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MEGASPORE MORPHOLOGY OF CHINESE SELAGINELLACEAE AND ITS TAXONOMIC SIGNIFICANCE

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Abstract

The megaspore morphology of 15 species of the genus *Selaginella* P. Beauv. (Selaginellaceae Willk.) using scanning electron microscopy was carried out. Of the 15 species, megaspore morphology *S. arbuscula* and *S. drepanophylla* are reported for the first time. On the basis of megaspore surface ornamentations five major megaspore types are recognized: granulate, tuberculate, verrucate, verrucate-rugulate and reticulate. The morphological characteristics of megaspores are useful in the subgeneric classification. However, the classification using megaspore morphological characteristics showed disagreement with the classifications reported in the literature. Therefore, the subgeneric relationships of the genus *Selaginella* need to be further studied.

Key words: Megaspore, Ornamentation type, Subgenus classification.

Introduction

Selaginella P. Beauv. (Selaginellaceae Willk.) is the most diverse group in lycophytes. It has about 770 species worldwide, accounting for about 70% of the extant lycophytes (Frey, 2009). Among them, 72 species are distributed in China (Zhang *et al.*, 2013a).

Previously, taxonomists classified *Selaginella* into multiple taxonomic units based on different taxonomic approaches (Spring, 1849; Braun, 1857, 1865; Baker, 1883; Hieronymus, 1901; Walton & Alston, 1938; Soják, 1992). Based on the most widely applied system proposed by Jermy (1986), *Selaginella* was categorized into five subgenera. Hieronymus (1901) classified the genus *Selaginella* into two subgenera *Homoeophyllum* and *Heterophyllum*, a taxonomic category widely used in China (Wu & Qin, 1991; Zhang, 2001). However, this classification has not yet been fully accepted by researchers in the field of molecular systematics (Korall & Kenrick, 2002, 2004; Zhou & Zhang, 2015; Weststrand & Korall, 2016a, 2016b; Zhou *et al.*, 2016).

Weststrand & Korall (2016a, 2016b) defined seven subgenera within the genus *Selaginella* based on molecular and morphological analyses: *Rupestrae*, *Gymnogynum*, *Stachygynandrum*, *Ericetorum*, *Exaltatae*, *Lepidophyllae* and *Selaginella*. Although they sampled as many as 223 species worldwide, the species distributed in China are rarely involved.

Zhou & Zhang (2015) and Zhou *et al.*, (2016) reconstructed the phylogenetic tree of approximately 200 species of *Selaginella* with a strong focus on taxa in China based on chloroplast gene *rbcL* and nuclear ITS. Based on the phylogeny and morphological data, they divided the genus *Selaginella* into six subgenera: *Ericetorum*, *Boreoselaginella*, *Heterostachys*, *Pulviniella*, *Stachygynandrum* and *Selaginella*.

The family Selaginellaceae is heterosporous and has ornamentation on the surface of both megaspores and microspores (Tryon & Lugardon, 1991; Moore *et al.*, 2006; Setyawan, 2011; Schulz *et al.*, 2013; Singh *et al.*, 2014; Zhou *et al.*, 2015). Thus, palynological studies on Selaginellaceae should take account of the morphological characteristics of both megaspores and microspores to avoid one-sided conclusion. Compared with those of other families, palynological studies on Selaginellaceae are more difficult, which also implies the vital systematic significance in Selaginellaceae.

Spore morphology is of great significance in taxonomy and phylogeny (Warren & Wagner, 1974; Wu & Qin, 1991; Zhou *et al.*, 2015; Savaroglu *et al.*, 2016). The exine of megaspores of Selaginellaceae is composed of sporopollenin and silicon dioxide, and its morphology has taxonomic significance (Kempf, 1970; Tryon & Lugardon, 1978; Korall & Taylor, 2006; Blackmore, 2007). The ornamentation diversity of Selaginellaceae megaspore exine is sufficient enough to distinguish those closely related species (Tryon & Lugardon, 1991; Valdespino, 1993; Morbelli *et al.*, 2001; Schulz *et al.*, 2013; Singh *et al.*, 2014; Zhou *et al.*, 2015). Therefore, studies on megaspores are valuable.

Morphological structures of megaspores in Selaginellaceae have been extensively studied outside China (Tryon, 1949; Hellwig, 1969; Nasu & Seto, 1980; Tryon & Tryon, 1982; Minaki, 1984; Bajpai & Maheshwari, 1986; Dahlen, 1988; Tryon & Lugardon, 1991; Valdespino, 1993; Giorgi *et al.*, 1997; Morbelli *et al.*, 2001; Moore *et al.*, 2006; Korall & Taylor, 2006; Huaylla *et al.*, 2010; Zavialova *et al.*, 2010; Schulz *et al.*, 2013; Singh *et al.*, 2014; Valdespino, 2015; Valdespino *et al.*, 2015).

Although Chinese researchers have been working on the morphology of megaspores of Selaginellaceae for many years (Liu *et al.*, 1989; Liu & Bao, 1990; Xiao *et al.*, 2001; Chang *et al.*, 2002; Chu, 2006; Zhou *et al.*, 2012; Xia *et al.*, 2013), especially Zhou *et al.*, (2015) observed ca. 70 species of Selaginellaceae in China, several species have not yet been investigated. With the revision of Chinese Selaginellaceae in recent years (Zhang & Zhang, 2004; Zhang *et al.*, 2013a, 2013b), more palynological works on megaspores need to be supplemented.

