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Spore SEM studies on the genus *Polystichum*, Dryopteridaceae (Polypodiale, Pteridophyta)

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ABSTRACT: Palynological data is helpful at all levels of the taxonomic hierarchy. Therefore, we used SEM to examine spores of all Taiwan *Polystichum* species. The spore ornamentation types are almost consistent with the most recent classification hypothesis and can differentiate subgenera, sections, and species. Except for a few cases, each current species has one ornamentation type, and different species have different spore morphology. Development variation and cryptic species are possibilities for those species with more than one ornamentation type.

KEY WORDS: Infrageneric classification, *Polystichum*, spore SEM, Taiwan.

INTRODUCTION

Palynological information is beneficial at all levels of the taxonomic hierarchy (Wagner, 1974; Stuessy *et al.,* 2014). Since the 19th century, Waldheim (1864), Tchistiakoff (1874), and Hanning (1911) conducted studies on the morphology of pteridophyte spores, proposing the hypothesis that these pieces of information could be used as evidence for classification. In Taiwan, Huang (1981) employed LM (light microscope) to observe the morphology of spores and published "Spore Flora of Taiwan (Pteridophyta)" documenting the morphology of 535 species from 159 genera and 38 families. This work provided essential information for the phylogeny of pteridophytes. With the advancement of EM (electron microscope), ornamentation not observable under LM, can be seen through SEM (scanning electron microscope), leading to a more vigorous development in pteridophyte spore palynology. Wagner (1974) explored the structure and phylogeny of spores, while Devi (1977) delved into the spore morphology and perispore development of pteridophytes. In general, spore ornamentation varies from species to species. Thus, the spore ornamentation types could tell which species the taxon studied belongs to and construct phylogenetic hypotheses.

Many studies have examined the morphology of spores, such as size, shape, aperture, and surface ornamentation, in various taxa belonging to the Dryopteridaceae. The differences observed in these characteristics have proven valuable for classifying most Dryopteridaceae species (Gorrer *et al.*, 2020; Shan *et al.*, 2021). For example, in the genus *Polystichum*, the morphology of the spores of several taxa has been examined for their identification characteristics in various places such as Japan, China, and Pakistan. (Crane, 1953; Nayar, 1964; Mitui, 1973; Devi, 1977; Zhang and Kung, 1994; Dominic Rajkumar and Manickam, 1999; Chen, 2007; Ghanbari Hamedani *et al.*, 2008; Shah *et al.*, 2021).

Nayar (1964) employed LM to examine 14 species of *Polystichum* and discussed and elucidated the systematic position of the *Polystichum* genus within the Aspidiaceae from the perspective of spore morphology. Mitui (1973) conducted a study utilizing SEM to examine the spores of 40 *Polystichum* species collected from Daigobo's specimens and classified them into eight groups based on ornamentation patterns. Zhang and Kung (1994) studied the spores of species of *Polystichum* Sect. *Metapolystichum* (now Sect. *Hypopeltis*; Zhang and Barrington, 2013) under LM and SEM. They classified into six types and supposed the spores' evolutionary trends in this section were from other types to reticulate.

In previous studies, the *Polystichum* spores are monolete, with the shape ellipsoidal or elliptic viewed from the polar plane and plano-convex or ellipsoidal viewed from the equatorial plane (Mitui, 1973; Xiang, 1992; Zhang and Kung, 1994; Shah *et al.*, 2021). However, using perispore characteristics to make phylogenetic analyses, as in the related genus *Elaphoglossum* (Moran *et al.,* 2007), is not available in *Polystichum*. Recently, numerous new species of cave *Polystichum* ferns have been reported, and the identification and documentation of these new species relied on SEM images of spores (Zhang and He, 2010, 2011).

The Dryopteridaceae family is one of the most diverse pteridophytes, and the *Polystichum* Roth. is one of the largest and most complex genera, with ca. 500 worldwide (PPG I, 2016). Due to its high diversity and cosmopolitan distribution, each of the morphology-based infrageneric classifications proposed in the past (Tagawa, 1940; Daigobo, 1972; Fraser-Jerkins, 1997; Kung *et al.*, 2001, Zhang and Barrington, 2013) are all regional. Its diversity center is in southwest China and adjacent regions. In Taiwan, 38 species are known (TPG, 2019) and could be allocated into two subgenera and 15 sections based on the recent infrageneric classification by Zhang and Barrington (2013). Therefore, it may be inferred that *Polystichum* in Taiwan contains high morphological variation. Le Péchon *et al.* (2016) applied a multilocus phylogeny to examine the classification hypotheses by Zhang and Barrington (2013) and call for more data, including molecular data, to obtain a complete evolutionary picture and new taxonomic framework of this genus.

The present study uses a SEM to observe the spore ornamentation types of Taiwan *Polystichum* species. The resulting spore morphology, especially perispore characteristics, is combined with the information from the previous publications. The combined data then infer the possible phylogenetic relationship of the genus *Polystichum* and the taxonomic position of each species. Then, how the data is compatible with previous infrageneric schemes is assessed. By the way, building upon the information outlined above, the shape of spores in this genus is no obvious difference; each taxon has no description.

MARERIALS AND METHODS

Studied materials

The spore samples were prepared based on Liu *et al.* (2000) collected from the dry specimens deposited in the herbarium of the National Chiayi University. In addition, some materials were obtained from the Taiwan Forestry Research Institute (TAIF). The *Polystichum* in Taiwan contains 38 taxa (TPG, 2019), and we examined 42 taxa of this genus from Taiwan, including four unknown species (Table 1).

The species delimitation is according to Zhang and Barrington (2013), but the species nomenclature follows TPG (2019). The infrageneric treatment is referred to Tagawa (1940), Daigobo (1972), Fraser-Jenkins (1997), Kung *et al.* (2001), Zhang and Barrington (2013) (Table 2).

Preparation of samples

After collecting spores from dry specimens, the ornamentation was examined by scanning electron microscope (SEM). The untreated spores were fixed on aluminum stubs, coated with gold about 15 nm by the iron sputter (Hitachi E–101), and then examined using SEM (Hitachi S2400) at 12–18 kV. 1000–2000 X magnification was used for the whole spores, and 6000 X for the details. Photos were taken by NIKON D40.

Terminology

The terminology of spore surface ornamentation followed Tryon and Lugardon (1990), Lellinger and Taylor (1997), Moran *et al.* (2007), Wang and Dai (2010), and Shah *et al.* (2021).

RESULTS

In this study, we observed 42 *Polystichum* taxa. The spores are monolete, and the shape is ellipsoidal or elliptic when viewed from the polar plane and semicircular (plano-convex) or ellipsoidal from the equatorial plane. The exospore is plain, with ornamentation formed by the perispore. The general perispore classes is muriform. Five types of perispore macro-ornamentation are found:

1. Retate type

Perispore sculptured with network formed by anastomosing folds. Six species in three sections are in this type.

Subg. ***Haplopolystichum*** (Tagawa) Li Bing Zhang
Sect. ***Adenolepia*** Daigobo

Polystichum formosanum Rosenstock (Fig. 1A)

Perispore muri-bearing with thin, wing-like folds. The surface micro-ornamentation is rugate. There are numerous microstructures on the perispore surface that are pits and echinae.

Polystichum hookerianum (C.Presl) C.Christensen (Fig. 1B)

Perispore muri-bearing with broad, regular folds. The surface micro-ornamentation is rugate. There are numerous microstructures on the perispore surface that are grains and echinae.

Polystichum obliquum (D. Don) T. Moore (Fig. 1C)

Perispore muri-bearing with broad folds. The areolae are irregular. There are numerous microstructures on the perispore surface that are echinae.

Sect. ***Cyrtomiopsis*** Tagawa

Polystichum lepidocaulon (Hook.) J. Smith (Fig. 1D)

Perispore muri-bearing with thin, wing-like folds. The areolae are irregular. There are numerous microstructures on the perispore surface that are grains and pits.

Sect. ***Haplopolystichum*** Tagawa

Polystichum acutidens Christ (Fig. 1E)

Perispore muri-bearing with thin, narrow folds. The areolae are irregular. The folds are dense. There are numerous microstructures on the perispore surface that are echinae and pits.

Polystichum deldoton (Baker) Diels (Fig. 1F)

Perispore muri-bearing with broad folds. The areolae are irregular. There are numerous microstructures on the perispore surface that are grains.

2. Rugate type

Perispore sculptured with wrinkles and the folds discontinuously. One species is in this type.

Subg**. *Haplopolystichum***
Sect. ***Crucifilix*** Tagawa

Polystichum hancockii (Hance) Diels (Fig. 2A)

Table 1. Taxa used in this study, with voucher information.

Taxon	Voucher information	Source localities	Relative synonym
P. acanthophyllum	*N Liu0132* (CHIA) *ct 727* (CHIA) *Lu 8144* (TAIF)	Taichung, Tao Sai Peak Kaohsiung, Guanshan Nantou, Patungkuan	*P. horridipinnum*
P. acutidens	*N Liu0097* (CHIA)	Hualien, Mt. Pingfengshan	
P. atkinsonii	*N Liu0015* (CHIA) *ZQ264* (CHIA)	Kaohsiung, Nanheng Taichung, Mt. Nanhu	*P. morri*
P. attenuatum	*Hung 2013-0702-006* (TAIF)	Nantou, Chunta Logging Trail	
P. biaristatum	*N Liu0022* (CHIA)	Nantou, Patungkuan	
P. capillipes	*Hsu9543* (TAIF) *Hsu4556* (TAIF)	Nantou, Chengkung Shelter Sichuan, Mt. Xilingxue	
P. chunii	*Hsu2856* (TAIF)	Taichung, Chiliehting	
P. deltodon	*N Liu0030* (CHIA)	Kaohsiung, Nanheng	
P. erosum	*Hsu11929* (TAIF) *Hsu04469* (TAIF)	Taichung, Chungyangchien Stream shelter Sichuan, Mt. Xilingxue	
P. formosanum	*N Liu0159* (CHIA)	Chiayi, Bantianyan	
P. fraxinellum	N Liu0065 (CHIA)	Hualien, Mt.Chingshuishan	*Cyrtogonellum fraxinellum*
P.glaciale	*N Liu0136* (CHIA)	Taichung, Mt. Nanhu	*Sorolepidium glaciale*
P.grandifrons	*N Liu0161* (CHIA)	Taichung,Nanshantsun	*P. kiusiuense*
P. hancockii	*ZQ319* (CHIA) *N Liu0012* (CHIA)	Chiayi, Alishan Highway Kaohsiung, Nanheng	
P. hecatopterum	*N Liu0110* (CHIA)	Chiayi, Mt. Lulin	
P. herbaceum	*N Liu0104* (CHIA)	Hualien, Mt. Pingfengshan	
P. hookerianum	*SW044* (CHIA)	Hualien, Mt. Pingfengshan	*P. integripinnum*
P. lachenense	*ZQ 272* & *368* (CHIA)	Taichung, Mt. Nanhu	
P. lepidocaulon	*N Liu0024* (CHIA)	Nantou, Patungkuan	
P. manmeiense	*N Liu0007* (CHIA)	Kaohsiung, Nanheng	*P. falcatipinnum*
P. mayebarae	*Hsu7487* (TAIF)	Pingtung, Mt. Chihpen	
P. mucronifolium	*N Liu0058* (CHIA)	Chiayi, Tefuyeh	*P. tacticopterum*
P. neolobatum	*Lu8141* (TAIF)	Nantou, Patungkuan	
P. sp1	*N Liu0192*, *0198* & 0199 (CHIA)	Nantou, Patungkuan	
P. sp2	*N Liu0076* (CHIA)	Chiayi, Mt. Yushan	
P. sp3	*N Liu0105* (CHIA) *N Liu0006* (CHIA)	Hualien, Dayuling Kaohsiung, Nanheng	
P. sp4	*Hsu6053*(TAIF)	Miaoli, Mt. Dabajenshan	
P. nepalense	*N Liu0009* (CHIA)	Kaohsiung, Nanheng	
P. obliquum	*YC. Liu s.n.* (CHIA) *N Liu0153* (CHIA)	Pingtung, Mt. Peitawu	
P. parvipinnulum	*N Liu0034* (CHIA) *N Liu0087* (CHIA)	Kaohsiung, Nanheng Taipei, Erziping Trail	
P. piceopaleaceum	*ZQ325* (CHIA)	Chiayi, Mt. Lulin	
P. prescottianum	*Hsu9474* (TAIF)	Taichung, Mt. Nanhu	
P. prionolepis	*N Liu0037* (CHIA)	Kaohsiung, Nanheng	
P. pseudostenophyllum	*N Liu0125 & 165* (CHIA)	Taichung, Mt. Nanhu	
P. scariosum	*N Liu0152* (CHIA)	Pingtung, Woilososhan	*P. eximium*
P. sinense	*N Liu134* (CHIA)	Taichung, Mt. Nanhu	*P. wilson*
P. stenophyllum	*ZQ0337* (CHIA) *N Liu0138* (CHIA)	Chiayi, Mt. Yushan Taichung, Mt. Nanhu	
P. taizhongense	*N Liu0141* (CHIA)	Taichung, Mt. Nanhu	
P. tenuius	*N Liu0064* & 0070 (CHIA)	Hualien,Mt. Chingshuishan	*Cyrtogonellum tenuius*
P. thomsonii	*N Liu0005* (CHIA) *N Liu0112* (CHIA)	Kaohsiung, Nanheng Chiayi, Mt. Lulin	
P. tsus-simense	*ZQ378* (CHIA) *N Liu0013* (CHIA) *Hsu0210* (TAIF)	Hualien, Mt. Pingfengshan Kaohsiung, Nanheng Taichung, Chungyangchien Stream	
P. xiphophyllum	N Liu0151 (CHIA) Hung0796(TAIF)	Nantou, Shanlinhsi Forest Recreation Area Pingtung, Mt. Chihpen	

