopogon intermedius D. Don	Chiayi County: Yushan	Chao 4147
	O. reversus C.C. Huang	Pingtung County: Mutan	Chao 3333
	Peliosanthes arisanensis Hayata	Pingtung County: Wutai	Chao 4018
	P. kaoi Ohwi	Kaohsiung City: Tenchih	Chao 2125
Polygonateae	*Disporopsis pernyi* (Hua) Diels	Nantou County: Highway no. 14 A, 19 km	Chao 3296
	Heteropolygonatum altelobatum (Hayata) Y.H. Tseng, H.Y. Tzeng, C.T. Chao	Hsinchu County: Lupi trail	Chao 1273
	Maianthemum formosanum (Hayata) LaFrankie	Taichung City: Mt. Sylvia	Chao 3788
	M. harae Y.H. Tseng & C.T. Chao	Chiayi County: Tefuyeh ancient trail	Chao 1385
	Polygonatum arisanensis Hayata var. *arisanensis*	Nantou County: Chuntashan	Chao 4131
	P. arisanensis var. *chingshuishanianum* (S.S. Ying) C.T. Chao & Y.H. Tseng	Hualien County: Chingshuishan	Chao 4128
	P. arisanensis var. *formosanum* (Hayata) C.T. Chao & Y.H. Tseng	Taipei City: Tatun main peak	Chao 4105
Lomandroideae	*Thysanotus chinensis* Benth.	Kinmen County: Tienpu	Chao 3098
Scilloideae	*Barnardia japonica* (Thunb.) Schult. & Schult.	Miaoli County: Tunghsiao	Chao 3826

Table 2. Pollen morphology of Asparagaceae of Taiwan.

Subfamily	Taxon	Equatorial axis (E, µm)	Polar axis (P, µm)	P/E	Shape	Size	Aperture	Sculpture Non-aperture	Sculpture Aperture
Asparagoideae	*Asparagus cochinchinensis*	16.84 ± 1.01	16.55 ± 0.99	0.98	os	s	Monosulcate	Reticulate	Foveola
Convallarioideae	*Aspidistra attenuata*	29.26 ± 1.04	28.05 ± 1.12	0.96	os	m	Omniaperture	Microgemmate-microreticulate	–
Convallarieae	*A. daibuensis*	29.53 ± 0.57	27.11 ± 1.42	0.92	os	m	Omniaperture	Microgemmate-microreticulate	–
	A. mushaensis	26.92 ± 0.37	26.67 ± 0.99	0.99	os	m	Omniaperture	Microgemmate-microreticulate	–
	Rohdea fargesii	33.73 ± 0.44	30.03 ± 0.81	0.89	os	m	Monosulcate	Foveola	Foveola
Dracaeneae	*Dracaena angustifolia*	49.35 ± 0.89	11.2	0.23	s	m	Ulcus	Foveola	Foveola
Ophiopogoneae	*Liriope graminifolia*	24.61 ± 1.76	19.20 ± 0.94	0.78	s	s	Insulae	Fossula	Foveola
	L. muscari	25.78 ± 1.80	21.32 ± 0.56	0.83	s	m	Insulae	Fossula	Foveola
	L. spicata	30.41 ± 0.30	24.10 ± 0.41	0.79	s	m	Insulae	Fossula	Foveola
	Ophiopogon intermedius	31.09 ± 1.50	26.79 ± 0.70	0.86	s	m	Insulae	Granulate	Foveola
	O. reversus	27.33 ± 0.88	22.73 ± 0.46	0.83	s	m	Insulae	Perforate-psilate	Foveola
	Peliosanthes arisanensis	27.70 ± 0.80	24.13 ± 0.79	0.87	os	m	Diffuse	Granulate	Granulate
	P. kaoi	26.59 ± 1.24	22.60 ± 1.64	0.85	s	m	Diffuse	Granulate	Granulate
Polygonateae	*Disporopsis pernyi*	40.24 ± 2.52	38.09 ± 1.58	0.95	os	m	Insulae	Rugulae	Granulate
	Heteropolygonatum altelobatum	31.83 ± 1.60	24.18 ± 0.67	0.76	s	m	Insulae	Reticulate	Psilate
	Maianthemum formosanum	29.19 ± 0.76	22.95 ± 1.36	0.79	s	m	Monosulcate	Reticulate	Foveola
	M. harae	27.88 ± 1.26	23.38 ± 0.72	0.84	s	m	Monosulcate	Foveola	Foveola
	Polygonatum arisanensis var. *arisanensis*	28.03 ± 0.66	24.07 ± 0.83	0.86	s	m	Monosulcate	Perforate-psilate	Foveola
	P. arisanensis var. *chingshuishanianum*	38.03 ± 3.03	32.81 ± 3.37	0.77	s	m	Monosulcate	Foveola	Foveola
	P. arisanensis var. *formosanum*	40.87 ± 0.26	31.34 ± 0.81	0.89	s	m	Monosulcate	Foveola	Foveola
Lomandroideae	*Thysanotus chinensis*	32.28 ± 0.17	31.89 ± 1.10	0.99	os	m	Omniaperture	Reticulate	–
Scilloideae	*Barnardia japonica*	25.84 ± 0.41	24.44 ± 0.66	0.95	os	m	Monosulcate	Foveola	Granulate

os: oblate spheroidal; s: spheroidal; s: small; m: medium.

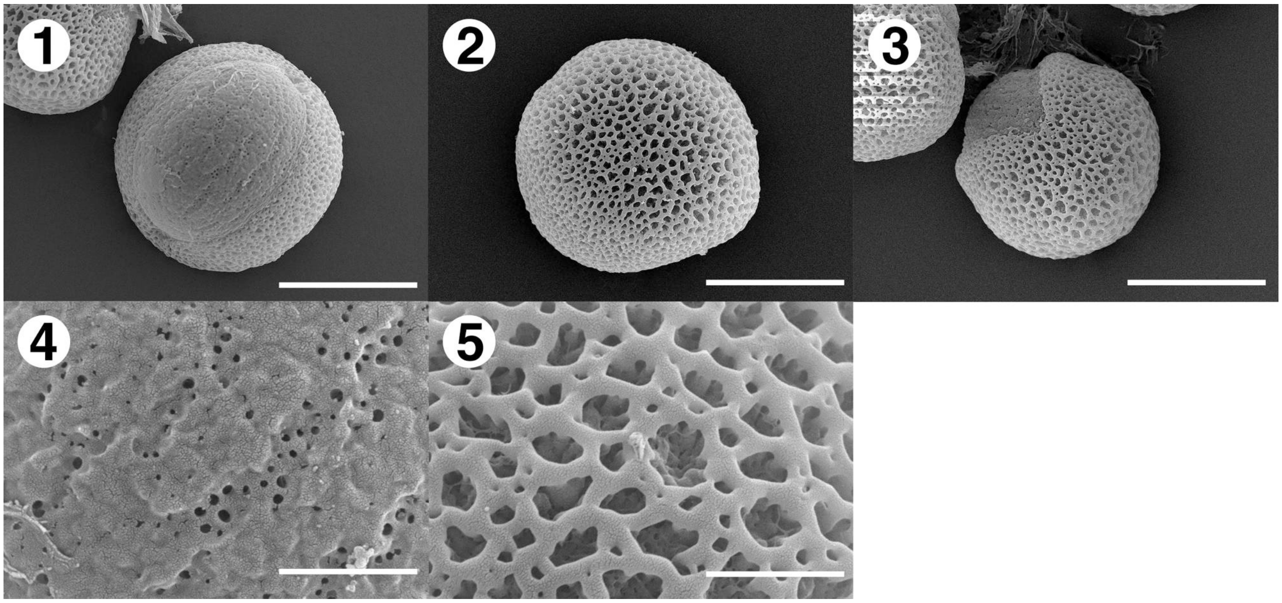

Plate 1. Pollen morphology of *Asparagus cochinchinensis*. 1: distal polar view; 2: proximal polar view; 3: equatorial view; 4: sculpture of distal polar view; 5: sculpture of proximal polar view. Scale bar: 1, 2, 3 = 20 µm, 4, 5 = 2 µm.