To provide more palynological data for Selaginellaceae classification, identification and systematic studies, the color, polar axis, equatorial axis, shape, polar view, equatorial view, surface ornamentation, sutura and cingulum of megaspores of 15 species of Selaginellaceae were observed and examined in details using optical microscopy and scanning electron microscopy. Of them, *S. arbuscula* and *S. drepanophylla* are reported for the first time.

Materials and Methods

Materials: The megaspores of 15 species of Selaginellaceae were collected from the specimens in the Herbarium Institute of Botany (PE), Chinese Academy of Science. Among them, 13 species were from China, one from India and one from Hawaii (Table 1). Their names are accepted in the International Plant Names Index (IPNI) and Tropicos.

Optical microscopy: The color of 20–30 megaspores was observed under a Nikon E600 optical microscope at 600 magnification and their polar axis length (P) and equatorial axis length (E) were measured using an ocular micrometer. Their three-dimensional shape was defined based on the ratio of P/E as proposed by Punt *et al.,* (2007) and further quantitatively classified.

Scanning electron microscopy: The naturally dried megaspores were scattered evenly on the sample stage affixed with double-sided, transparent adhesive, sprayed with a thin layer of gold, and observed and photographed under a HITACHIS-800 scanning electron microscope at 10.0 kV. Palynological terms in this paper followed those in previous studies (Zhang *et al.*, 1976; Punt *et al.*, 2007; Wang & Dai, 2010).

Results

The megaspore morphological characteristics of the 15 species in Selaginellaceae exhibited certain heterogeneity (Table 1; Figs. 1-4).

Following Hieronymus (1901) and the recent phylogenic relationship based on molecular and morphological analyses (Zhou & Zhang, 2015; Zhou *et al.*, 2015, 2016; Weststrand & Korall, 2016a, 2016b), we summarized the major achievements of researches on megaspores of Chinese Selaginellaceae (Liu *et al.*, 1989; Liu & Bao, 1990; Xiao *et al.*, 2001; Chang *et al.*, 2002; Zhou *et al.*, 2012; Xia *et al.*, 2013) for comparative analyses (Table 2).

Megaspores of Selaginellaceae are white, light yellow, light red, light pink, pale brown, yellow, orange or red, and 319.9 (229.3–389.3) μm and 348.9 (253.1–424.0) μm long at polar and equatorial axes, respectively, with P/E of 0.88–1.00. They are nearly spheroidal, subcircular or subtriangular at the polar view and subcircular, nearly semicircular or nearly fan-shaped at the equatorial view.

The megaspore surface ornamentation of Selaginellaceae is diverse and can be divided into five major types, namely granulate, tuberculate, verrucate, verrucate-rugulate and reticulate types. The granulate ornamentation has spherical fine particles with diameter of 3–18 μm, as shown in *S. kurzii* (Fig. 2F-H) and *S. scabrifolia* (Fig. 4D-F). The tuberculate ornamentation has larger, semicircular protrusions on the top with height slightly longer than width, as shown in *S. drepanophylla* (Fig. 1K, L). The verrucate ornamentation has large, flat, uniformly distributed protrusions with diameter of 9–43 μm and height shorter than width, as shown in *S. pennata* (Fig. 3J-L).

The verrucate-rugulate ornamentation is short-strip or reticulate. It is formed by flat and large rugulate ornamentation with particles of varying density and size distributed on the surface of the verrucate-rugulate ornamentation, as shown in *S. amblyphylla* (Fig. 1B) and *S. ornata* (Fig. 3H).

The reticulate ornamentation is formed by the projections of megaspore exine and has particles of different amount, size as well as projection width and height distributed on the surface, as shown in *S. arbuscula* (Fig. 1E-G), *S. indica* (Fig. 2C-E) and *S. vardei* (Fig. 4J-L). Among them, *S. indica* and *S. vardei* have more spherical particles with wider and higher projections on the surface compared with other species. In addition to the five major surface ornamentations, other fine structures such as echinulate (Fig. 1D) and granulate (Fig. 4K) are also found on the megaspore surface ornamentation of Selaginellaceae.

The megaspores are trilete at the *proximal side with obvious* profiles and straight forms. Their length is about 2/3 or 3/4 of the spore radius or almost reaches the equator.

In trilete megaspores, the exine thickens along the equatorial region, forming the cingulum (Zhang *et al.*, 1976). Among all the megaspores, cingulum is only present in those from *S. commutata* (Fig. 1H) and *S. rolandi-principis* (Fig. 4A).

Discussion

Morphological features of megaspores: Most megaspores in the present study have light colors such as white and pale yellow. Although the results are basically consistent with those of Liu *et al.,* (1989), previous studies on the color of the same species are inconsistent (Tryon, 1949; Hellwig, 1969; Liu *et al.*, 1989; Zhou *et al.*, 2012, 2015; Xia *et al.*, 2013). Yan *et al.,* (2016) proposed that the color of microspores is diverse and difficult to distinguish by the naked eyes. In addition, the color of microspores is also related to their degree of maturity and sometimes could be bleached. We believe that these factors may also affect color stability of megaspores of Selaginellaceae, making it insignificant in taxonomic analysis. Moreover, the color of megaspores was also not used as a basis for taxonomic classification in the study by Alston *et al.,* (1955).