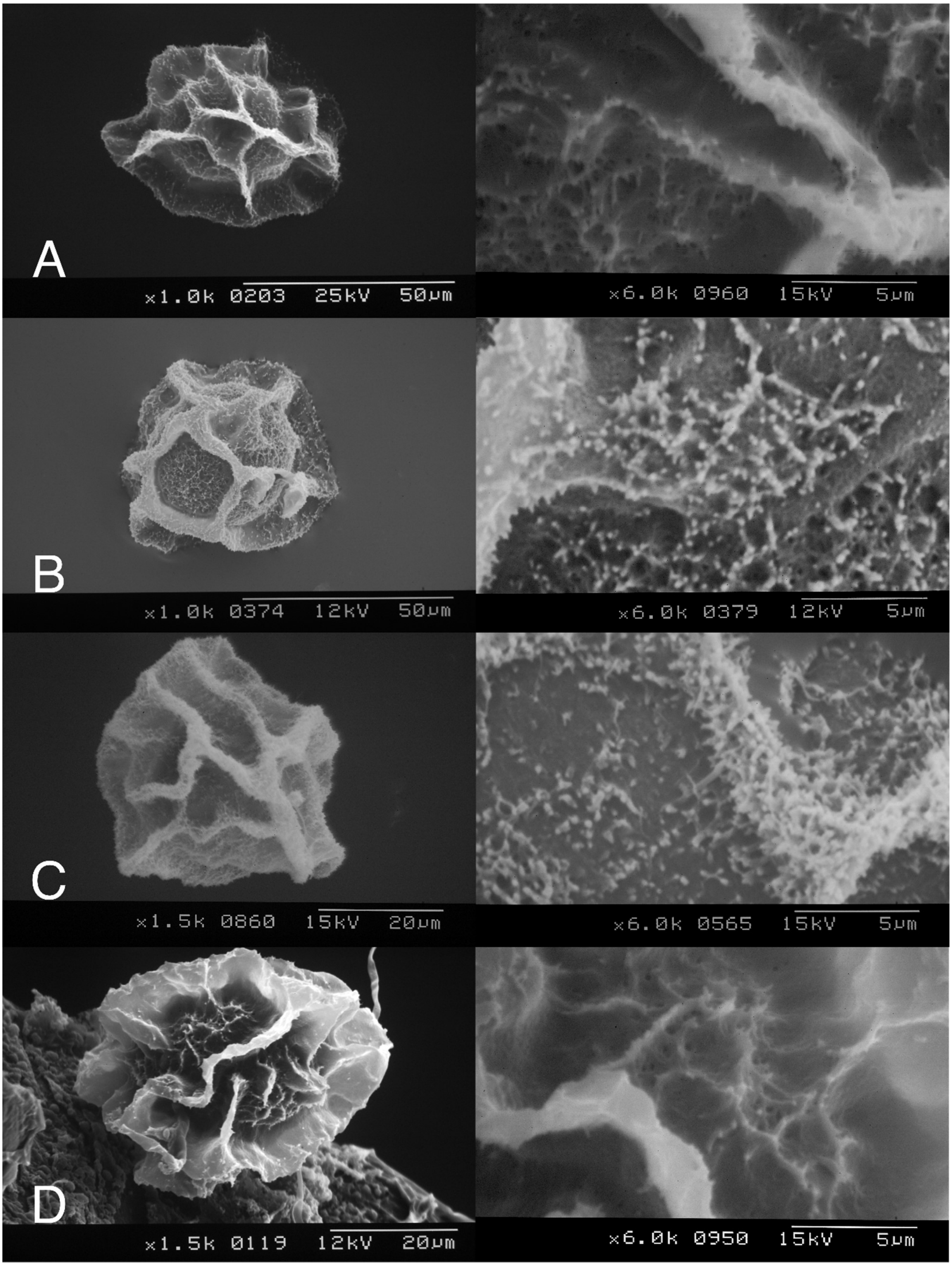

Fig. 1. Scanning electron micrographs of ***Polystichum*** with Retate type in Taiwan. The left images depict a proximal view of spores, while the right images show a distal view of spore surfaces. **A.** ***P. formosanum***; **B.** ***P. hookerianum***; **C.** ***P. obliquum***; **D.** ***P. lepidocaulon***.

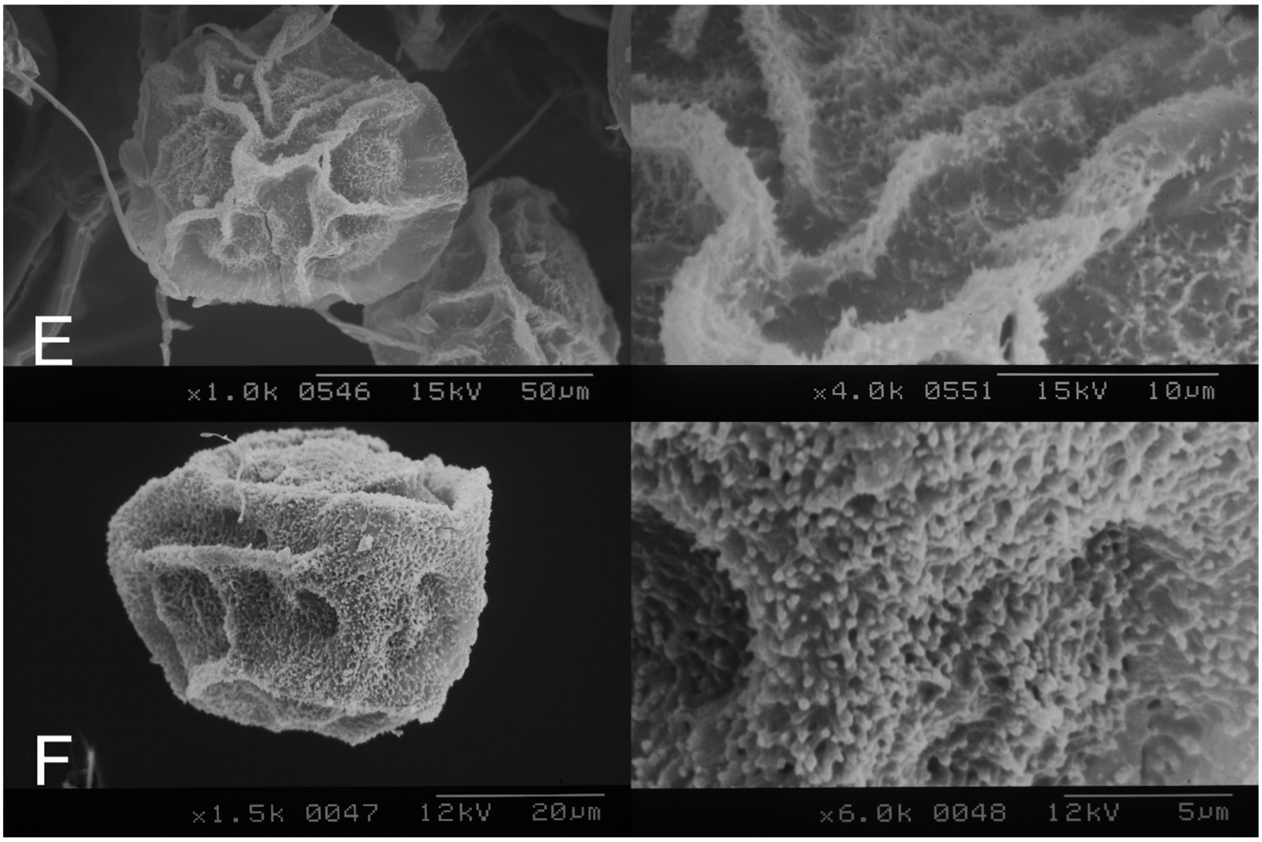

Fig. 1. Continued. **E.** ***P. acutidens***; **F.** ***P. deltodon***.

Perisore muri-bearing folds are thin and irregular. The surface micro-ornamentation is fenestrate. There are numerous microstructures on the perispore surface that are pits.

3. Rugulate type

Perispore sculptured with coarse structures and without micro-ornamentation on the surface. Three species in two sections are in this type.

Subg. ***Haplopolystichum***
Sect. ***Basigemmifera*** (W.M.Chu & Z.R.He) Li Bing Zhang
Polystichum erosum Ching & K.H.Shing (Figs. 2B & C)

Perispore muri-bearing folds are unobvious. There are numerous microstructures on the perispore surface that are grains.

Subg. ***Polystichum*** (Roth) Li Bing Zhang
Sect. ***Duropolystichum*** Fraser-Jenkins
Polystichum *sp*1 (Fig. 2D)
(*cf.* ***Polystichum acanthophyllum*** (Franch.) Christ.)

Perispore muri-bearing folds are mottled and unobvious. There are numerous microstructures on the perispore surface that are grains.

Polystichum *sp*2 (Fig. 2E)
(*cf.* ***Polystichum neolobatum*** Nakai.)

Perispore muri-bearing folds are wide, rounded and dense. There are numerous microstructures on the perispore surface that are grains.

4. Rivulate type

Perispore sculptured with elongated and parallel structures narrower than furrows, and the folds discontinuously. Two species in one section are in this type.

Subg. ***Haplopolystichum***
Sect. ***Cyrtogonellum*** (Ching) Li Bing Zhang
Polystichum fraxinellum (Christ) Diels. (Fig. 2F)

Perispore muri-bearing folds are wide, sparse and irregular. There are numerous microstructures on the perispore surface that are echinae.

Polystichum tenuius (Christ) Li Bing Zhang. (Figs. 2G & H)

Perispore muri-bearing folds are wide, dense and irregular. There are numerous microstructures on the perispore surface that are echinae or grains.

5. Cristate type

Perispore sculptured with elevated and sharp structures. There are two micro-ornamentations in this type. One is fenestrate, and the other is without fenestrate structures on the surface. Thirty species in twelve sections are in this type.