Lour., and others showing suboblate shapes (Plates 5 and 9), while the rest demonstrated oblate spheroidal forms (Plate 10). Regarding size, all pollen grains except for *A. cochinchinensis* were of medium size. The sculpture of pollen grains, observed from both proximal polar and distal polar views, displayed a variety of types including reticulate, verrucate, rugulate, fossulate, and granulate, with specific patterns associated with certain species (Plate 1, Figure 5; Plate 8, Figure

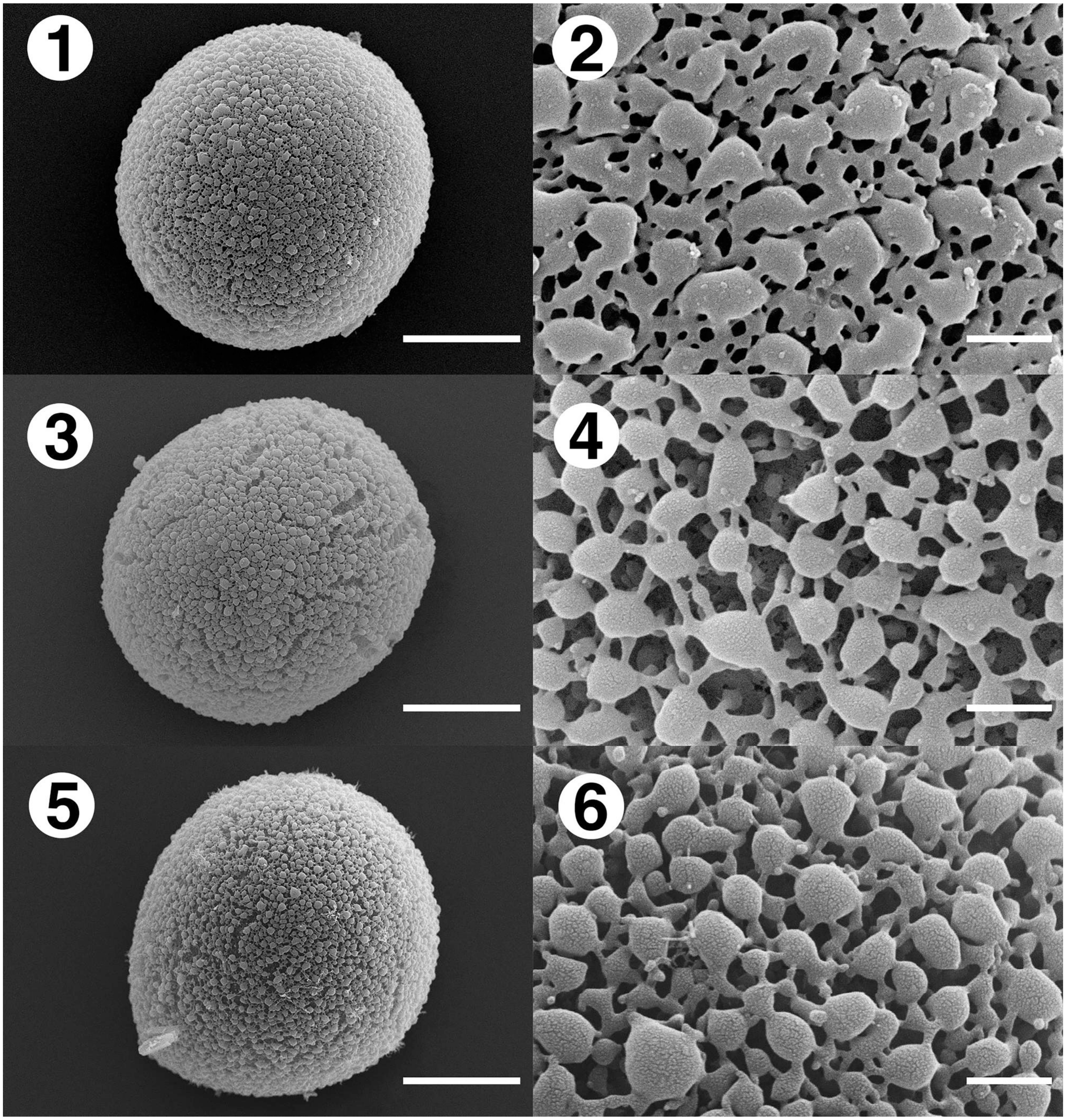

Plate 2. Pollen morphology of *Aspidistra*. 1, 2: *A. attenuata*; 3, 4: *A. daibuensis*; 5, 6: *A. mushaensis*. 1, 3, 5: pollen grain; 2, 4, 6: sculpture. Scale bar 1, 3, 5 = 10 μm; 2, 4, 6 = 1 μm.

10; Plate 9, Figure 5; and Plate 11). The aperture analysis showed a predominance of monosulcate apertures, with variations such as inaperture and ulcus types also present, illustrating the diversity within the studied species (Plates 2 and 11).

However, the morphology of apertures of dry pollen varied due to the harmomegathic effect. Pollen grains with a monosulcate aperture would close after dehydration, resulting in a boat-shaped overall appearance (Plate 13, Figure 1). The aperture of *D. angustifolia* was flat or slightly concave after drying, resulting in a hemispherical appearance (Plate 12, Figure 2). Species without aperture, viz. *Aspidistra* and *Thysanotus*, had different states after drying. Although the pollen of *Aspidistra* still appeared spherical after drying, the surface had numerous irregular folds (Plate 13, Figure 3). The pollen of *Thysanotus*, on the other hand, had the groove portion folded inward, causing such structures to connect with each other, but the overall appearance remained spherical (Plate 13, Figure 2).

The cluster analysis identified three clusters at a height of 0.7. Cluster 1 included *Asparagus*, *Aspidistra*, *Rohdea*, *Thysanotus*, and *Barnardia*, all of which have spheroidal pollen grains with either omniaperturate or monosulcate apertures. Cluster 2 comprised species of *Polygonatum*, *Maianthemum*, and *Dracaena*, which all featured oblate spheroidal pollen grains with apertures that were either monosulcate or ulcus (in the case of *D. angustifolia*). Cluster 3 contained *Liriope*, *Ophiopogon*, *Peliosanthes*, *Disporopsis*,

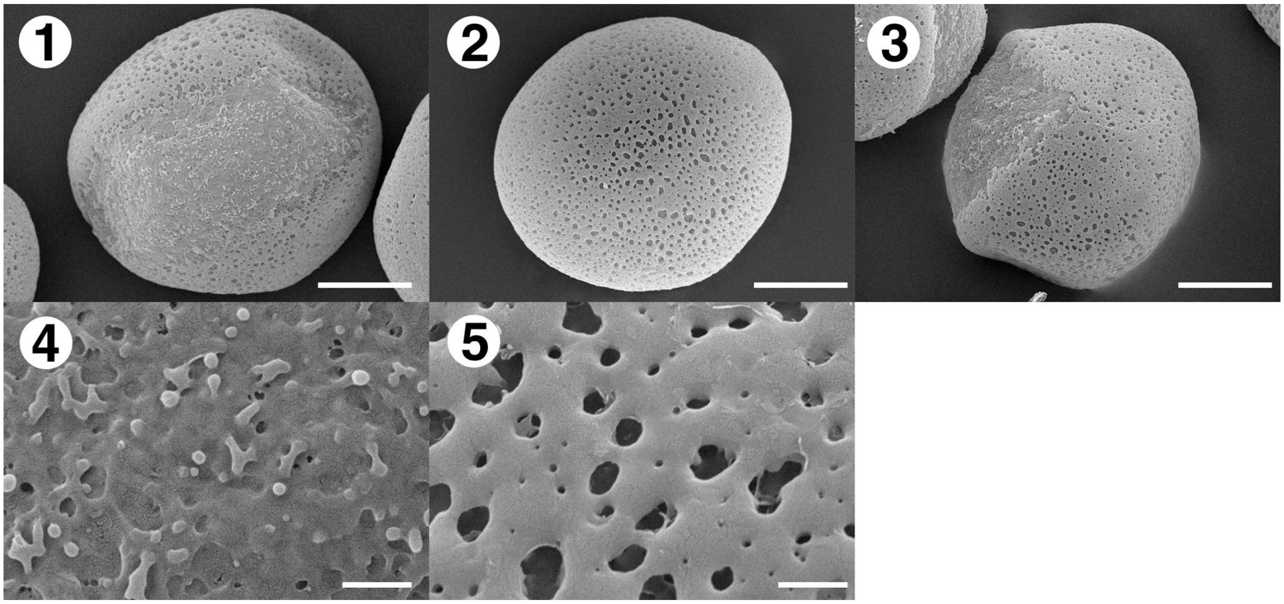

Plate 3. Pollen morphology of *Rohdea fargesii*. 1: distal polar view; 2: proximal polar view; 3: equatorial view; 4: sculpture of distal polar view; 5: sculpture of proximal polar view. Scale bar: 1, 2, 3 = 10 µm, 4, 5 = 1 µm.