Previous studies found that the megaspores of Selaginellaceae are 158–593 μm long in polar axis and 189–675 μm long in equatorial axis (Liu *et al.*, 1989; Liu & Bao, 1990; Zhou *et al.*, 2012; Xia *et al.*, 2013). Our results showed that the megaspores of Selaginellaceae are 229.3–389.3 μm long in polar axis and 253.1–424.0 μm long in equatorial axis. Although the results are within the range of those in previous studies, we believe that the megaspores of Chinese Selaginellaceae vary greatly in their lengths at polar axis and equatorial axis and could not be used as a basis for taxonomic classification.

Liu *et al.*, (1989) pointed that most megaspores of Selaginellaceae are tetrahedral-globose and only megaspores of *S. uncinata* are both tetrahedral-globose and bean-shaped. Tryon & Lugardon (1991) and Xia *et al.,* (2013) considered that the megaspores of Selaginellaceae are tetrahedral-globose. According to Punt *et al.,* (2007), we calculated the ratio of polar axis length to equatorial axis length (P/E) of the megaspores and considered that their three-dimensional shape be spheroidal.

Table 1. Morphological features of *Selaginella* megaspores.

Taxon	Voucher specimen	Color	Polar axis (P)/μm	Equatorial axis (E)/μm	Shape (P/E)	Polar view	Equatorial view	Ornamentation		Trilete		Cingulum	Figure
								Proximal surface	Distal surface	Profile	Length		
S. amblyphylla	Zhenkang, Yunnan, PE 15204	White/Pale red	229.3 (186.7–300.0)	253.1 (173.4–340.0)	Spheroidal (0.91)	Subcircular	Nearly fan-shaped	Granulate	Verrucate-rugulate	Obvious	About 3/4 of spore radius	Absence	Fig. 1A-D
S. arbuscula	Hawaii, PE 1262579	White	265.3 (213.3–333.3)	294.7 (240.0–373.3)	Spheroidal (0.90)	Subtriangular	Subcircular	Reticulate	Reticulate	Blurry	About 3/4 of spore radius	Absence	Fig. 1E-G
S. commutata	Guangxi, PE 7995	White/Yellow	325.3 (280.0–346.7)	350.7 (306.7–373.3)	Spheroidal (0.93)	Subcircular	Nearly semicircular	Reticulate	Reticulate	Obvious	Almost reaching the equator	Presence	Fig. 2H-J
S. drepanophylla	Guilin, Guangxi, PE 3827	Orange	237.3 (200.0–266.7)	253.3 (200.0–306.7)	Spheroidal (0.94)	Subcircular	Nearly semicircular	Tuberculate	Tuberculate	Obvious	Almost reaching the equator	Absence	Fig. 1K-L; Fig. 2A-B
S. indica	India, PE 17039	Pale yellow	308.7 (266.7–333.3)	350.7 (320.0–373.3)	Spheroidal (0.88)	Subcircular	Nearly fan-shaped	Reticulate	Reticulate	Obvious	Almost reaching the equator	Absence	Fig. 2C-E
S. kurzii	Mengliancheng, Yunnan, PE 15682	Pale yellow	260.0 (173.3–293.3)	260.0 (200.0–306.7)	Spheroidal (1.00)	Subcircular	Nearly fan-shaped	Granulate	Granulate	Obvious	Almost reaching the equator	Absence	Fig. 2F-I
S. mairei	PE 299	Pale yellow	376.0 (266.7–466.7)	397.3 (280.0–520.0)	Spheroidal (0.95)	Subcircular	Nearly fan-shaped	Tuberculate	Tuberculate	Blurry	About 2/3 of spore radius	Absence	Fig. 2J-L
S. megaphylla	Medog, Tibet, PE 03816	Pale brown	350.7 (280.0–386.7)	396.0 (280.0–440.0)	Spheroidal (0.89)	Subtriangular	Nearly semicircular	Tuberculate	Verrucate-rugulate	Blurry	About 2/3 of spore radius	Absence	Fig. 3A-C
S. nummularifolia	Yarlung Zangbo River, Tibet, PE 4233	Pale yellow	377.3 (333.3–413.3)	413.3 (386.7–466.7)	Spheroidal (0.91)	Subtriangular	Nearly fan-shaped	Granulate	Granulate	Obvious	About 3/4 of spore radius	Absence	Fig. 3D-F
S. ornata	Xichou, Yunnan, PE 596	Pale pink	313.3 (186.7–373.3)	341.3 (240.0–413.3)	Spheroidal (0.92)	Subtriangular	Nearly semicircular	Verrucate	Verrucate–rugulate	Obvious	About 2/3 of spore radius	Absence	Fig. 3G-I
S. pennata	Cangyuan, Yunnan, PE 15422	White	389.3 (346.7–413.3)	424.0 (373.3–453.3)	Spheroidal (0.92)	Subcircular	Subcircular	Verrucate	Verrucate	Obvious	About 2/3 of spore radius	Absence	Fig. 3J-L
S. rolandi–principis	Hainan, PE 1114	White/Pale yellow	310.7 (226.7–373.3)	330.7 (226.7–400.0)	Spheroidal (0.94)	Subcircular	Subcircular	Reticulate	Reticulate	Obvious	Almost reaching the equator	Presence	Fig. 4A-C
S. scabrifolia	Wuzhi Mt., Hainan, PE 1704	White	345.3 (240.0–386.7)	385.3 (280.0–426.7)	Spheroidal (0.90)	Subcircular	Subcircular	Granulate	Granulate	Obvious	About 2/3 of spore radius	Absence	Fig. 4D-F
S. siamensis	Lancang, Yunnan, PE 15741	White/Red	348.0 (226.7–400.0)	368.0 (280.0–413.3)	Spheroidal (0.95)	Subtriangular	Nearly fan-shaped	Tuberculate	Tuberculate	Obvious	About 3/4 of spore radius	Absence	Fig. 4G-I
S. vardei	Qiaojia, Yunnan, PE 24548	Orange	362.3 (333.3–400.0)	414.7 (373.3–453.3)	Spheroidal (0.88)	Subcircular	Nearly fan-shaped	Reticulate	Reticulate	Obvious	About 3/4 of spore radius	Absence	Fig. 4J-L