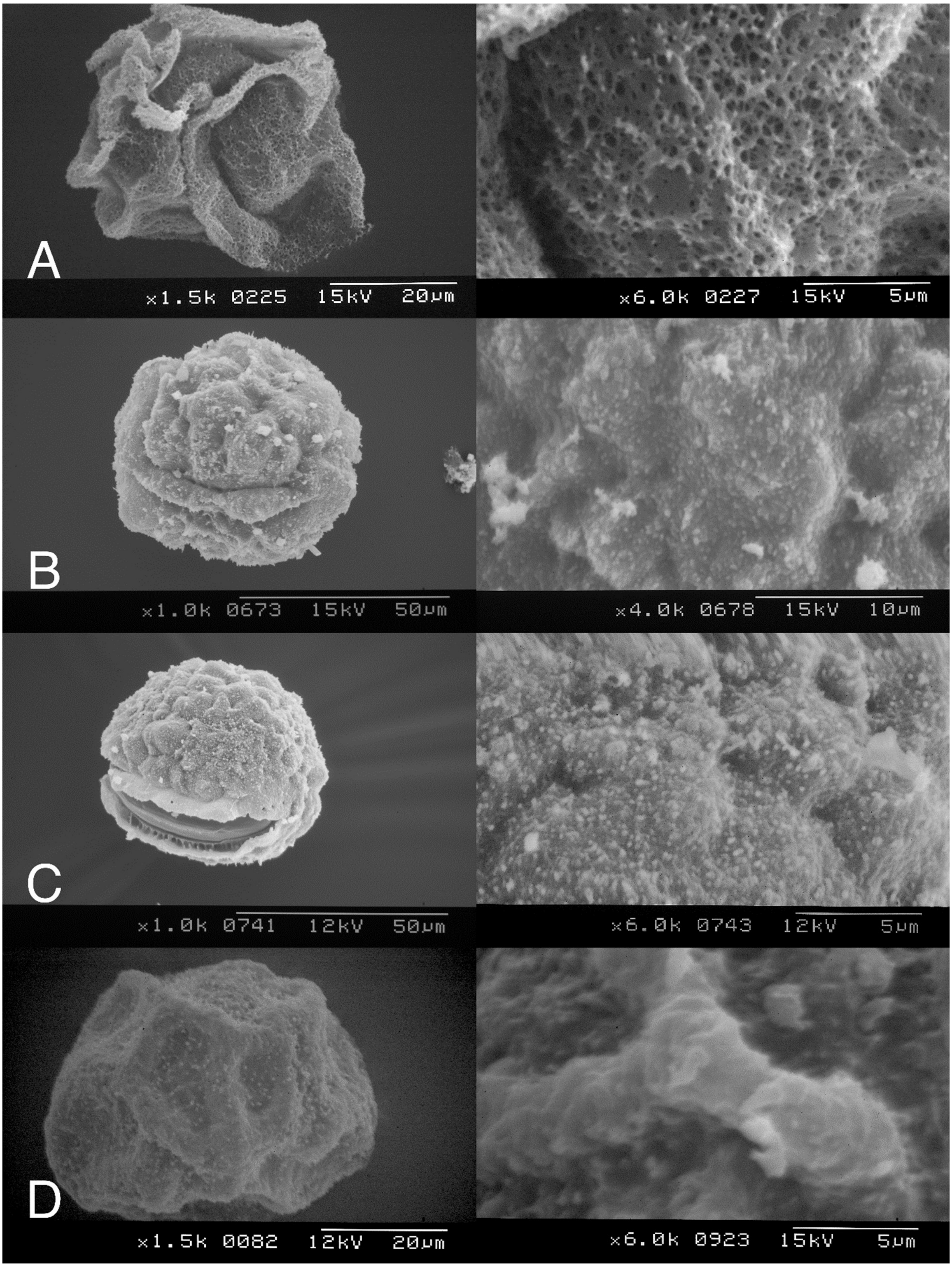

Fig. 2. Scanning electron micrographs of ***Polystichum*** with Rugate, Rugulate or Rivulate type in Taiwan. The left images depict a proximal view of spores, while the right images show a distal view of spore surfaces. **A. *P. hancockii*** (Rugate); **B. *P. erosum*** sample collected from Taiwan (Rugulate); **C. *P. erosum*** sample collected from China (Rugulate); **D. *P.*** *sp*1 (Rugulate).

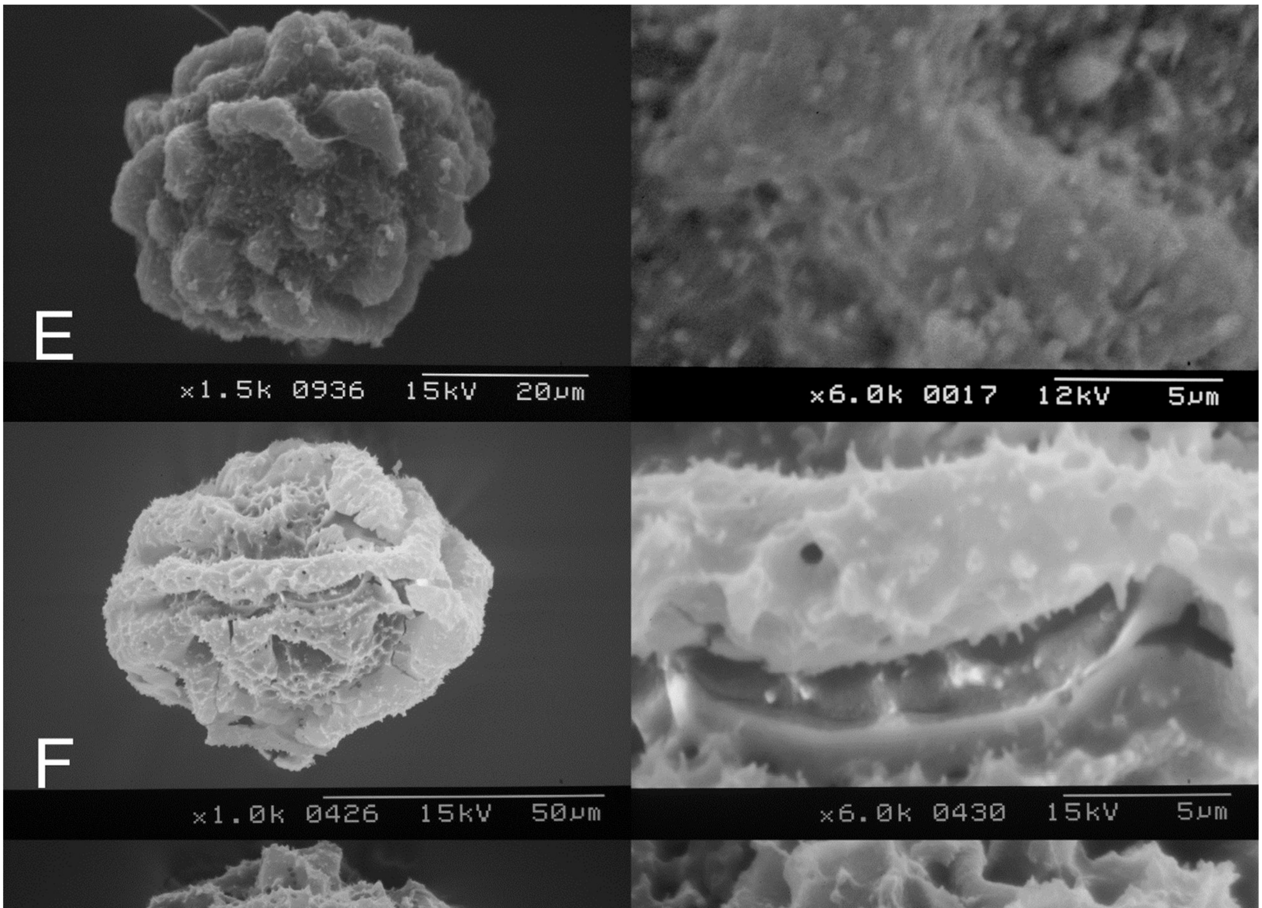

Fig. 2. Continued. **E.** ***P***. *sp*2 (Rugulate); **F. *P. fraxinellum*** (Rivulate); **G. *P. tenuius*** (Rivulate); **H. *P. tenuius*** with another microstructures on the surface in this study (Rivulate).

Form I

The surface micro-ornamentation is fenestrate. Twenty-five species in ten sections are in this form.

Subg. ***Polystichum***

Sect. ***Achroloma*** Tagawa

Polystichum manmeiense (Christ) Nakaike. (Fig. 3A)

Perispore muri-bearing cristae are unobvious on the surface. There are numerous microstructures on the perispore surface that are tiny pits and echinae. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are small pits, and the pits are smaller than *P. nepalense*.

Polystichum nepalense (Sprengel) C. Christense (Fig. 3B)

Perispore muri-bearing cristae are unobvious on the surface. There are numerous microstructures on the perispore surface that are tiny pits and echinae. The outer perispore surfaces are fenestrate, and the inner perispore are numerous microstructures on the surface that are pits, which are larger than *P. manmeiense*.

Sect. ***Duropolystichum*** Fraser-Jenkins

Polystichum acanthophyllum (Franch.) Christ. (Fig. 3C)

Perispore muri-bearing cristae are obvious, irregular and with echinae on the surface. There are numerous microstructures on the perispore surface that are large pits.

Polystichum *sp*3 (Fig. 3D)

(*cf.* ***Polystichum acanthophyllum*** (Franch.) Christ.)

Perispore muri-bearing cristae are obvious, irregular and with echinae on the surface. There are numerous microstructures on the perispore surface that are small pits.

Polystichum neolobatum Nakai. (Fig. 3E)

Perispore muri-bearing cristae are low and irregular on the surface. There are numerous microstructures on the perispore surface that are pits. The outer perispore surfaces are obvious fenestrate structures.

Sect. ***Hypopeltis*** (Michaux) T. Moore

Polystichum parvipinnulum Tagawa (Fig. 3F)

Perispore muri-bearing cristae are unobvious on the surface. There are numerous microstructures on the perispore surface that are large pits.

***Polystichum piceopaleaceu*m** Tagawa (Fig. 3G)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. The outer perispore surfaces are obvious fenestrate structures.

Polystichum prescottianum (Wall. ex Mett.) T. Moore (Fig. 3H)

Perispore muri-bearing cristae are irregular on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are large pits.

Polystichum sinense (Christ) Christ (Fig. 3I)

Perispore muri-bearing cristae are irregular on the surface. There are numerous microstructures on the perispore surface that are pits and echinae.

Sect. ***Lasiopolystichum*** Daigobo

Polystichum lachenense (Hooker)Beddome (Figs. 3J & K)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. There is another microstructure character on the perispore in this taxon. Perispore muri-bearing cristae are unobvious on the surface. There are numerous microstructures on the perispore surface that are large pits and high echinae.

Polystichum taizhongense H. S. Kung (Fig. 3L.)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are large pits.

Polystichum *sp*4 (Fig. 3M)

(*cf.* ***Polystichum lichiangense*** (C.H.Wright) Ching ex H.S.Kung)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and high echinae.

Sect. ***Sorolepidium*** (Christ) Tagawa

Polystichum glaciale Christ. (Fig. 3N)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are large pits and high echinae. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are pits.

Sect. ***Micropolystichum*** Tagawa

Polystichum thomsonii (J.D.Hooker) Beddome (Fig. 3O)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and short echinae. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are small pits.

Sect. ***Stenopolystichum*** Daigobo

Polystichum atkinsonii Beddome (Fig. 3P)

Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are small pits.