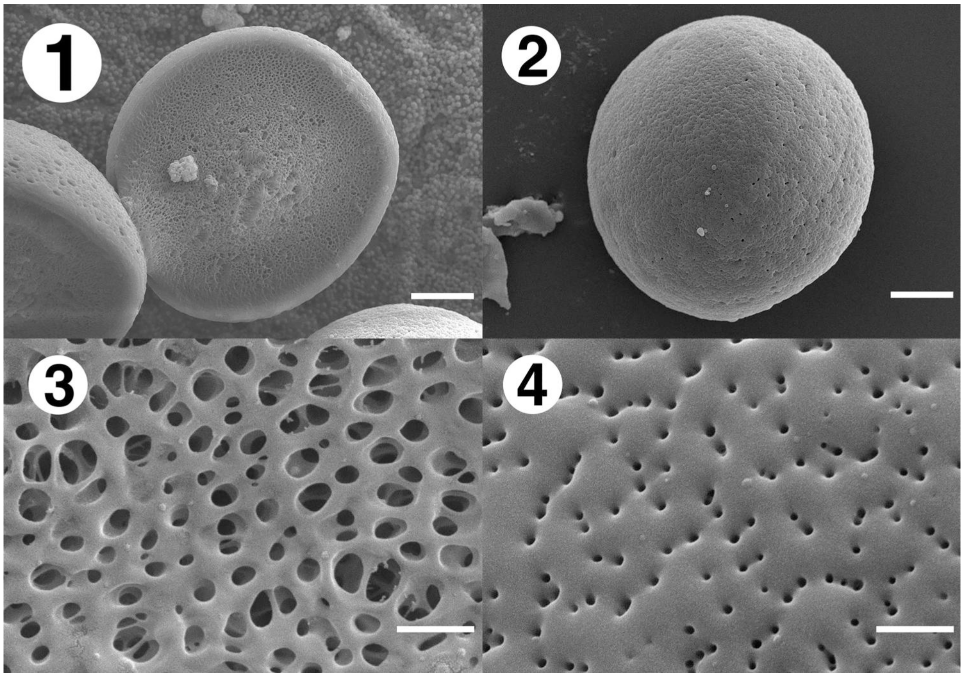

Plate 4. Pollen morphology of *Dracaena angustifolia*. 1: distal polar view; 2: proximal polar view; 3: sculpture of distal polar view; 4: sculpture of proximal polar view. Scale bar: 1, 2 = 10 µm, 3, 4 = 1 µm.

and *Heteropolygonatum*. These species generally possessed oblate spheroidal pollen grains with either insulae or a diffuse type of aperture (Figure 1).

4. Discussion

This study is the first to examine the pollen morphology of most Asparagaceae species in Taiwan using SEM, incorporating DMP as a pretreatment. Traditionally, acetolysis or no pretreatment has been employed, often leading to the collapse of pollen grains or their encasement in thick pollenkitt, which could cause character misidentification. In contrast, the DMP method has proven more effective in preserving detailed information about the shape, aperture, and sculpture of pollen grains.

4.1. Systematic value of pollen morphology on Asparagaceae

Taiwan hosts four of the seven subfamilies of Asparagaceae: Asparagoideae, Lomandroideae, Nolinoideae, and Scilloideae. The pollen grains of these subfamilies are monads, which are small to medium in size, with omniaperture to various aperture types and have various sculpture types. In the present

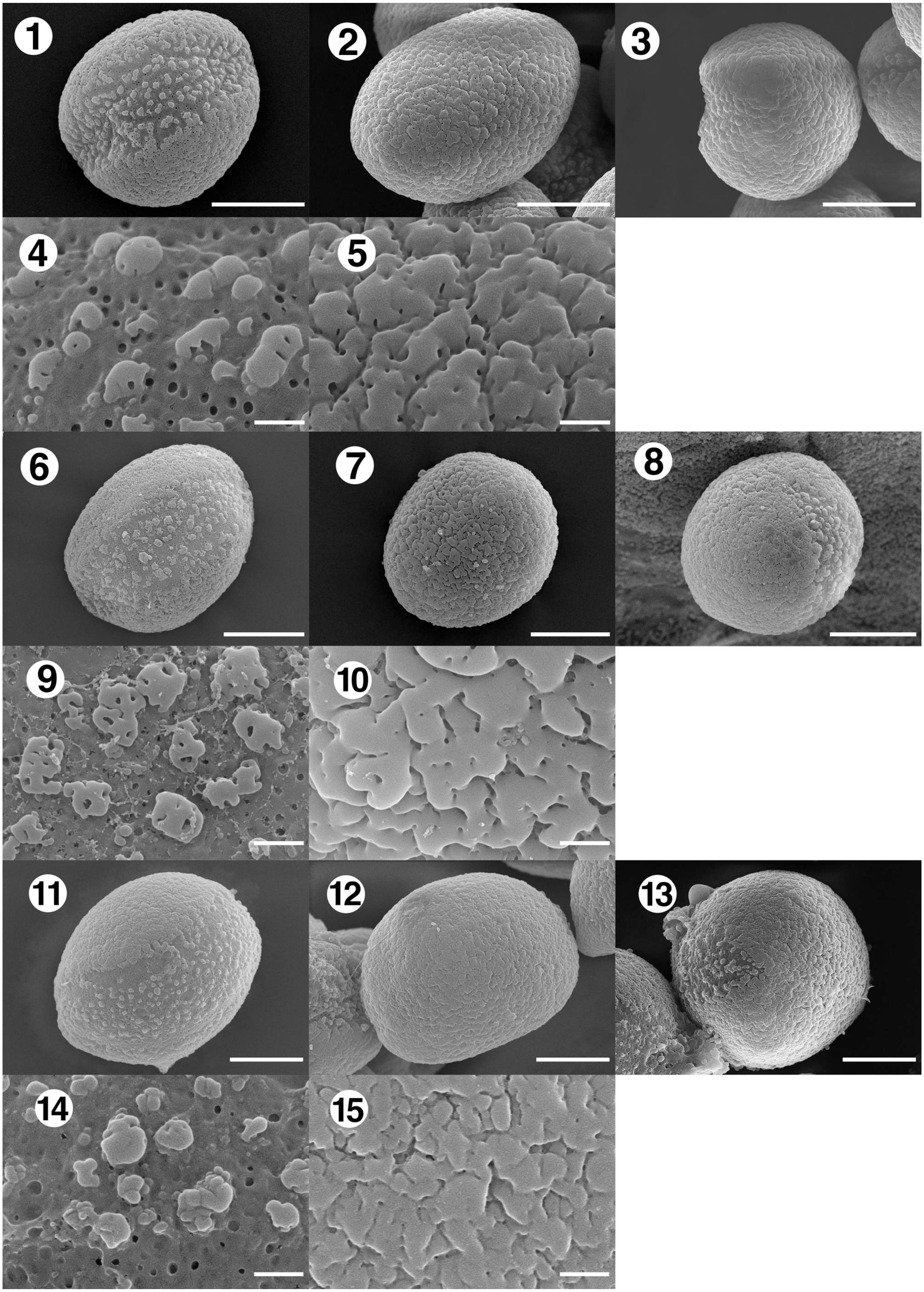

Plate 5. Pollen morphology of *Liriope*. 1–5: *L. graminifolia*; 6–10: *L. muscari*; 11–15: *L. spicata*. 1, 6, 11: distal polar view; 2, 7, 12: proximal polar view; 3, 8, 13: equatorial view; 4, 9, 14: sculpture of distal polar view; 5, 10, 15: sculpture of proximal polar view. Scale bar: 1, 2, 3, 6, 7, 8, 11, 12, 13 = 10 μm, others = 1 μm.