Table 2. Earlier reports on ornamentation of *Selaginella* megaspores.

Classifications: Hieronymus (1901)	Classifications: Weststrand & Korall (2016a, 2016b)	Classifications: Zhou & Zhang (2015); Zhou *et al.*, (2015, 2016)	Taxon	Authors	The name accepted in the IPNI and Tropicos	Megaspores – Ornamentation in the report: Proximal surface	Megaspores – Ornamentation in the report: Distal surface	Megaspores – Ornamentation we defined: Proximal surface	Megaspores – Ornamentation we defined: Distal surface
subg. *Homoeophyllum*	subg. *Rupestrae*	subg. *Ericetorum*	*S. indica*	In this study	+			Reticulate	Reticulate
			S. sibirica	Liu *et al.*, (1989)	*S. indica*	Cerebroid		Reticulate	Reticulate
			S. vardei	Liu *et al.*, (1989)	+	Cerebroid		Reticulate	Reticulate
				Xia *et al.*, (2013)		Verrucate		Verrucate	Verrucate
				In this study				Reticulate	Reticulate
subg. *Heterophyllum*	subg. *Gymnogynum*		*S. remotifolia*	Liu *et al.*, (1989)	+	Armor-like lamella		Reticulate	Reticulate
				Xia *et al.*, (2013)		Reticulate		-	Reticulate
	subg. *Stachygynandrum*	subg. *Boreoselaginella*	*S. nummularifolia*	In this study	+			Granulate	Granulate
			S. rossii	Liu *et al.*, (1989)	+	Granulate		Granulate	-
			S. sanguinolenta	Liu *et al.*, (1989)	+	Granulate		Granulate	-
				Xiao *et al.*, (2001)		Tuberculate or rugulate	Tuberculate or rugulate	Verrucate-rugulate	-
			S. borealis	Liu *et al.*, (1989)	*S. sanguinolenta*	Granulate		Granulate	-
		subg. *Heterostachys*	*S. albocincta*	Liu *et al.*, (1989)	+	Tuberculate		Tuberculate	-
			S. amblyphylla	In this study	+			Granulate	Verrucate-rugulate
			S. arbuscula	In this study	+			Reticulate	Reticulate
			S. bisulcata	Chang *et al.*, (2002)	+	Large round particles	Large round particles	-	Verrucate
			S. bodinieri	Xia *et al.*, (2013)	+	Verrucate-rugulate		Verrucate	Verrucate-rugulate
			S. omeiensis	Liu *et al.*, (1989)	*S. bodinieri*	Verrucate		Verrucate	-
				Xiao *et al.*, (2001)		Granulate	Granulate	Granulate	-
			S. braunii	Liu *et al.*, (1989)	+	Verrucate		Verrucate	-
				Zhou *et al.*, (2012)		Short baculate and papillate	Papillate and baculate	Tuberculate	Tuberculate
				Xia *et al.*, (2013)		Verrucate-rugulate		Verrucate	Verrucate-rugulate
subg. *Heterophyllum*	subg. *Stachygynandrum*	subg. *Heterostachys*	*S. chaetoloma*	Zhou *et al.*, (2012)	+	Tuberculate	Tuberculate and vermiculate	Tuberculate	Verrucate-rugulate
			S. chrysocaulos	Liu *et al.*, (1989)	+	Granulate		Granulate	-
			S. ciliaris	Chang *et al.*, (2002)	+	Micro-granulate	Reticulate	Granulate	-
			S. decipiens	Xia *et al.*, (2013)	+	Verrucate-rugulate		Verrucate-rugulate	Verrucate-rugulate
			S. delicatula	Liu *et al.*, (1989)	+	Papillate		Tuberculate	-
				Chang *et al.*, (2002)		Micro-granulate	Large granulate and short baculate	Verrucate	-
			S. drepanophylla	In this study	+			Tuberculate	Tuberculate
			S. effusa	Xia *et al.*, (2013)	+	Reticulate		Verrucate	Reticulate
			S. helferi	Liu *et al.*, (1989)	+	Plicate		-	Reticulate

Table 2. (Cont'd.).