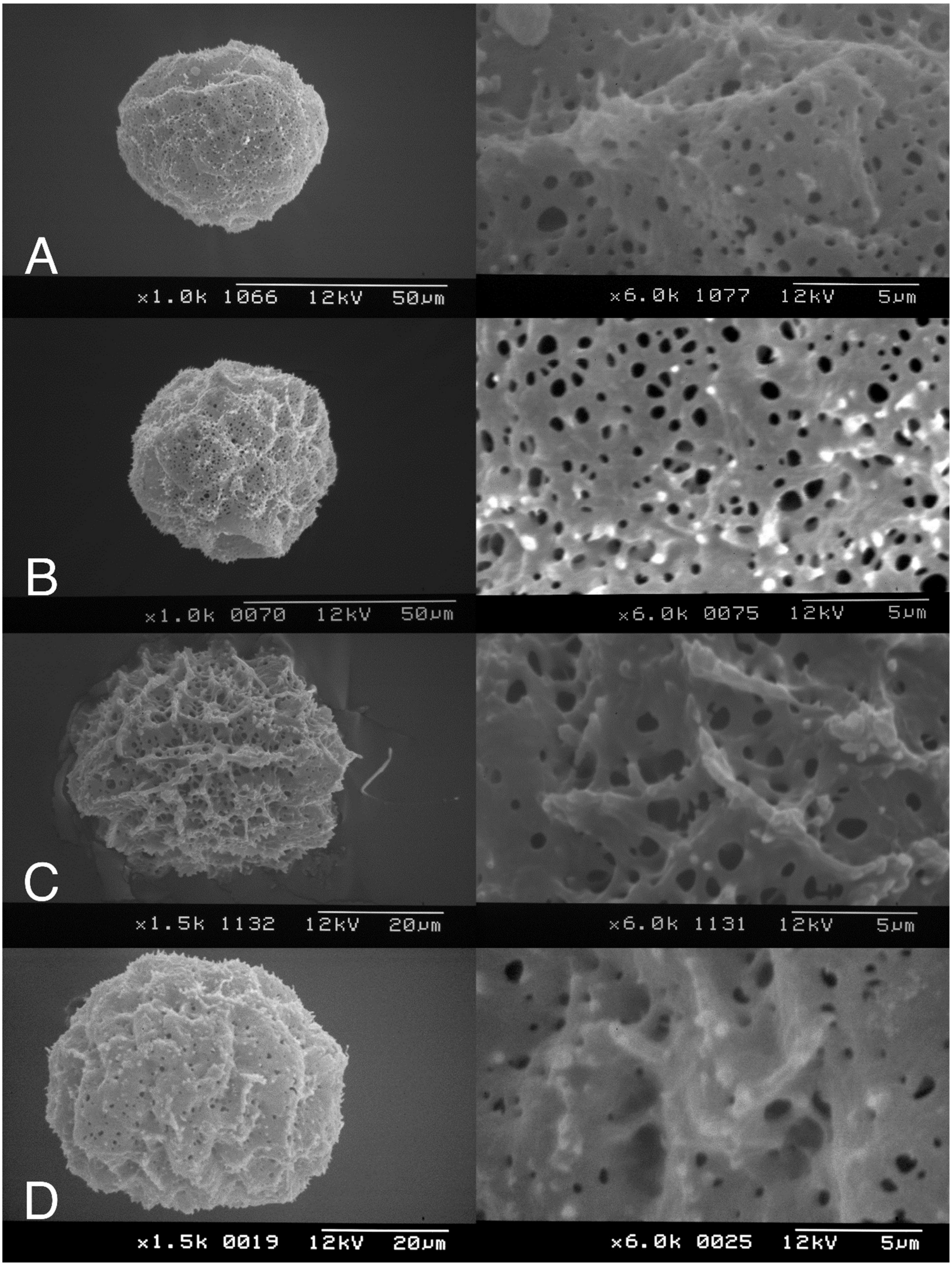

Fig. 3. Scanning electron micrographs of ***Polystichum*** with Cristate type in Taiwan. The left images depict a proximal view of spores, while the right images show a distal view of spore surfaces. **A.** ***P. manmeiense***; **B.** ***P. nepalense***; **C.** ***P. acanthophyllum***; **D.** ***P.*** *sp*3.

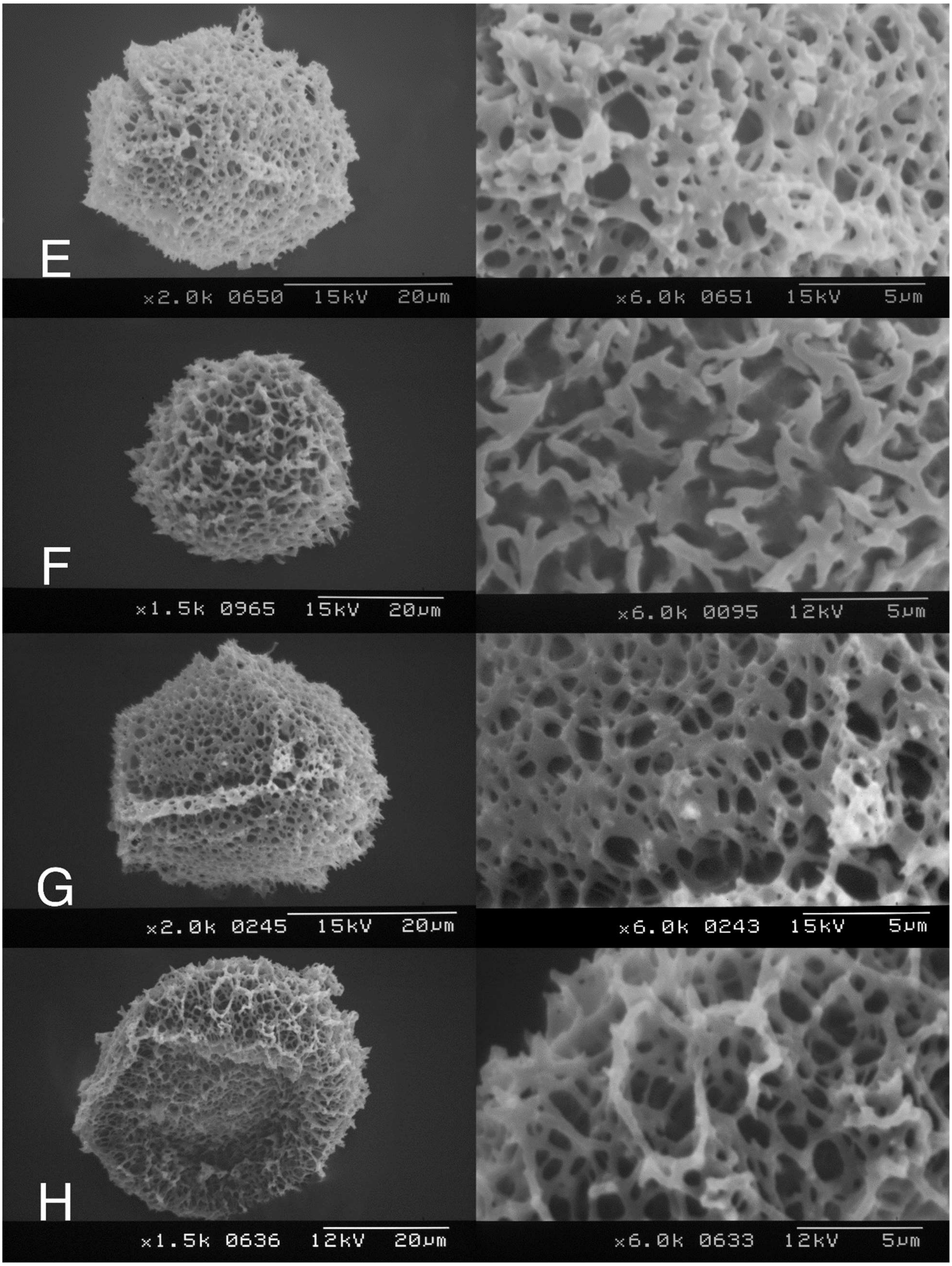

Fig. 3. Continued. **E.** ***P. neolobatum***; **F.** ***P. parvipinnulum***; **G.** ***P. piceopaleaceum***; **H.** ***P. prescottianum***.

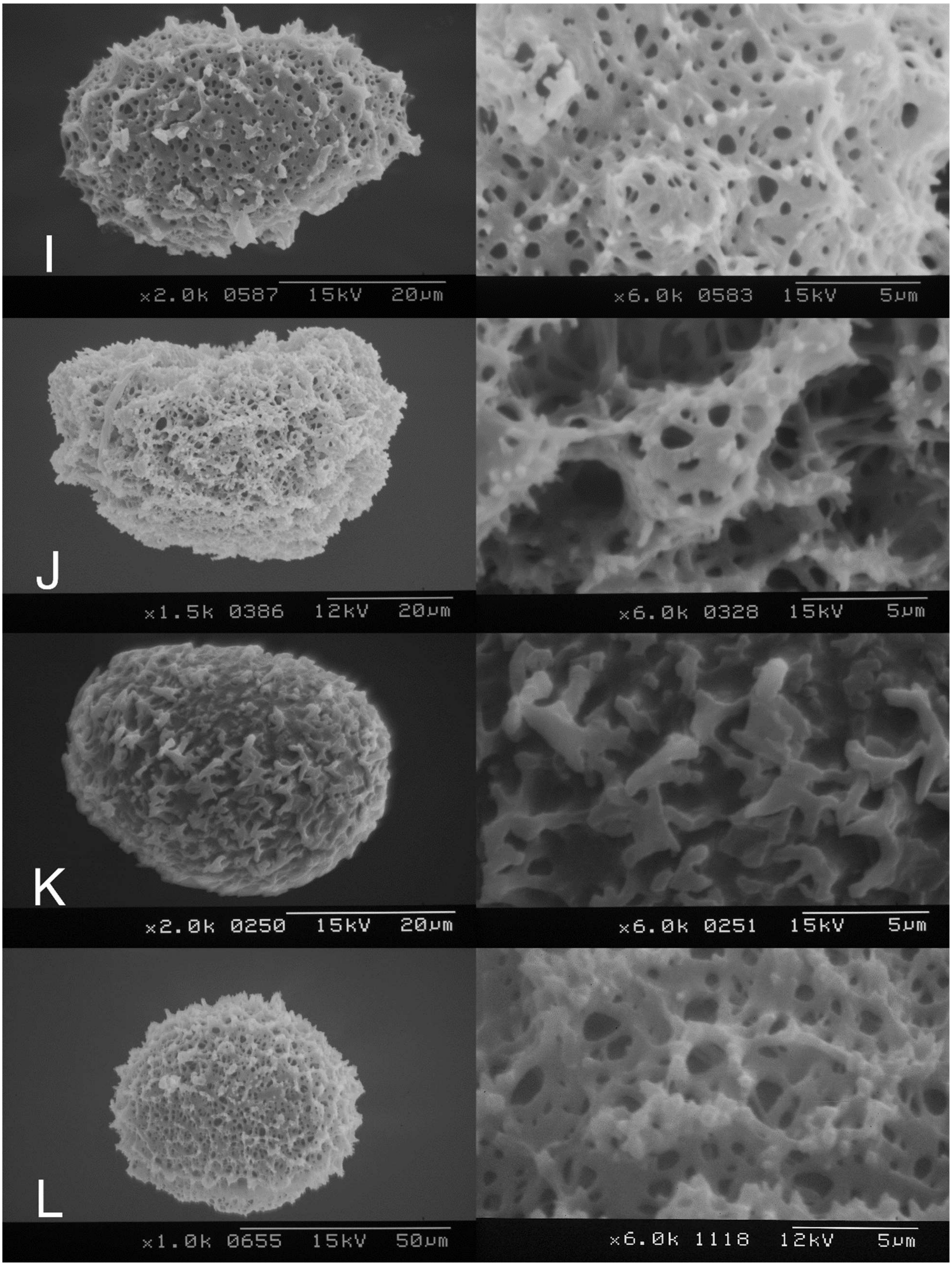

Fig. 3. Continued. **I.** ***P. sinense***; **J.** ***P. lachenense***; **K.** ***P. lachenense*** with another microstructures on the surface in this study; **L.** ***P. taizhongense***.