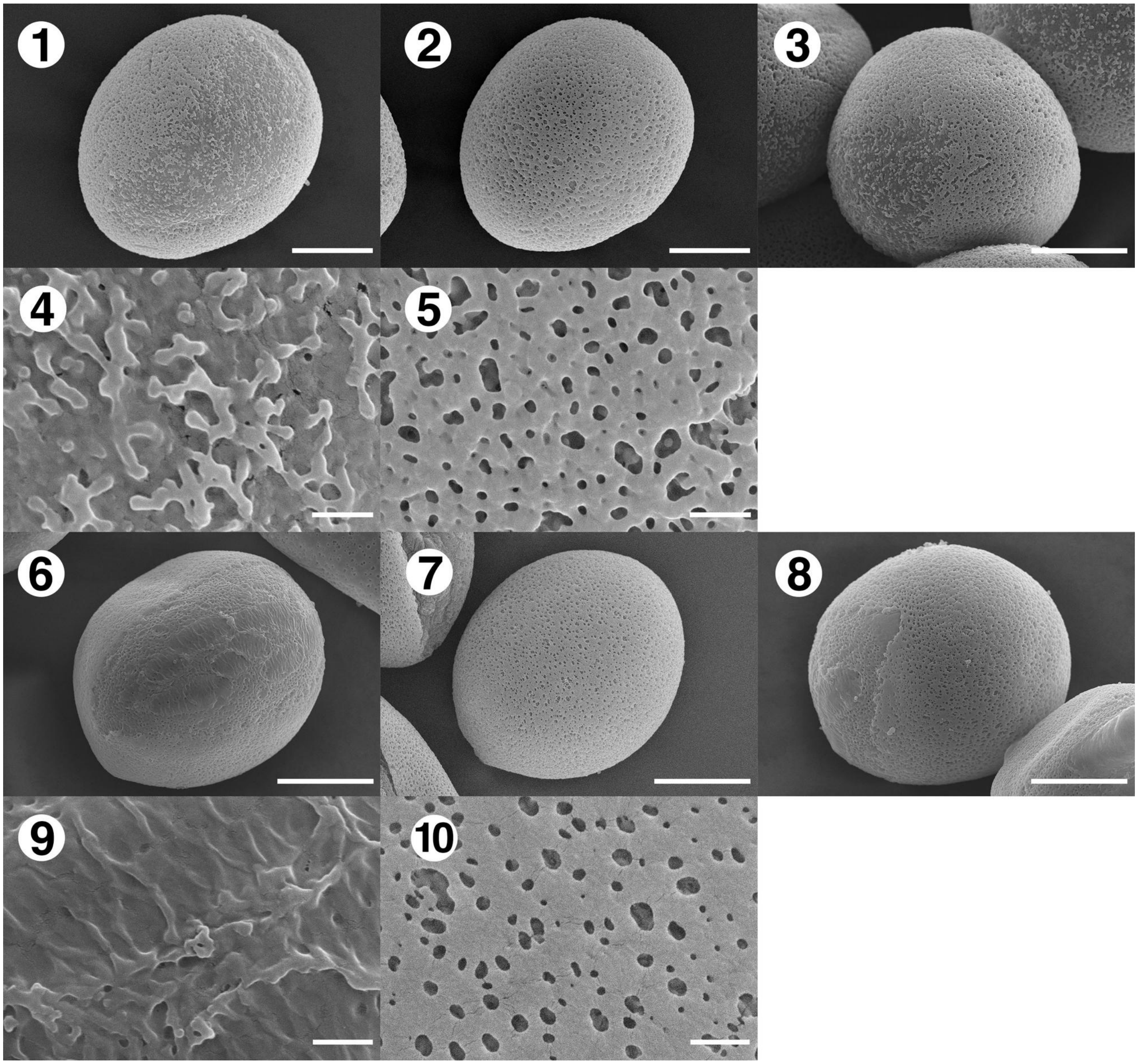

Plate 6. Pollen morphology of *Ophiopogon*. 1–5: *O. intermedium*; 6–10: *O. reversus*. 1, 6: distal polar view; 2, 7: proximal polar view; 3, 8: equatorial view; 4, 9: sculpture of distal polar view; 5, 10: sculpture of proximal polar view. Scale bar: 1, 2, 3, 6, 7, 8 = 10 μm, others = 1 μm.

study, the most common pollen type (clusters 2 and 3) has also been found in other taxa such as *Lilium* L., *Tricyrtis* Wall. (Liliaceae), *Disporum* Salisb. (Colchicaceae) (Wang et al. 2018; Chao and Tseng 2019), *Veratrum* L. (Melanthiaceae), and *Petrosavia* Hutch. (Petrosaviaceae). The study of Dahlgren and Clifford (1982) showed this type of pollen appearing in some monocot orders, such as Liliales, Asparagales, Comelinales, Bromeliales, Arecales, and Orchidales. They suggested that this pollen type might be a symplesiomorphy of monocots.

4.2. Significance of pollen morphology on the intra-familial level for Asparagaceae

Consistency in pollen morphology was found to be more pronounced at the intra-familial and even genus level, as opposed to the family level. Taiwanese Asparagaceae can be classified into four subfamilies. Among these, Asparagoideae, Lomandroideae, and Scilloideae have only one species each, while there are multiple species of Convallarioideae. Each subfamily is discussed in turn below.

4.2.1. Asparagoideae (Plate 1)

Asparagus cochinchinensis is the only species of Asparageae distributed in Taiwan. Pollen morphology was found to be similar to that of *Barnardia* and *Rohdea* (all in cluster 1), with reticulate sculpture in distal polar view. Ozler and Pehlivan (1970) observed eight *Asparagus* species of Turkey and identified three exine types, viz. reticulate, reticulate-rugulae, and retipilate. However, these authors did not discuss the significance of pollen morphology in the taxonomy of *Asparagus*.