Classifications: Hieronymus (1901)	Classifications: Weststrand & Korall (2016a, 2016b)	Classifications: Zhou & Zhang (2015); Zhou *et al.*, (2015, 2016)	Taxon	Authors	The name accepted in the IPNI and Tropicos	Megaspores – Ornamentation in the report: Proximal surface	Megaspores – Ornamentation in the report: Distal surface	Megaspores – Ornamentation we defined: Proximal surface	Megaspores – Ornamentation we defined: Distal surface
subg. *Heterophyllum*	subg. *Stachygynandrum*	subg. *Heterostachys*	*S. heterostachys*	Liu *et al.*, (1989)	+	Papillate		Verrucate	-
				Chang *et al.*, (2002)		Round processes	Round and short baculate processes	Granulate	Verrucate-rugulate
				Xia *et al.*, (2013)		Tuberculate		Tuberculate	Tuberculate
			S. hezhangensis	Zhou *et al.*, (2012)	*S. heterostachys*	Tuberculate densely and vermiculate sparsely	Tuberculate sparsely and vermiculate	Granulate	Verrucate-rugulate
			S. kurzii	In this study	+			Granulate	Granulate
			S. labordei	Liu *et al.*, (1989)	+	Granulate		Granulate	Verrucate-rugulate
				Xia *et al.*, (2013)		Granulate		Granulate	Granulate
			S. leptophylla	Xia *et al.*, (2013)	+	Reticulate		Reticulate	-
			S. mairei	In this study	+			Tuberculate	Tuberculate
			S. megaphylla	In this study	+			Tuberculate	Verrucate-rugulate
			S. minutifolia	Zhou *et al.*, (2012)	+	Tuberculate	Tuberculate	Granulate	Granulate
			S. monospora	Chang *et al.*, (2002)	+	Large granulate	Granulate	-	Granulate
			S. nipponica	Liu *et al.*, (1989)	+	Papillate		Verrucate	-
				Xia *et al.*, (2013)		Verrucate-rugulate		Verrucate	Verrucate-rugulate
			S. ornata	In this study	+			Verrucate	Verrucate-rugulate
			S. pennata	In this study	+			Verrucate	Verrucate
			S. picta	Chang *et al.*, (2002)	+	Micro-granulate	Reticulate	-	Reticulate
				Xia *et al.*, (2013)		Granulate		Granulate	Granulate
			S. prostrata	Xiao *et al.*, (2001)	+	Micro-spinate	Reticulate	Reticulate	Reticulate
			S. pseudopaleifera	Xia *et al.*, (2013)	+	Tuberculate		Tuberculate	Tuberculate
			S. helvetica	Liu *et al.*, (1989)	*S. pseudonipponica*	Granulate		Granulate	-
			S. repanda	Chang *et al.*, (2002)	+	Reticulate and granulate densely	Reticulate and micro-granulate densely	Verrucate	-
			S. siamensis	In this study	+			Tuberculate	Tuberculate
			S. sinensis	Liu *et al.*, (1989)	+	Granulate		Granulate	-
				Xiao *et al.*, (2001)		Tuberculate or short baculate	Tuberculate or short baculate	Tuberculate	-
			S. trichoclada	Xiao *et al.*, (2001)	+	Granulate or short baculate	Baculate and spinate	Tuberculate	-
				Xia *et al.*, (2013)		Verrucate-rugulate		-	Verrucate-rugulate
			S. uncinata	Liu *et al.*, (1989)	+	Plicate		Verrucate	Verrucate-rugulate
				Liu & Bao (1990)		Plicate		Verrucate	Reticulate
				Xiao *et al.*, (2001)		Tuberculate or granulate	Blunt-spinate or rugulate irregularly	Granulate	-

Table 2. (Cont'd.).