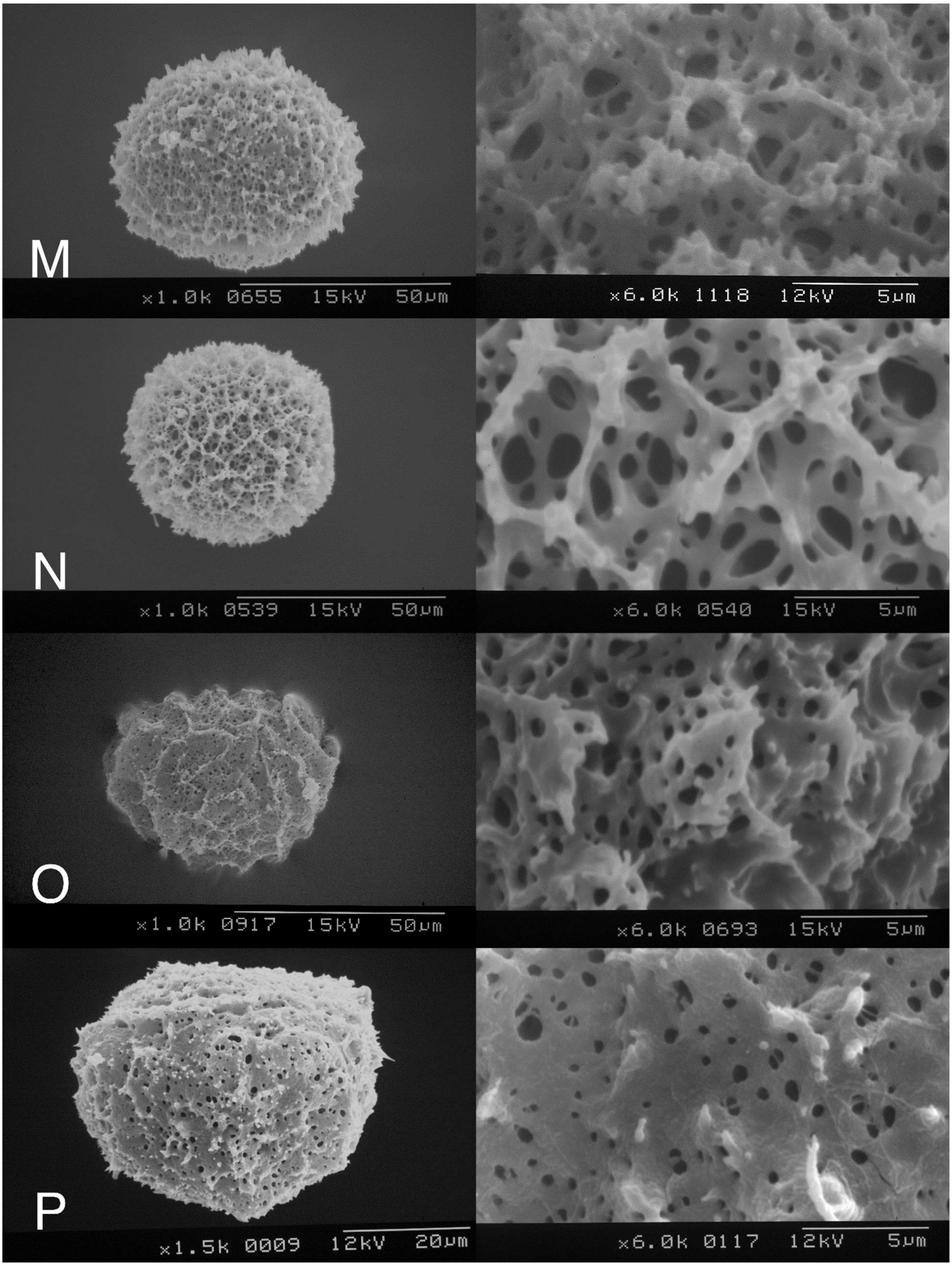

Fig. 3. Continued. **M.** ***P.*** *sp*4; **N.** ***P. glaciale***; **O.** ***P. thomsonii***; **P.** ***P. atkinsonii***.

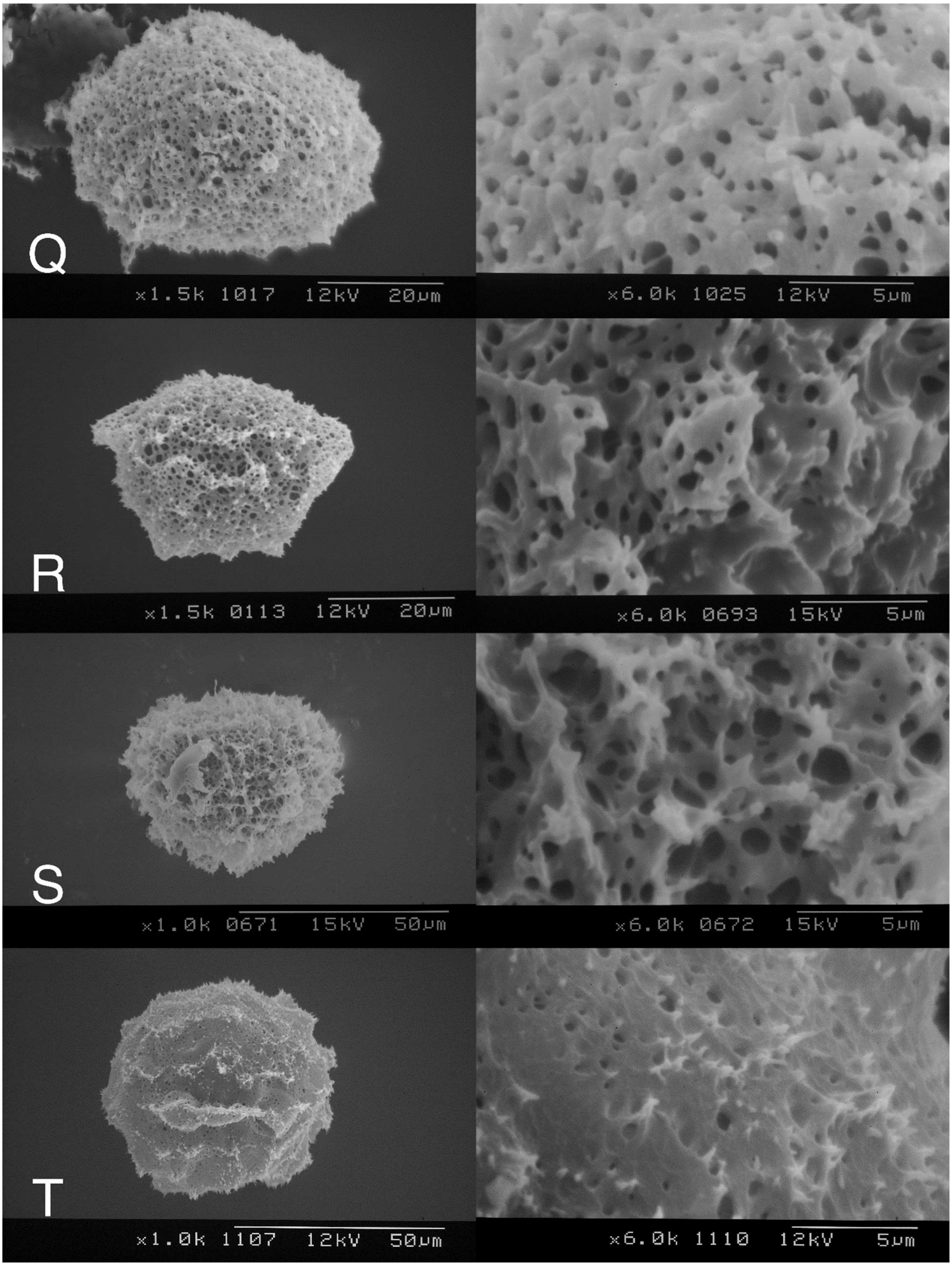

Fig. 3. Continued. **Q.** ***P. pseudostenophyllum***; **R.** ***P. stenophyllum***; **S.** ***P. attenuatum***; **T.** ***P. biaristatum***.

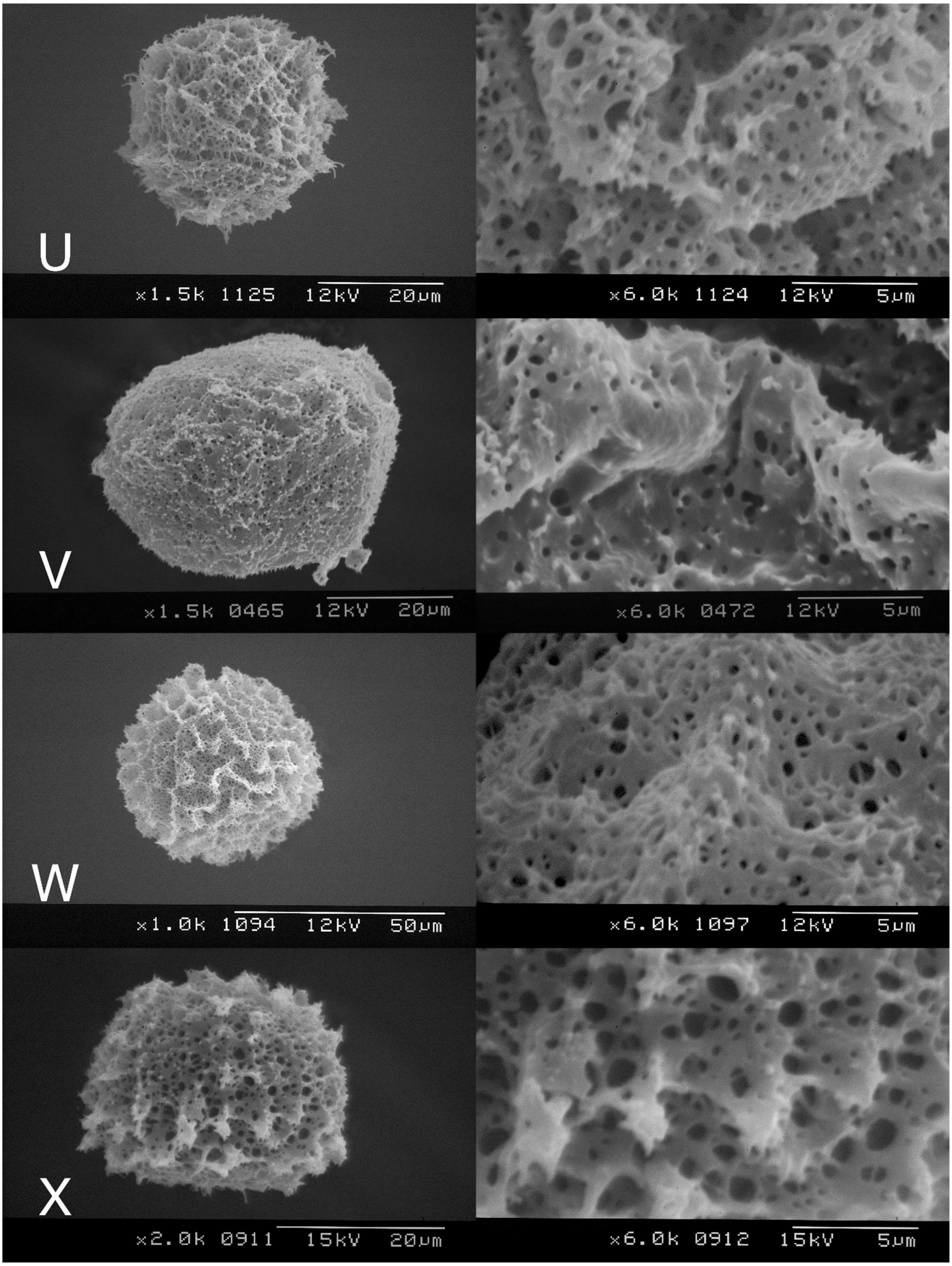

Fig. 3. Continued. **U.** ***P. chunii***; **V.** ***P. mucronifolium***; **W.** ***P. prionolepis***; **X.** ***P. scariosum***.