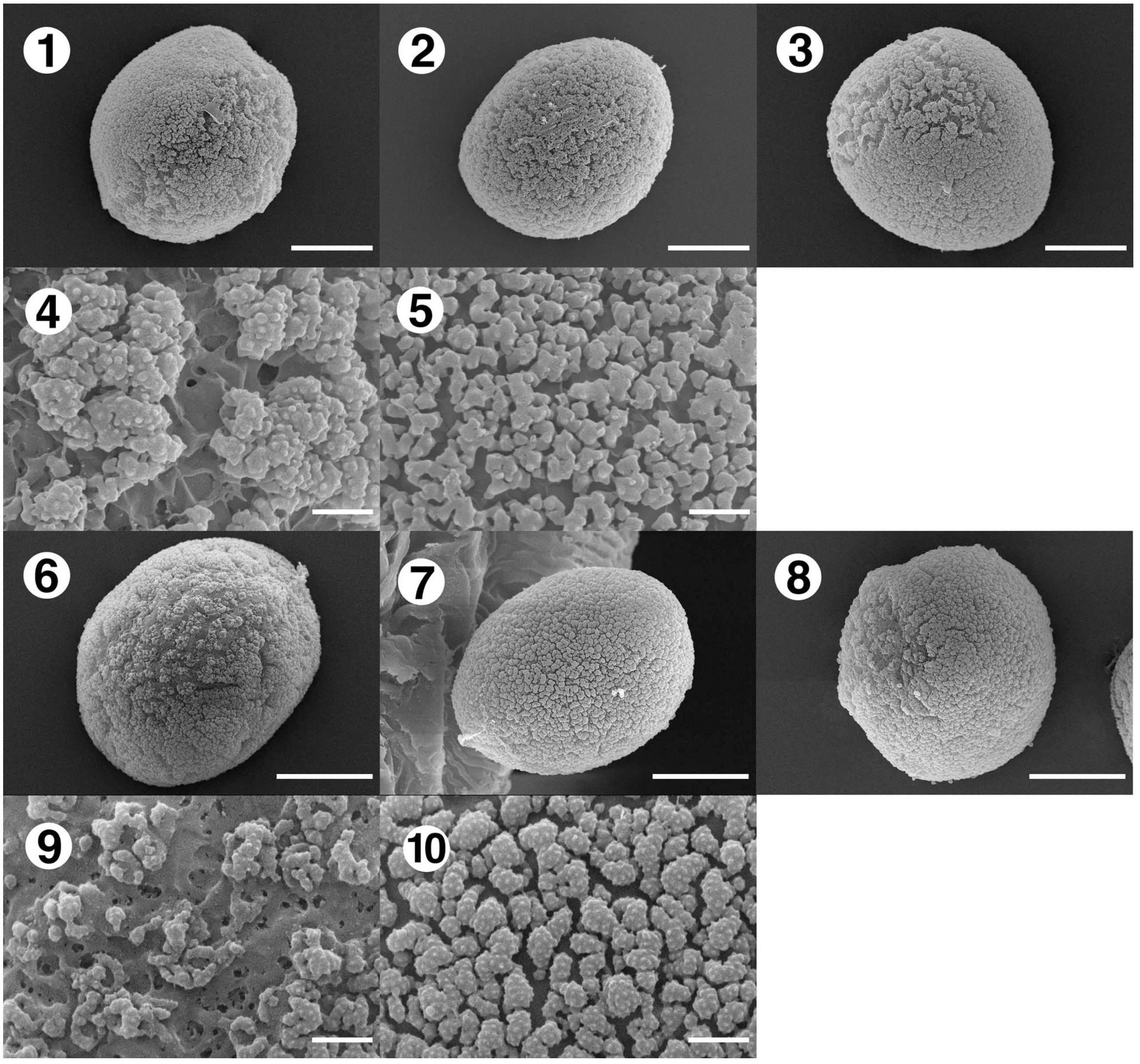

Plate 7. Pollen morphology of *Peliosanthes*. 1–5: *P. arisanensis*; 6–10: *P. kaoi*. 1, 6: distal polar view; 2, 7: proximal polar view; 3, 8: equatorial view; 4, 9: sculpture of distal polar view; 5, 10: sculpture of proximal polar view. Scale bar: 1, 2, 3, 6, 7, 8 = 10 µm, others = 1 µm.

4.2.2. Convallrioideae

The subfamily Convallrioideae includes the following tribes classified by Krause (1930).

4.2.2.1 Convallarieae (Plates 2 and 3). Two genera and four species of Convallarieae were reported here. Among them, pollen of both *Aspidistra* and *Rohdea* Roth was in cluster 1, indicating they all had spheroidal pollen. Two genera could be separated according to whether the aperture was present or not. However, three species of *Aspidistra* could not be separated solely by pollen morphology; a previous study (Wang 2004) supported this opinion.

4.2.2.2. Dracaeneae (Plate 4). *Dracaena angustifolia* is the only member of Dracaeneae in Taiwan, and it exhibits the ulcus aperture type, which is unique to Asparagaceae. Related genera like *Chrysodracon* and *Sansevieria* Petagna also have ulcus type pollen (Klimko et al. 2017, 2018; Halbritter et al. 2018). Also, Halbritter and Hesse (1993) reported that *Ginkgo biloba* L. (Ginkgoaceae), a gymnosperm, had ulcus-type pollen, similar to these angiosperm species. Hence, understanding the distribution, origin, and evolution of ulcus-type pollen in both gymnosperms and angiosperms is an interesting issue and an area for further research.

4.2.2.3. Ophiopogoneae (Plates 5–7). Three genera and seven species of Ophiopogoneae were found in Taiwan. Pollen from *Liriope*, *Ophiopogon*, and *Peliosanthes* was grouped into cluster 3, with *Ophiopogon* showing similarity to *Liriope*. The aperture of *Peliosanthes* was diffuse type, and that of the other two genera was insulae type. The different aperture type might imply *Liriope* and *Ophiopogon* had a

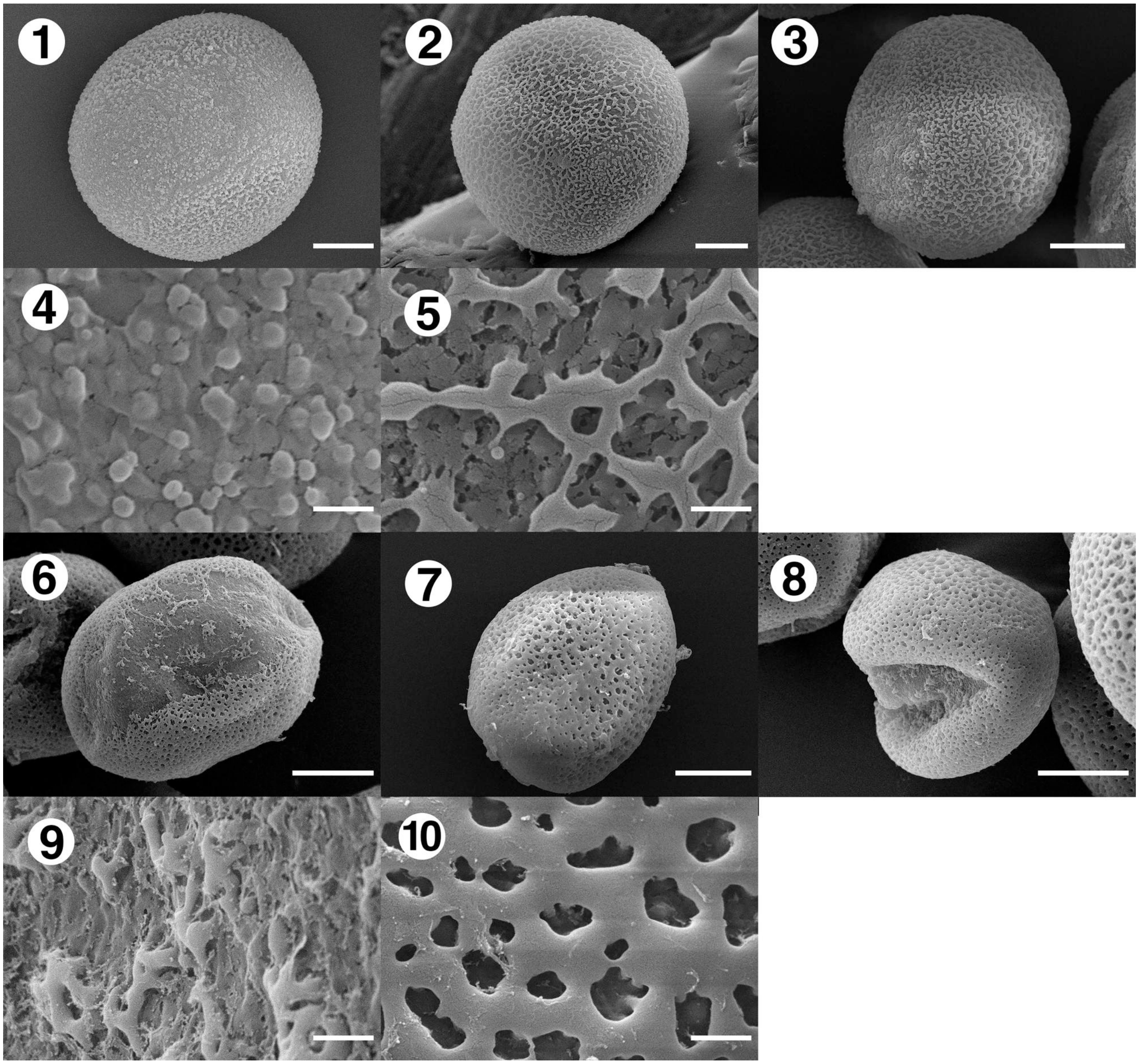

Plate 8. Pollen morphology of *Disporopsis* and *Heteropolygonatum*. 1–5: *D. pernyi*; 6–10: *H. altelobatum*. 1, 6: distal polar view; 2, 7: proximal polar view; 3, 8: equatorial view; 4, 9: sculpture of distal polar view; 5, 10: sculpture of proximal polar view. Scale bar: 1, 2, 3, 6, 7, 8 = 10 μm, others = 1 μm.

closer relationship than *Peliosanthes*; the same inference was also reported in an epidermal and morphological study (Chao et al. 2022). This opinion is also supported by the study of Liang and Dai (1992).