Classifications			Taxon	Authors	The name accepted in the IPNI and Tropicos	Megaspores			
						Ornamentation in the report		Ornamentation we defined	
Hieronymus (1901)	Weststrand & Korall (2016a, 2016b)	Zhou & Zhang (2015); Zhou *et al.*, (2015, 2016)				Proximal surface	Distal surface	Proximal surface	Distal surface
				Xia *et al.*, (2013)	+	Verrucate-rugulate		Verrucate-rugulate	-
			S. vaginata	Xia *et al.*, (2013)	+	Verrucate-rugulate		-	Verrucate-rugulate
			S. compta	Liu *et al.*, (1989)	*S. vaginata*	Granulate		Granulate	-
		subg. *Pulviniella*		Liu *et al.*, (1989)		Rugulate		Verrucate-rugulate	-
			S. pulvinata	Xiao *et al.*, (2001)	+	Rugulate irregularly	Rugulate irregularly	Verrucate-rugulate	-
				Xia *et al.*, (2013)		Verrucate		-	Verrucate
			S. tamariscina	Liu *et al.*, (1989)	*S. pulvinata*	Rugulate		Granulate	-
				Xiao *et al.*, (2001)		Tuberculate or granulate	Tuberculate or granulate	Granulate	-
			S. stauntoniana	Xiao *et al.*, (2001)	+	Verrucate	Verrucate or granulate	Verrucate	-
		subg. *Stachygynandrum*	*S. biformis*	Liu *et al.*, (1989)	+	Reticulate		-	Reticulate
			S. commutata	In this study	+			Reticulate	Reticulate
subg. *Heterophyllum*	subg. *Stachygynandrum*	subg. *Stachygynandrum*	*S. davidii*	Xiao *et al.*, (2001)	+	Verrucate, irregularity	Rugulate irregularly, looklike cloud	Verrucate	Verrucate-rugulate
				Xia *et al.*, (2013)		Verrucate-rugulate		Verrucate	Verrucate-rugulate
			S. gebaueriana	Liu *et al.*, (1989)	*S. davidii*	Verrucate		Verrucate	-
				Liu *et al.*, (1989)		Rugulate and reticulate		Verrucate-rugulate	-
			S. doederleinii	Xiao *et al.*, (2001)	+	Verrucate or granulate	Verrucate or granulate	Verrucate	-
				Xia *et al.*, (2013)		Verrucate-rugulate		Reticulate	Reticulate
				Liu *et al.*, (1989)		Equator membrane		Reticulate	-
			S. involvens	Xiao *et al.*, (2001)	+	Micro-granulate and finely verrucate	Ornate	Granulate	-
				Xia *et al.*, (2013)		Reticulate		Reticulate	Reticulate
				Liu *et al.*, (1989)		Reticulate		Reticulate	Reticulate
			S. moellendorffii	Xiao *et al.*, (2001)	+	Micro-granulate and verrucate	Reticulate	Scabrate	Reticulate
				Xia *et al.*, (2013)		Reticulate		Scabrate	Reticulate
			S. rolandi-principis	In this study				Reticulate	Reticulate
			S. scabrifolia	In this study	+			Granulate	Granulate
			S. doederleinii subsp. *scabrifolia*	Xia *et al.*, (2013)	*S. scabrifolia*	Verrucate-rugulate		Reticulate	Reticulate

Note: "+" indicates the name is accepted in the IPNI and Tropicos; "-" indicates that related micrograph is not available