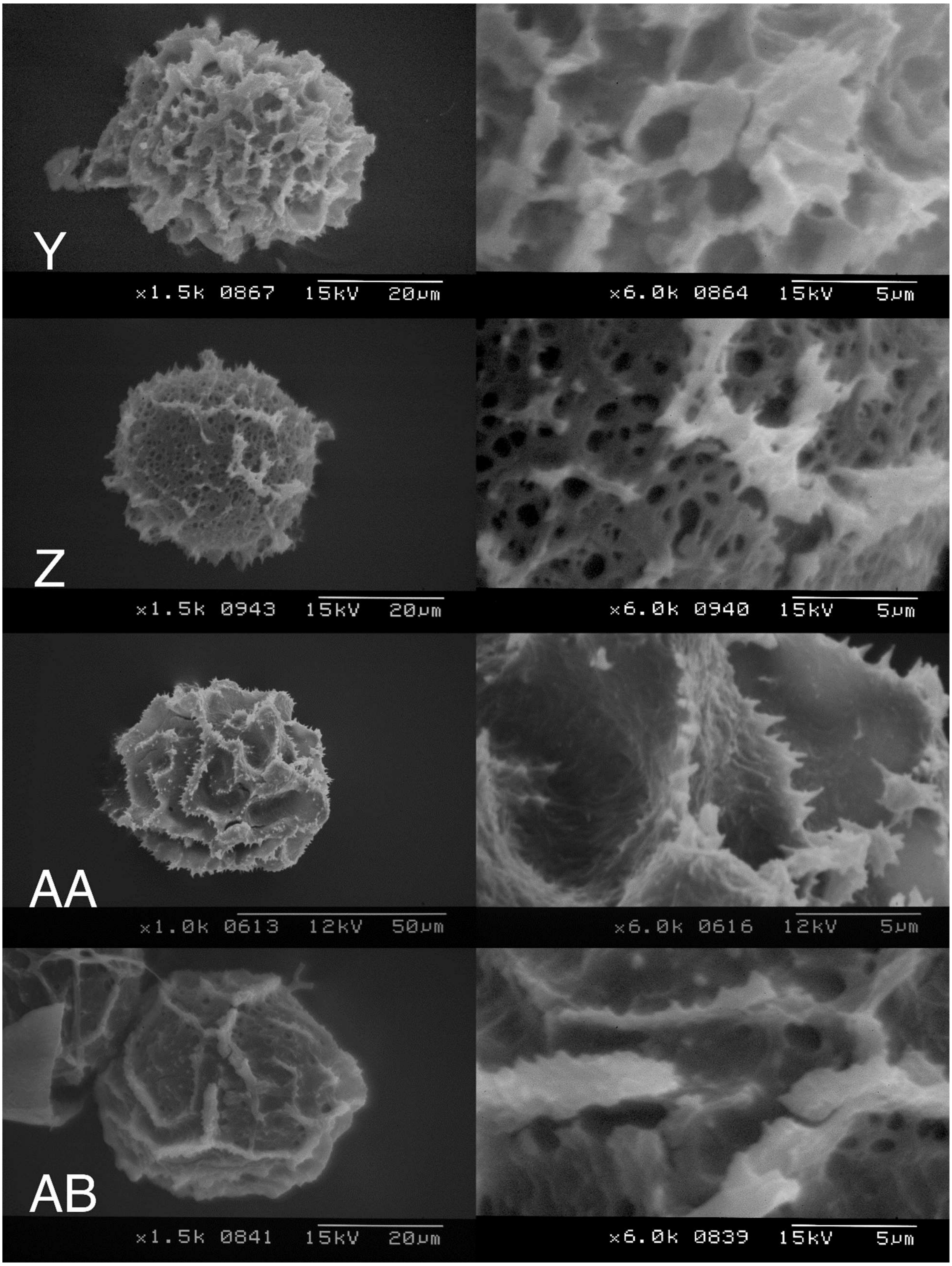

Fig. 3. Continued. **Y.** ***P. grandifrons***; **Z.** ***P. hecatopterum***; **AA.** ***P. capillipes***; **AB.** ***P. herbaceum***.

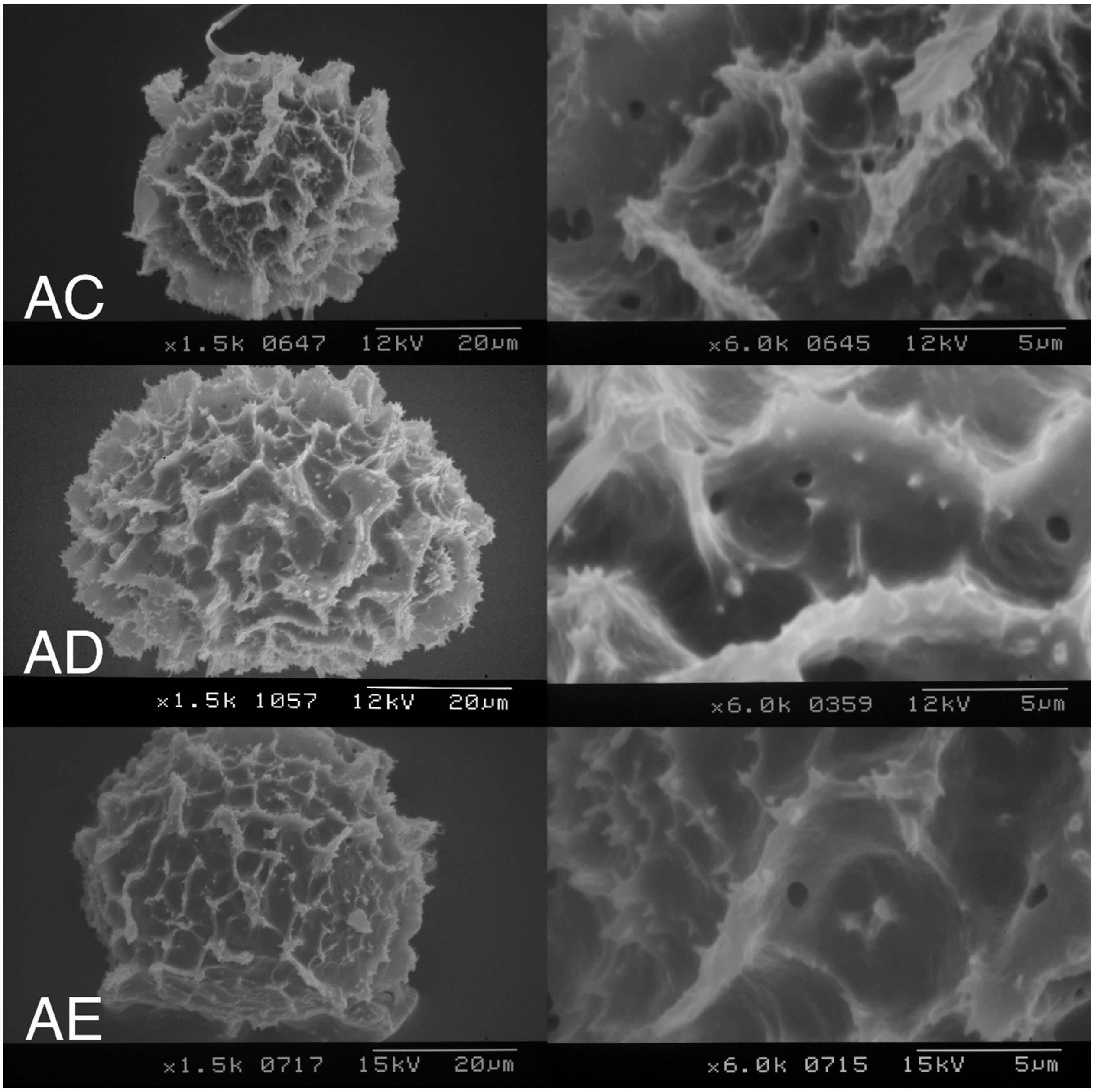

Fig. 3. Continued. **AC.** ***P. mayebarae***; **AD.** ***P. tsus-simemse***; **AE.** ***P. xiphophyllum***.

Polystichum pseudostenophyllum Tagawa (Fig. 3Q)
Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. The pits are fewer, and the cristae are narrower than *P. stenophyllum*.

Polystichum stenophyllum (Franchet) Christ (Fig. 3R)
Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. There are morepits, and the cristae are sparser than *P. pseudostenophyllum*.

Sect. ***Macropolystichum*** Daigobo

Polystichum attenuatum Tagawa & K. Iwatsuki. (Fig. 3S)
Perispore muri-bearing cristae are high, sharp, and with numerous pits and echinae on the surface. The echinae on the cristae are higher and more than *P. chunii*. There are numerous microstructures on the perispore surface that are pits.

Polystichum biaristatum (Blume) T. Moore (Fig. 3T)
Perispore muri-bearing cristae are obvious on the surface. There are numerous microstructures on the perispore surface that are pits and echinae.

Polystichum chunii Ching (Fig. 3U)
Perispore muri-bearing cristae are obvious on the

surface. The echinae on the cristae are narrower and lower than *P. attenuatum*. There are numerous microstructures on the perispore surface that are pits. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are large pits.

Polystichum mucronifolium (Blume) C. Presl. (Fig. 3V)

Perispore muri-bearing cristae are unobvious, with numerous small pits, echinae, and grains on the surface. There are numerous microstructures on the perispore surface that are pits.

Polystichum prionolepis Hayata. (Fig. 3W)

Perispore muri-bearing cristae are high, with numerous pits and echinae on the surface. There are numerous microstructures on the perispore surface that are pits.

Polystichum scariosum (Roxburgh) C.V Morton (Fig. 3X)

Perispore muri-bearing cristae are high, sharp, spare, and with numerous pits and echinae on the surface. There are numerous microstructures on the perispore surface that are pits.

Sect. ***Neopolystichum*** Ching ex Li Bing Zhang & H.S.Kung

Polystichum grandifrons C. Christensen (Fig. 3Y)

Perispore muri-bearing cristae are irregular on the surface. The echinae on the cristae are unobvious. The surface micro-ornamentation transitions from rugate to fenestrate.

Sect. ***Hecatoptera*** (L. L. Xiang) Li Bing Zhang

Polystichum hecatopterum Diels (Fig. 3Z)

Perispore muri-bearing cristae are sparse and high on the surface. There are numerous microstructures on the perispore surface that are pits and echinae. The outer perispore surfaces are fenestrate, and the inner perispore has numerous microstructures on the surface that are pits.

Form II

The surface micro-ornamentation without fenestrate structures. Five species in two sections are in this form.

Subg. ***Haplopolystichum***

Sect. ***Basigemmifera*** (W.M.Chu & Z.R.He) Li Bing Zhang

Polystichum capillipes (Baker) Diels (Fig. 3AA.)

Perispore muri-bearing cristae are irregular and fimbriate on the surface. There are numerous microstructures on the perispore surface that are echinae.

Subg. ***Polystichum***

Sect. ***Xiphopolystichum*** Daigobo

Polystichum herbaceum Ching & Z. Y. Liu. (Fig. 3AB)

Perispore muri-bearing cristae are irregular, scattered, and low on the surface. The microstructures on the cristae are short and less noticeable. There are numerous microstructures on the perispore surface that are echinae and grains.

Polystichum mayebarae Tagawa (Fig. 3AC)

Perispore muri-bearing cristae are irregular on the surface. There are numerous microstructures on the perispore surface that are echinae and grains.

Polystichum tsus-simense (Hook.) J. Smith (Fig. 3AD)

Perispore muri-bearing cristae are irregular and dense on the surface. The echinae on the cristae are short and less noticeable. There are numerous microstructures on the perispore surface that are echinae and grains.

Polystichum xiphophyllum (Baker) Diels (Fig. 3AE)

Perispore muri-bearing cristae are irregular. The echinae on the cristae are high. There are numerous microstructures on the perispore surface that are echinae and grains.