4.2.2.4. Polygonateae (Plates 8–10). Four genera and seven species of the tribe Polygonateae were documented in Taiwan. Within this group, the pollen of *Disporopsis* and *Heteropolygonatum* was classified into cluster 3, while *Maianthemum* and *Polygonatum* fell into cluster 2. Notably, the two species of *Maianthemum* exhibited distinct sculptures in proximal polar view, suggesting that pollen morphology could serve as a diagnostic characteristic for these species.

Our observation of *H. altelobatum* determined that the pollen of this species had an insulae-type aperture, which differed from all the analyzed *Polygonatum* species. This finding supports the classification of *H. altelobatum* in *Heteropolygonatum* rather than *Polygonatum*. However, Deng et al. (2007) studied 13 species of Chinese *Polygonatum* and found that the sculpture types matched the classification of Tang (1978) for this genus. They also discussed the taxonomic status of *H. ginfushanicum* (F.T. Wang & Tang) M.N. Tamura, S.C. Chen & Turland based on pollen morphology and suggested that it should be treated as a *Polygonatum* species rather than a species of *Heteropolygonatum*. This opinion differed from most recent studies on *H. ginfushanicum* (Wang et al. 2018; Gu et al. 2021), which classified it as a *Heteropolygonatum* species. These results might reveal the close relationship of these two genera, and further sampling of *Heteropolygonatum* is necessary to better evaluate the systematic value of pollen morphology in the Polygonateae.

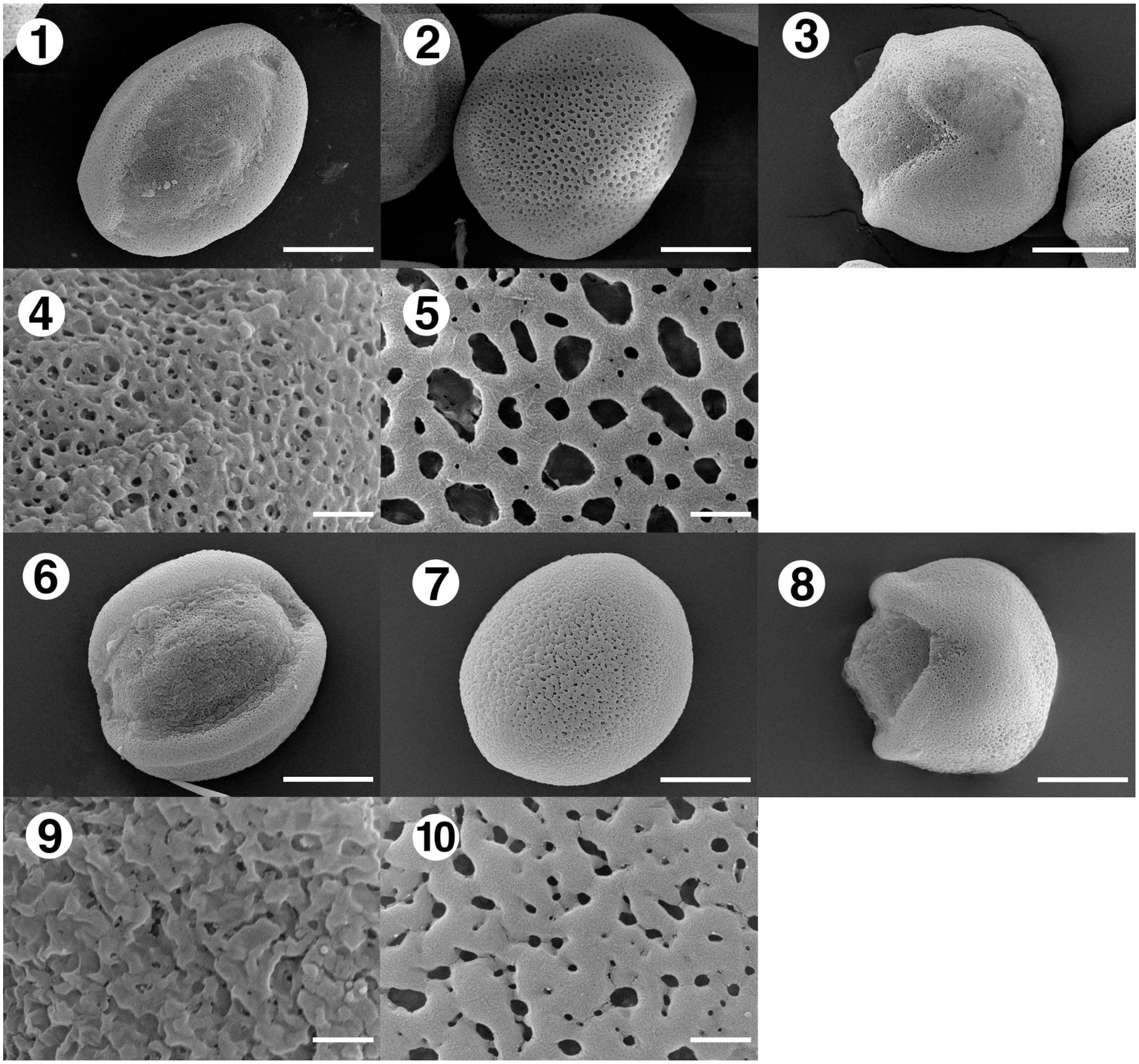

Plate 9. Pollen morphology of *Maianthemum*. 1–5: *M. formosana*; 6–10: *M. harae*. 1, 6: distal polar view; 2, 7: proximal polar view; 3, 8: equatorial view; 4, 9: sculpture of distal polar view; 5, 10: sculpture of proximal polar view. Scale bar: 1, 2, 3, 6, 7, 8 = 10 μm, others = 1 μm.

4.2.3. Lomandroideae (Plate 11)

In Taiwan, Lomandroideae is represented by only one species, *Thysanotus chinensis*, which falls into cluster 1. This species features omniaperture pollen with the exine divided into shield-like structures, a characteristic seemingly unique to *Thysanotus* within the Asparagaceae family thus far. Clypeate pollen, similar to that of *Thysanotus*, is also found in several other taxa, including *Phyllanthus* L. (Euphorbiaceae), Berberidaceae, Iridaceae, and *Smilax* L. (Smilacaceae), and is likely more widespread, as suggested by Halbritter and Hesse (1995). The emergence of clypeate pollen across different angiosperm groups may be attributed to their analogous germination and harmomegathy modes, as proposed by Halbritter and Hesse (1995).

4.2.4. Scilloideae (Plate 12)

The sole species of Scilloideae found in Taiwan is *Barnardia japonica*, whose pollen falls into cluster 1, characterized by a perforate exine sculpture in distal polar view. Historically, *Barnardia* has often been classified within the broader *Scilla* L. *sensu lato*, as noted by Speta (1998) and Mabberley (2017). Nevertheless, subsequent research has challenged this genus' monophyly, leading to its division into several distinct genera, including *Pfosser* Speta, *Fessia* Speta, *Prospero* Salisb., *Barnardia*, and *Zagrosia* Speta, among others (Kubitzki and Huber 1998; Speta 1998; Mabberley 2017). Ghavami et al. (2009) analyzed the pollen morphology of 13 species within *Scilla* s.l., finding that variations in exine sculpture supported the genus's subdivision.

Our observations suggest that the pollen morphology of *B. japonica* closely resembles that of *Fessia* and *Zagrosia*, particularly in terms of lumen size. However, *B. japonica* is distinguished by its broader muri, potentially serving as a defining trait for *Barnardia* and its related genera. Future studies should include an examination of the pollen from *B. numidica* (Poir.) Speta to further clarify these relationships.