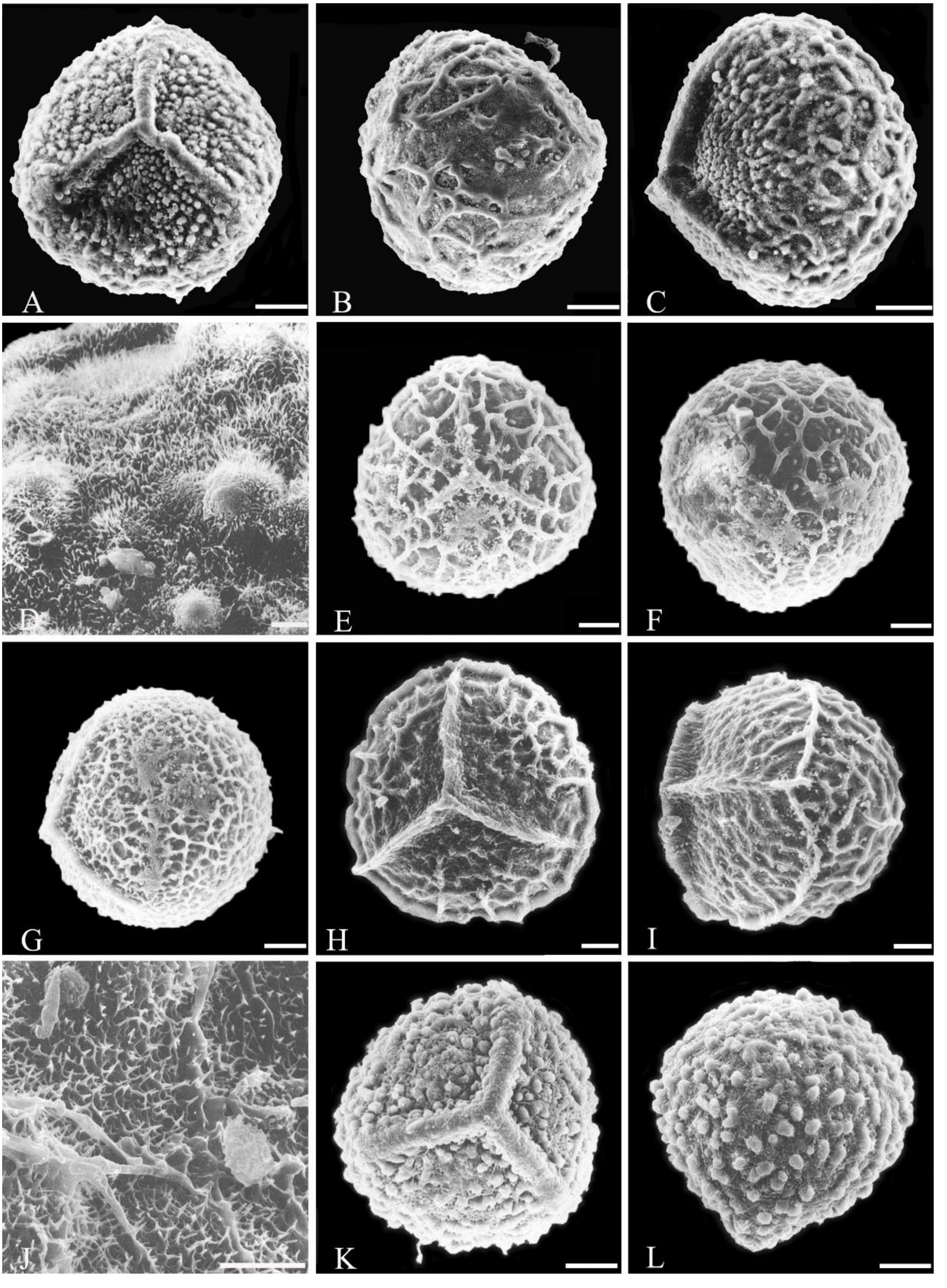

Fig. 1. Scanning electron microscope (SEM) micrographs of *Selaginella* megaspores. A-D. *Selaginella amblyphylla*, polar view (A, B), equatorial view (C), surface ornamentation (D). E-G. *Selaginella arbuscula*, polar view (E, F), equatorial view (G). H-J. *Selaginella commutate*, polar view (H), equatorial view (I), surface ornamentation (J). K, L. *Selaginella drepanophylla*, polar view (K, L). Scale bars: 5 µm (D, J), 50 µm (A-C, E-I, K, L).