Key to the spores of *Polystichum* in Taiwan

1. Muri anastomosing …………………………………………………… 2
1. Muri non-anastomosing …………………………………………… 7
2. Surface micro-ornamentation rugate ………………………….…… 3
2. Surface micro-ornamentation not rugate ……………..……….…… 4
3. Microstructures on the surface echinate ………….… ***P. formosanum***
3. Microstructures on the surface granular …………… ***P. hookerianum***
4. Microstructures on the surface echinate ……………….……….…… 5
4. Microstructures on the surface granular ……….…..…… ***P. deldoton***
5. Microstructures on the surface with small pits ……..…… ***P. acutidens***
5. Microstructures on the surface without small pits ……….…..…… 6
6. Muri on the perispore wing-like ………..……..…… ***P. lepidocaulon***
6. Muri on the perispore irregular and broad ……..…… ***P. obliquum***
7. Muri wide and rounded ………………………………………..…… 8
7. Muri narrow and sharp …………………………..…………..…… 12
8. Muri parallel ……………………..……………………………..……9
8. Muri not parallel …………………..………………………………10
9. Sparse muri with echinae on the surface ………..…… ***P. fraxinellum***
9. Dense muri with grains or echinae on the surface ………… ***P. tenuius***
10. Microstructures on the surface with pit ………..……… ***P. hancockii***
10. Microstructures on the surface without pits ……………..…..…… 11
11. Muri obvious and high ………………………..……………… ***P. sp2***
11. Muri unobvious and low ……………………..…….……..…… 12
12. The granular microstructures on the surface dense and small ………. ……………..………...…………………………..…… ***P. erosum***
12. The granular microstructures on the surface sparse and large . ***P. sp1***
13. Microstructures on the surface with pits ………..……………..…… 14
13. Microstructures on the surface without pits ……….………..……… 37
14. Surface fenestrate micro-ornamentation unobvious ……………... 15
14. Surface fenestrate micro-ornamentation obvious ………….…… 21
15. Muri high …………………..………………………………..…… 16
15. Muri low ……………………..…………..…… ***P. mucronifolium***
16. Perispore sculpture exhibits dense cristae ………………………… 17
16. Perispore sculpture exhibits sparse cristae ………..………..…… 20
17. Microstructures on the surface with numerous pits ……..……… 18
17. Microstructures on the surface with few pits ……..…… ***P. atkinsonii***
18. Slope of the cristae high and sharp, with echinae and grains …… 19
18. Slope of the cristae low and blunt, only with echinae … ***P. nepalense***
19. Microstructures on the surface with small pits and low echinae …….. …………………………………..……………..…… ***P. thomsonii***
19. Microstructures on the surface with large pits and high echinae ……. ……………………………………………..…… ***P. acanthophyllum***
20. Microstructures on the surface with numerous and high echinae, mainly distributed on the cristae ……………..…… ***P. biaristatum***
20. Microstructures on the surface with few and low echinae and distributed everywhere ………………………………..…...…… 21
21. Microstructures on the surface with numerous pits ... ***P. manmeiense***

21. Microstructures on the surface with few pits ………………… ***P. sp3***
22. Outer perispore surfaces echinate and it formed to reticular structure .. 23
22. Outer perispore surfaces echinate and it not formed to reticular structure …………………………………………………………… 25
23. Fenestrate micro-ornamentation on the surface unobvious ……………………………………………………………………… ***P. grandifrons***
23. Fenestrate micro-ornamentation on the surface obvious ………. 24
24. Outer reticular structure sparse, inner dense …… ***P. prescotttianum***
24. Outer reticular structure dense, inner sparse ……………. ***P. glaciale***
25. Perispore sculpture discontinuous, irregulate and sparse cristae ... 26
25. Perispore sculpture continuous, regulate and dense cristae …….. 27
26. The cristae wide and round ………………………… ***P. taizhongense***
26. The cristae sharp …………………………………… ***P. prionolepis***
27. Fenestrate micro-ornamentation on the surface dense ………… 28
27. Fenestrate micro-ornamentation on the surface sparse ………… 34
28. Fenestrate micro-ornamentation on the surface consist of sparse and high cristae ……………………………………………………… 29
28. Fenestrate micro-ornamentation on the surface consist of dense and low cristae ………………………………………………………… 31
29. The cristae blunt ……………………………………… ***P. sinense***
29. The cristae sharp ……………………………………………… 30
30. Microstructures on the surface with few and small pits ………………………………………………………… ***P. hecatopterum***
30. Microstructures on the surface with numerous and large pits ………………………………………………………… ***P. scariosum***
31. The connection of fenestrate structure thin ………………………… 32
31. The connection of fenestrate structure thick ……………………… 33
32. The pore size of the fenestrate structure large ……… ***P. lachenense***
32. The pore size of the fenestrate structure small ……………… ***P. sp4***
33. The pores numerous, and the muri sparse ……… ***P. stenophyllum***
33. The pores few, and the muri dense ……… ***P. pseudostenophyllum***
34. The connection of the fenestrate structure thin …………………… 35
34. The connection of the fenestrate structure thick …………………… 37
35. Fenestrate structure complicated (multilayers) ………………… 36
35. Fenestrate structure simple ……………………… ***P. piceopaleacum***
36. Perispore sculpture cristae with numerous and high echinae ……………………………………………………………… ***P. attenuatum***
36. Perispore sculpture cristae with few and low echinae …… ***P. chunii***
37. Fenestrate micro-ornamentation on the surface consist of high echinae, and the pores large ……………………… ***P. parvipinnulum***
37. Fenestrate micro-ornamentation on the surface consist of cristae, and the pores medium ………………………………… ***P. neolobatum***
38. The cristae sharp and narrow ……………………………………… 39
38. The cristae broad ……………………………………………………… 41
39. The cristae sparse …………………………………………………… 40
39. The cristae dense ………………………………… ***P. tsus-simense***
40. The cristae with numerous granulate microstructures . ***P. mayebarae***
40. The cristae with numerous spinely microstructures …. ***P. xiphophyllum***
41. The cristae high and fimbriate ……………………… ***P. capillipes***
41. The cristae low and not fimbriate ……………………… ***P. herbaceum***

DISCUSSION

The infrageneric classification, like subgenera or sections, is based on the morphological phylogenetic hypothesis. The first natural subdividing of the genus *Polystichum* was by Tagawa (1940), who placed the species of Korea, Japan, and Taiwan into eight sections. Daigobo used microscales to divide the species of Japan, Ryukyu, and Taiwan (Daigobo, 1972). Fraser-Jerkins placed the 45 species of the Indian Subcontinent into seven sections (Fraser-Jerkins 1997). Kung *et al.* (2001) put the 168 species of *Polystichum* in China into 13 sections. The species of Asian *Polystichum* were recently processed into two subgenera and 23 sections (Zhang and Barrington, 2013), with 14 strongly supported as monophyletic by the molecular study (Le Péchon *et al.*, 2016). Therefore, more data, including palynological data, is still needed to construct a better taxonomic framework. According to the results of Zhang and Barrington (2013), the species of Taiwan accounted for 15 sections, and their spore information will be beneficial in the future infrageneric classification constructing matter (Table 3).

There are two subgenera in *Polystichum*: *Haplopolystichum* and *Polystichum* (Zhang and Barrington, 2013), and palynological data in this study support this distinction except for a doubtful species, *P. capillipes*. The subg. *Haplopolystichum* spores are in retate, rugate, rivulate, or rugulate types, and there is no pit or pits unobvious on the micro-ornamentation. However, the subg. *Polystichum* spores are in cristate types, with many pits on the surface. The molecular data supports two subgenera hypotheses (Le Péchon *et al.*, 2016).

The taxonomic position of *P. capillipes* needs further investigation. This species was classified into *P.* sect. *Micropolystichum* by Daigobo (1972), Fraser-Jenkins (1997) and Kung *et al.* (2001), and later sunk into a synonym of *P. thomsonii* (Ebihara, 2017; Fraser-Jenkins, 2018). Zhang and Barrington (2013) placed *P. capillipes* in *P.* sect. *Basigemmifera* but retained *P. thomsonii* in *P.* sect. *Micropolystichum*. The section *Basigemmifera* is in Subg. *Haplopolystichum*, but Section *Micropolystichum* is in Subg. *Polystichum* (Zhang and Barrington, 2013). The relocation of *P. capillipes* into *P.* sect. *Basigemmifera* was hinted to be appropriate by later reports (Zhang and He, 2011; Le Péchon *et al.*, 2016), but no support data was shown. The perispore of *P. capillipes* is the cristate type, the same as other Subg. *Polystichum* species. However, no perforation is similar to Subg. *Haplopolystichum* species. The previous Chinese SEM study (Wang and Dai, 2010) on *P. capillipes* showed the same image. A further notice from our spore observation is that *P. capillipes* and *P. thomsonii* differ in echinae height and perforation size and should be two different taxa. A detailed analysis, including molecular data, is needed to resolve this taxon belonging.

The subgenus *Haplopolystichum* contains nine sections, with six (*Adenolepia*, *Basigemmifera*, *Crucifilix*, *Cyrtogonellum*, *Cyrtomiopdid*, and *Haplopolystichum*) studied herein. Four perispore types were found in this subgenus: rugulate in *Basigemmifera* (*P. capillipes* excluded), rivulate in *Cyrtogonellum*, rugate in *Crucifilix*, and retate in the remaining three sections. Recent reports had discovery of several new species of the genus *Polystichum* in China (Han *et al*., 2016, 2018), include SEM images of spores of these new species. According to the studies of Han *et al*. (2016, 2018), these species belong to the taxonomic position within the subgenus *Haplopolystichum* and inhabit limestone caves characterized by humid and shady conditions. The CCPC clade of the genus *Polystichum* encompasses *Polystichum*

Table. 3. List of spore ornamentation type of ***Polystichum*** in Taiwan. Form I represent with fenestrate structures, Form II represent without fenestrate structures.

Subgenus	Section	Taxon	Spore ornamentation type	Micro-ornamentation
Haplopolystichum	*Adenolepia*	*P. formosanum*	Retate	Form II
		P. hookerianum	Retate	Form II
		P. obliquum	Retate	Form II
	Basigemmifera	*P. capillipes*	Cristate	Form II
		P. erosum	Rugulate	Form II
	Crucifilix	*P. hancockii*	Rugate	Form I
	Cyrtogonellum	*P. fraxinellum*	Rivulate	Form II
		P. tenuius	Rivulate	Form II
	Cyrtomiopsis	*P. lepidocaulon*	Retate	Form II
	Haplopolystichum	*P. acutidens*	Retate	Form II
		P. deltodon	Retate	Form II
Polystichum	*Achroloma*	*P. manmeiense*	Cristate	Form I
		P. nepalense	Cristate	Form I
	Duropolystichum	*P. acanthophyllum*	Cristate	Form I
		P. neolobatum	Cristate	Form I
		P. sp1	Rugulate	Form II
		P. sp2	Rugulate	Form II
		P. sp3	Cristate	Form I
	Hecatoptera	*P. hecatopterum*	Cristate	Form I
	Hypopeltis	*P. parvipinnulum*	Cristate	Form I
		P. piceopaleaceum	Cristate	Form I
		P. prescottianum	Cristate	Form I
		P. sinense	Cristate	Form I
	Lasiopolystichum	*P. lachenense*	Cristate	Form I
		P. taizhongense	Cristate	Form I
		P. sp4	Cristate	Form I
	Macropolystichum	*P. attenuatum*	Cristate	Form I
		P. biaristatum	Cristate	Form I
		P. chunii	Cristate	Form I
		P. mucronifolium	Cristate	Form I
		P. prionolepis	Cristate	Form I
		P. scariosum	Cristate	Form I
	Micropolystichum	*P. thomsonii*	Cristate	Form I
	Neopolystichum	*P. grandifrons*	Cristate	Form I
	Sorolepidium	*P. glaciale*	Cristate	Form I
	Stenopolystichum	*P. atkinsonii*	Cristate	Form I
		P. pseudostenophyllum	Cristate	Form I
		P. stenophyllum	Cristate	Form I
	Xiphopolystichum	*P. herbaceum*	Cristate	Form II
		P. mayebarae	Cristate	Form II
		P. tsus-simense	Cristate	Form II
		P. xiphophyllum	Cristate	Form II

lepidocaulon (formerly *Cyrtomidictyum lepidocaulon*), two species in Sect. *Cyrtogonellum*, three species in Sect. *Adenolepia*, and a small number of other *Polystichum* species. These species are typically found in limestone environments (Li *et al*., 2008). Zhang and Barrington (2013) have reclassified most species of the CCPC clade under the subgenus *Haplopolystichum*. Similarly, species within the subgenus in Taiwan also exhibit a preference for such environments. The spore ornamentation types identified in these new species in China include retate, rugate, regulate, rivulate, and cristate, according to our classification, and the surface of the perispore characterized by numerous spinules or granules, is consistent with our study about this subgenus. However, one species (*P. pingbianense*) in their study exhibits cristate, a type less observed in this subgenus, also found in the sample of Taiwan (*P. capillipes*). Based on the above, spore ornamentation types found in the subgenus *Haplopolystichum* are present, and species within this subgenus tend to prefer limestone environments. It may be asserted that these species adapted to limestone environments often exhibit similar spore ornamentation types. However, because of the small sample, using these perispore features for section diagnosis is premature.