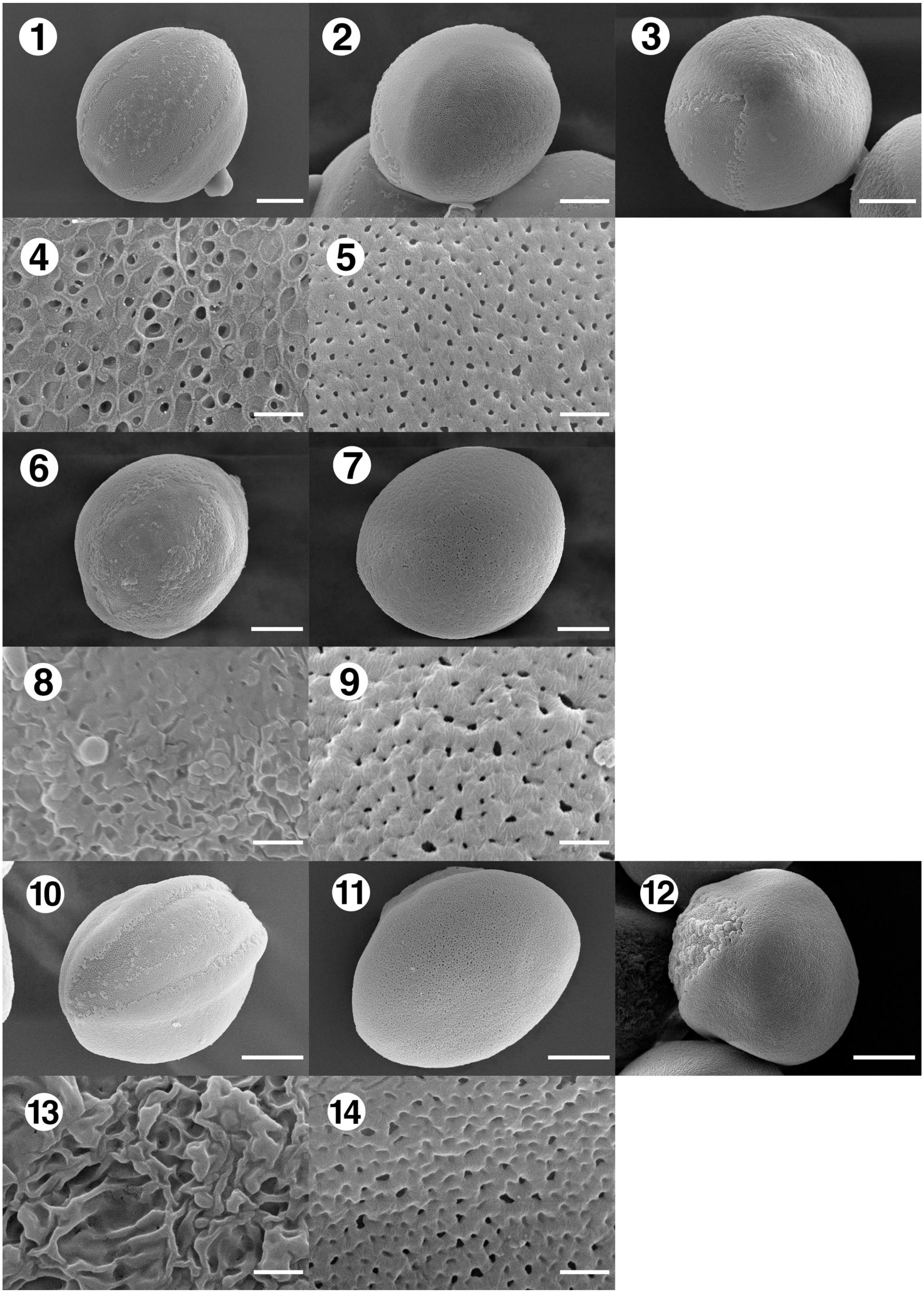

Plate 10. Pollen morphology of *Polygonatum*. 1–5: *P. arisanensis* var. *arisanensis*; 6–9: *P. arisanensis* var. *chingshuishanianum*; 10–14: *P. arisanensis* var. *formosanum*. 1, 6, 10: distal polar view; 2, 7, 11: proximal polar view; 3, 12: equatorial view; 4, 8, 13: sculpture of distal polar view; 5, 9, 14: sculpture of proximal polar view. Scale bar: 1, 2, 3, 6, 7, 10, 11, 12 = 10 μm, others = 1 μm.

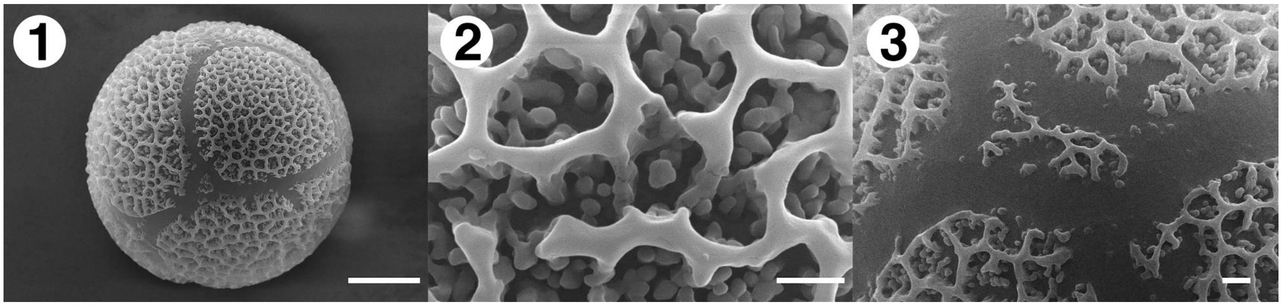

Plate 11. Pollen morphology of *Thysanotus chinensis*. 1: pollen grain; 2: sculpture of shield-like structure; 3: sculpture of grooves. Scale bar: 1 = 10 μm, 2, 3 = 1 μm.

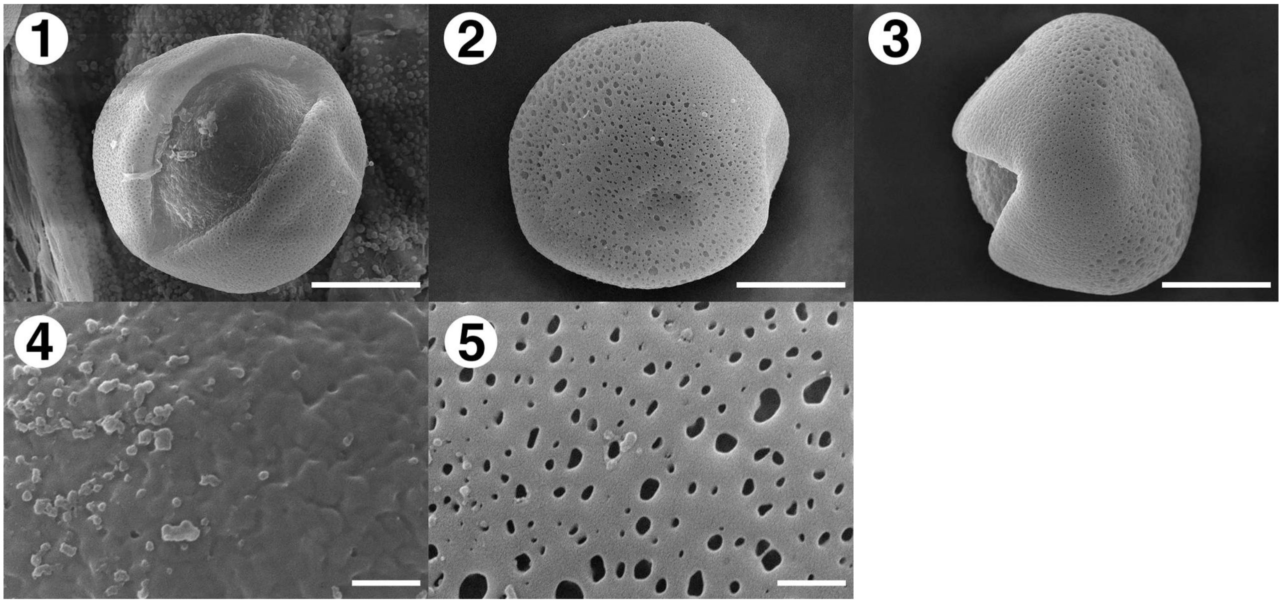

Plate 12. Pollen morphology of *Barnardia japonica*. 1: distal polar view; 2: proximal polar view; 3: equatorial view; 4: sculpture of distal polar view; 5: sculpture of proximal polar view. Scale bar: 1, 2, 3 = 10 μm, 4, 5 = 1 μm.

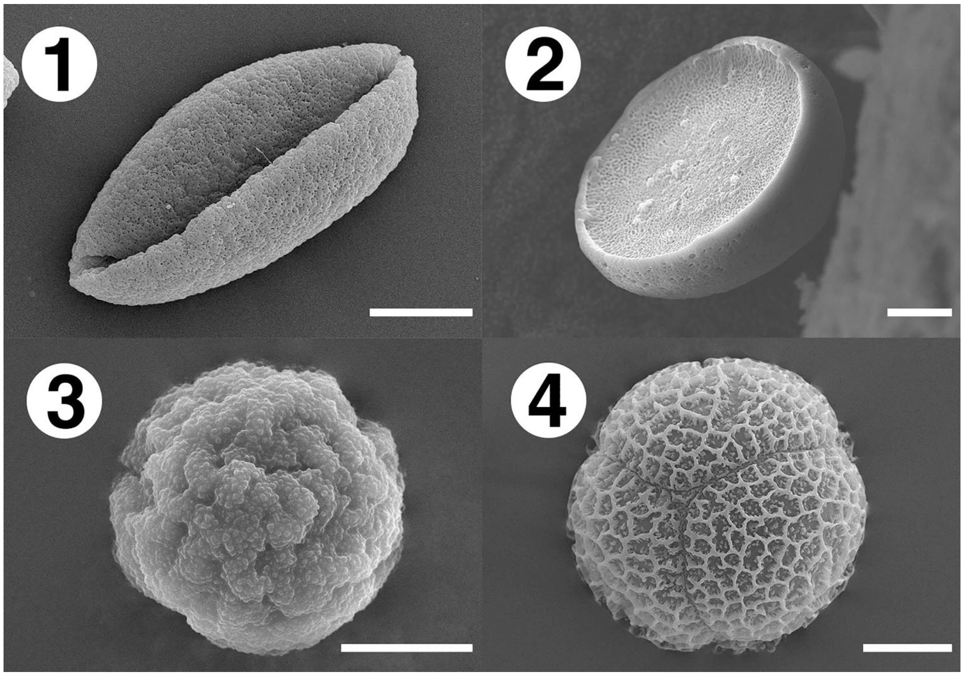

Plate 13. Morphology of dry pollen grain with different aperture types. 1: monosulcate (*Liriope spicata*); 2: ulcus (*Dracaena angustifolia*); 3: omniaperture (*Aspidistra attenuata*); 4: clypeate (*Thysanotus chinensis*). Scale bar = 10 μm.

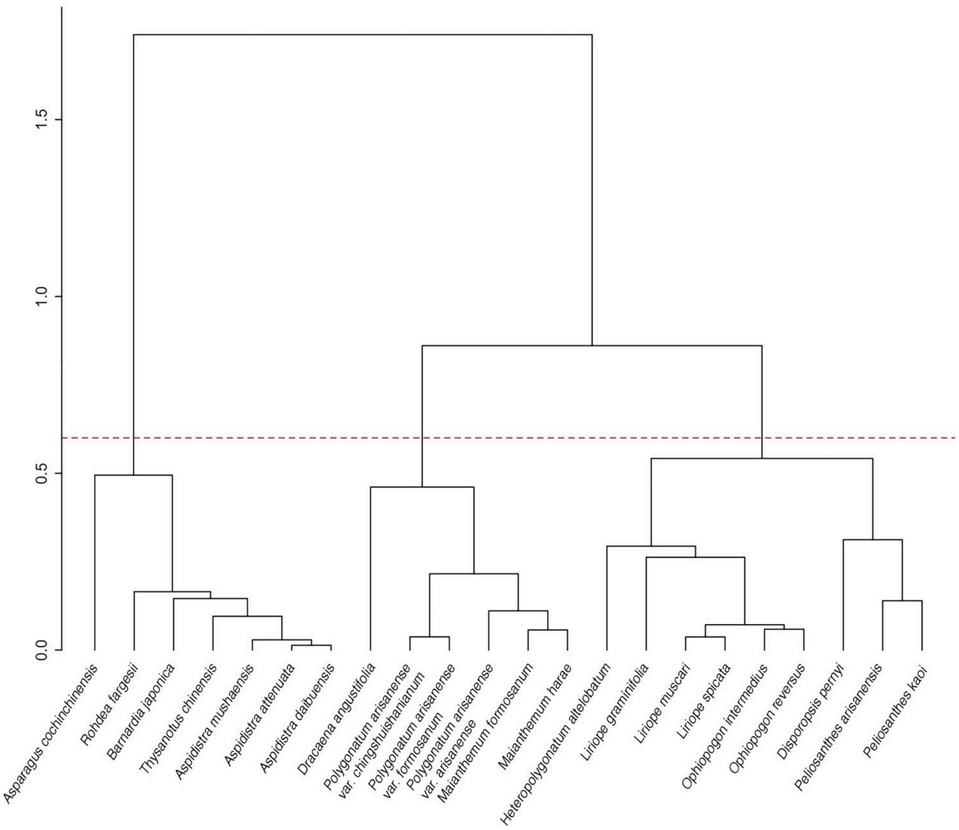

Figure 1. Hierarchical clustering dendrogram of Asparagaceae species from Taiwan. The analysis was based on Gower's distance and employed Ward's linkage method. The red dashed line at 0.6 represents a suggested threshold for three clusters' division.

4.3. The impact of harmomegathic effect on pollen morphology of Asparagaceae

The concept of the harmomegathic effect, introduced by Wodehouse (1935), describes how pollen walls adjust to variations in moisture content, leading to changes in shape. Specifically, in low moisture conditions (such as when pollen leaves the anther and before it reaches the stigma), the intine within the sulcus often folds inward, altering the pollen's shape. Conversely, in high moisture conditions (for example, when pollen is still in the anther or after reaching the stigma), the pollen resumes its swollen state. Previous studies often overlooked this phenomenon, missing critical information about the aperture. Our findings highlight that the aperture's sculpture frequently differs from that in other areas, underscoring the importance of examining aperture morphology, particularly in monosulcate pollen.

Moreover, the variations induced by the harmomegathic effect exhibit a certain regularity and can serve as taxonomic characters. Halbritter and Hesse's (1993) research into the differences in apertures before and after the harmomegathic effect across several monocot families categorized them into 11 types. Our study further demonstrates that aperture morphology has taxonomic significance at the genus level within the family Asparagaceae, for example in Ophiopogoneae. To maximize the information gleaned from pollen morphology, we advocate for the simultaneous observation of dry and hydrated pollen, along with dehydrated pollen grains treated with DMP solution to prevent intine collapse during the acetolysis process.

5. Conclusion

This study provides a comprehensive overview of the pollen morphology of Asparagaceae in Taiwan, revealing that the majority of the observed species possess monosulcate pollen grains, with a minority presenting alternative pollen types. The analysis highlighted that variations in aperture type and exine sculpture serve as characteristics useful for distinguishing between genera, noting that certain species display unique pollen morphologies. Future, more extensive observations of pollen within this family are essential for gaining a deeper insight into the diversity of pollen morphologies and their distribution across Asparagaceae. Such studies could further elucidate the connections between the evolution of pollen morphology and the phylogenetic relationships within the family Asparagaceae.

Acknowledgements

We thank Ms. Yuan-Chien Lin and Ms. Tai-Chen Ho for field assistance.

Disclosure statement

The authors declare no conflict of interest.

Funding

This study was supported by the National Science and Technology Council under Grant no. MOST 103-2313-B-005-009-MY3 to Yen-Hsueh Tseng and NSTC 112-2621-B-003-001-MY3 to Chien-Ti Chao.

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