Polystichum fraxinellum and *P. tenuius* were in a separate genus *Cyrtogonellum* (Kung *et al*., 2001). However, Zhang and Barrington (2013) downgraded *Cyrtogonellum* into a section under *Polystichum*, indicating it might not be monophyletic. This study showed that the perispore of the two species is rivulate and distinct from other *Polystichum* species. However, the perispore morphology of these species is very similar, and placing them in the same genus is also reasonable. Furthermore, *P. tenuius* from Taiwan has two microstructure types observed. This feature is due to ontogenetic variation, or evolutionary history may require further research to clarify. The same species with two microstructure types is also recorded from mainland Chinese material (Wang and Dai, 2010).

Six species are in the retate type, but their taxonomic position varies from author to author. *Polystichum hookerianum* and *P. lepidocaulon* have been placed into the genera *Cyrtomidictyum* and *Cyrtomium* or into *P.* sect. *Adenolepia* and *P.* sect. *Cyrtomiopsis* (Kung *et al.*, 2001; Zhang and Barrington, 2013). According to the spore observations in this and previous studies (Mitui, 1973; Wang and Dai, 2010), *P. hookerianum* and *P. lepidocaulon* might be close to *P.* sect. *Adenolepia* or *P.* sect. *Haplopolystichum*. More spore sampling, even a quantitative analysis, is needed to see whether this preliminary observation judgment could hold.

The macro-ornamentation of *P. erosum* spore is regulate type, micro-ornamentation with bulliform, but fenestrate and ridge in micro-ornamentation are wanted. The patterns of spore ornamentation are matched with Wang and Dai (2010). Herein, we also observed the specimens from mainland China, and the consequences of spore ornamentation are the same. Based on the scheme of subgeneric classification *Polystichum* proposed by Zhang and Barrington (2013), *P. erosum* is belonged to Section *Basigemmifera. P. sp*2 has the same pattern of spore ornamentation. The superficial morphology of *P. sp*2 resembles P. neolobatum, but the fenestrate and ridge are absent. The first author suggested the maturity of the spore wall might cause it, but with multiple sampling, the results are the same. The superficial morphology of *P. sp1* resembles *P. acanthophyllum*, and the macro-ornamentation is regulate type, too. The superficial morphology of *P. sp*1 and *sp*2 resemble Section *Xiphopolystichum* Zhang and Barrington (2013), but the section belongs to subgenus *Polystichum,* which has synapomorphy in regulate type of macro-ornamentation and fenestrate in micro-ornamentation. In the past significant studies on the spore ornamentation of *Polystichum*, no other species has been documented in this type of sculpturing (Xiang, 1992, Zhang and Kung, 1994). The various micro-ornamentations might be caused by ancestor hybridization and the consequences of hybrids due to apogamous species. The evolution of these taxa needs more detailed studies on polyploidization and phylogeny.

This study surveyed nine sections within the subgenus *Polystichum*. The cristate-fenestrate perispore is in all nine sections. However, Section *Xiphopolystichum* has four species (*P. tsus-simense*, *P. herbaceum*, *P. xiphophyllum,* and *P. mayebarae*) with cristate perispore. Besides, the molecular study showed these four species formed a distinct clade within *P.* sect. *Xiphopolystichum* (Le Péchon *et al*., 2016). The remaining studied species in the Section *Xiphopolystichum* are *P. acanthophyllum* and *P. neolobatum*. Their perispores are cristate-fenestrate type. These two species were in *P.* sect. *Scleropolystichum* (Daigobo, 1972; Kung *et al.*, 2001), but merged into *P.* sect. *Xiphopolystichum* by Zhang and Barrington (2013). The delimitation of *P.* sect. *Xiphopolystichum* needs reconsideration.

In previous studies, the taxonomists regarded the same species, *P. acanthophyllum*, as occurring in middle and high elevations. By the evidence of spore ornamentation, both taxa have similar macro-ornamentation, Cristate-fenestrate type, but minor differences between the two taxa. Taxon found in the high elevation has larger pits on the perispore, and ridges are closer, but taxon found in the middle elevation has smaller pits and looser ridges. Thus, we treat two taxa as different variations of *P. acanthophyllum* (*P. sp*3).

All other species have cristate macro-ornamentation and fenestrate micro-ornamentation. So, very few points can be said about the classification system and species delimitation. The synapomorphy of the Sect. *Achroloma* is a cartilaginous margin of the pinnae. There are only two species included, *P. mammeiense* and *P. nepalense*. The spore ornamentation is cristate-fenestrate type.

Christ (1911) established the genus *Sorolepidium* with the type species *S. glaciale* collected from Yunnan alpine region. Tryon and Tryon (1982) and Kramer *et al.* (1990) treated it as a synonym of *Polystichum*. Kung *et al.* (2001) reestablished the genus with an additional species, *S. ovale*. Finally, these two species were combined and placed in *P.* sect. *Sorolepidium* (Zhang and Barrington 2013). The spore ornamentation of *P. glaciale* is similar to other alpine *Polystichum* species (*P. glaciale, P. lachenense, P. prescotttianum, P. sinense, and P. taizhongense)*, so it is reasonable to place it in the section under the genus *Polystichum*. The same conclusion based on spore morphology was reported by Lu *et al.* (2007).

One alpine species, *P. lachenense,* has two types of spore ornamentation, different in perforation size and echinae length. A future collection of more morphological evidence will determine whether they are the same species or a new taxon to be named. The present study also includes another unknown species, *P. sp4*, from Dabajenshan, Taiwan (*T.C. Hsu, no.6053.* TAIF). The spore ornamentation of this novel species is similar to that of other alpine species, so we can assume that this species has some relationships with the alpine group.

In the section *Stenopolystichum,* Flora of China treated *P. pseudostenophyllum and P. stenophyllum* as the same (Kung *et al.*, 2001; Zhang and Barrington, 2013), but Taiwan floristic treatment kept these distinct (TPG, 2019). As the microstructures on the micro-ornamentation surface and the spore sizes are different, together with other morphological evidence, we consider them two separate species, and Wan *et al.* (2023) also accepted.

There are 17 taxa included in the *Macropolystichum* section (Zhang and Barrinton, 2013). In the present study, we sampled five taxa, which consist of 3 adventitious bud species and two adventitious bud free species. Based on the result proposed by Le Péchon *et al.* (2016), this section is a weak supported clade (MPJK=78, MLBS=73, BIPP=1), and there are no consequences between the adventitious bud and phylogeny. For the five taxa sampled herein, which are spread in different locations in the phylogenetic tree, the spore ornamentation is cristate-fenestrate type, which might be a synapomorphy of this section. However, the spore ornamentation of *P. chunii* from Taiwan (Hsu 2856, TAIF) is different from the samples from Quichu Prov., China (Wang and Dai, 2010). *P. chunii* has a disjunction distribution between S. Himalaya (China, N. Thailand, and Nepal) and Taiwan (Hsu *et al.*, 2014). In Taiwan, this species is only found (one population) in the high elevation area in Taichung Co. The spore ornamentations from China have no ridge on the surface, but the sample from Taiwan has obvious ridges. Thus, the material from Taiwan might need further studying for confirmation.

The section *Hypopeltis* is a large section composed of about 70 species worldwide (Kung *et al.*, 2001). Zhang and Barrington (2013) subdivided six series in this section. Herein, we sampled four species belonging to 3 different series. They are *P. sinense* and *P. prescottianum* for Ser. *Sinensia*; *P. parvipinnulum* for Ser. *Brauniana*; and *P. piceopaleaceum*, for Ser. *Makinoiana*. All of them have the same pattern of spore ornamentation, cristate-fenestrate type. However, according to the phylogenetic studies (Le Péchon *et al.*, 2016), the section *Hypopeltis* is polyphyletic and needs further studies.

The *P. parvipinnulum* usually grows in the middle to high elevation, but some occur in the low elevation. The latter was identified as *P. sozanense* because of the wider scales on the rachis. Zhang and Kung (1994) indicated *P. parvipinnulum* has a larger fenestrate than *P. sozanense* has a smaller fenestrate. Based on the present study, the samples from the two elevational zones have the same fenestrate pattern; thus, we consider that the samples from the two elevational zones belong to the same taxon.

Zhang and Kung (1994) studied spores of Sect. *Metapolystichum* (Kung *et al.*, 2001) classified into six types and supposed the evolutionary trends of spores in this section that Ser *Linearia* was guessed as the most primitive one in this section and Ser. *Exindusiata* is the most advanced one. According to the treatment by Zhang and Barrington (2013), Sect. *Metapolystichum* and Ser. *Sinensia* under Sect. *Lasiopolystichum* (Kung *et al.*, 2001) were merged into Sect. *Hypopeltis*. In this study, spores of each taxon within Sect. *Hypopeltis* in Taiwan were classified into the same type, cristate-fenestrate type. Based on SEM picture evidence and characteristics described by Zhang and Kung (1994), the features align with reticulate and cristate traits. In our study, after referencing the terminology used by Lellinger and Taylor (1997) and Wang and Dai (2010), the term "fenestrate" was deemed more suitable than "reticulate" to describe these spore characteristics.

The section *Neopolystichum* comprises four species, and we observed one species, *P. grandifrons*. The spore ornamentation is cristate-fenestrate type but with noticeable larger fenestrates than other *Polystichum* species. The section *Hecatoptera* is monospecific, *P. hecatopterum* included. The spore ornamentation is cristate-fenestrate type. We sampled different sizes of fronds, but the spore size and ornamentation are still coincident. We presume the frond size is a variation of superficial.

Previously, several new species of cave *Polystichum* ferns have been discovered and reported in China, such as *P. cavernicola*, *P. speluncicola*, and *P. fengshanense*. The identification and documentation of these new species relied on SEM images of spores (Zhang and He, 2010, 2011; He and Zhang, 2011). All of them belong to Sect. *Haplopolystichum*. *P. cavernicola* and *P. speluncicola* are similar morphologically. However, based on spore ornamentation, *P. cavernicola* resembles the regulate type in our study with coarse structures and lacks micro-ornamentation on the surface. In contrast, *P. speluncicola* has cristate sculpturing with numerous spinules, aligning more with the retate type in our study. These two species share the same type as the other species of Sect. *Haplopolystichum* in Taiwan. Different spore ornamentations allow the differentiation of *P. cavernicola* and *P. speluncicola*. Additionally, the spore ornamentation of *P. fengshanense* is also of the retate type, consistent with our study. All three species inhabit karst caves, and Li *et al.* (2008) indicated that Sect. *Haplopolystichum* prefers living in limestone caves. In our study, species with the regulate type also tended to inhabit such environments but don't belong to this section. Therefore, the relationship between the ornamentation type and the environment appears to be evolutionarily significant, requiring further research for clarification.

CONCLUSION

This study proved the taxonomic usefulness of spore morphology in the fern genus *Polystichum*. The palynological information in this genus can differentiate subgenera, sections, and species. Although the spore ornamentations are almost consistent with the classification system, we need more data, especially molecular data, to verify these preliminary judgments. For example, each species has a different spore morphology except for a few cases. Those exceptions might result from identification mistakes, cryptic species, or possible introgressive hybridization. Further research with more data should be able to clarify each case. The cryptic species can then be separated into taxa with closer relationships so that spore morphology could form the beginning basis of the species delimitation in this genus.

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