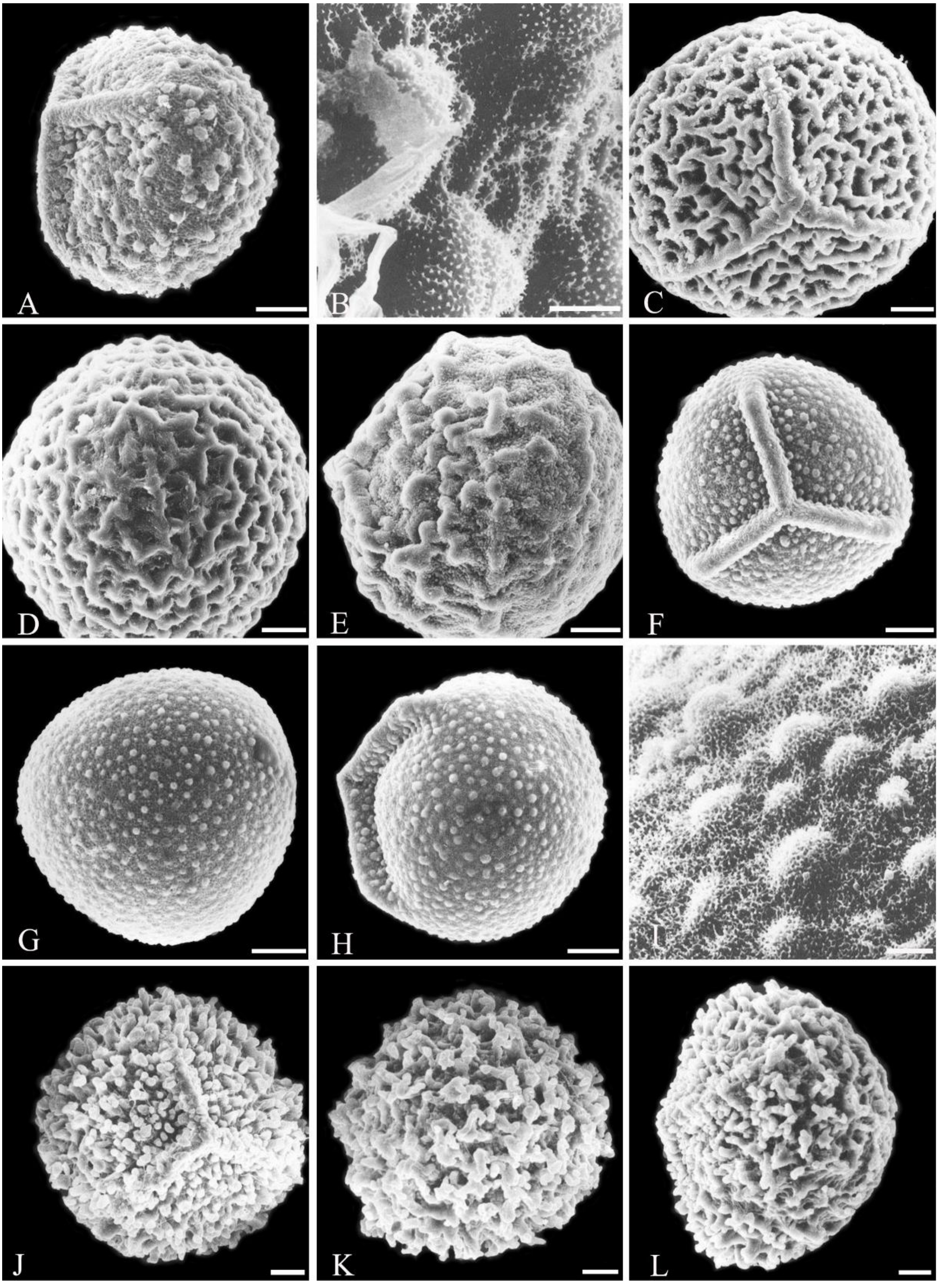

Fig. 2. Scanning electron microscope (SEM) micrographs of *Selaginella* megaspores. A, B. *Selaginella drepanophylla*, equatorial view (A), surface ornamentation (B). C-E. *Selaginella indica*, polar view (C, D), equatorial view (E). F-I. *Selaginella kurzii*, polar view (F, G), equatorial view (H), surface ornamentation (I). J-L. *Selaginella mairei*, polar view (J, K), equatorial view (L). Scale bars: 5 µm (B, I), 50 µm (A, C-H, J-L).

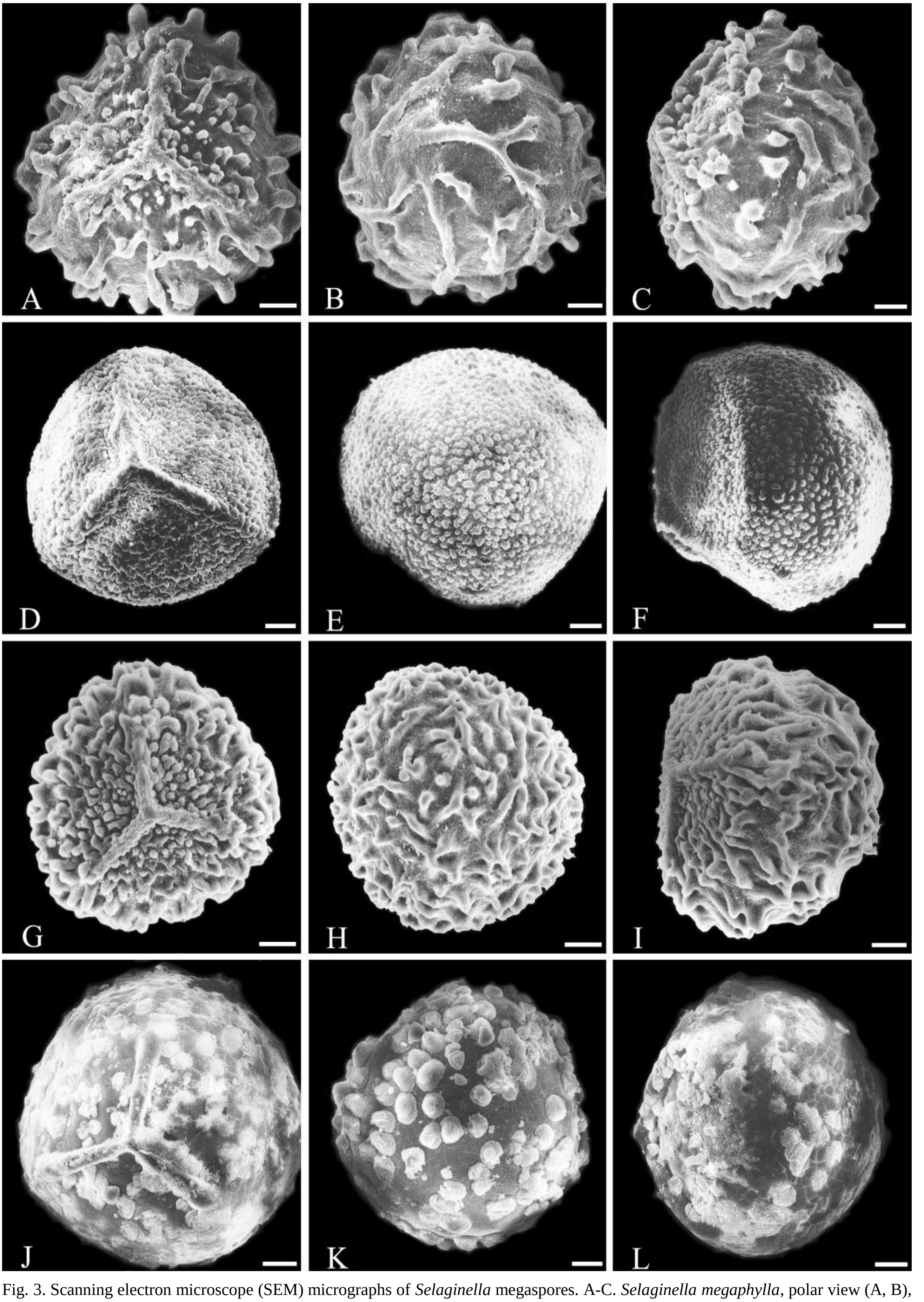

Fig. 3. Scanning electron microscope (SEM) micrographs of *Selaginella* megaspores. A-C. *Selaginella megaphylla*, polar view (A, B), equatorial view (C). D-F. *Selaginella nummularifolia*, polar view (D, E), equatorial view (F). G-I. *Selaginella ornata*, polar view (G, H), equatorial view (I). J-L. *Selaginella pennata*, polar view (J, K), equatorial view (L). Scale bars: 50 µm (A-L).

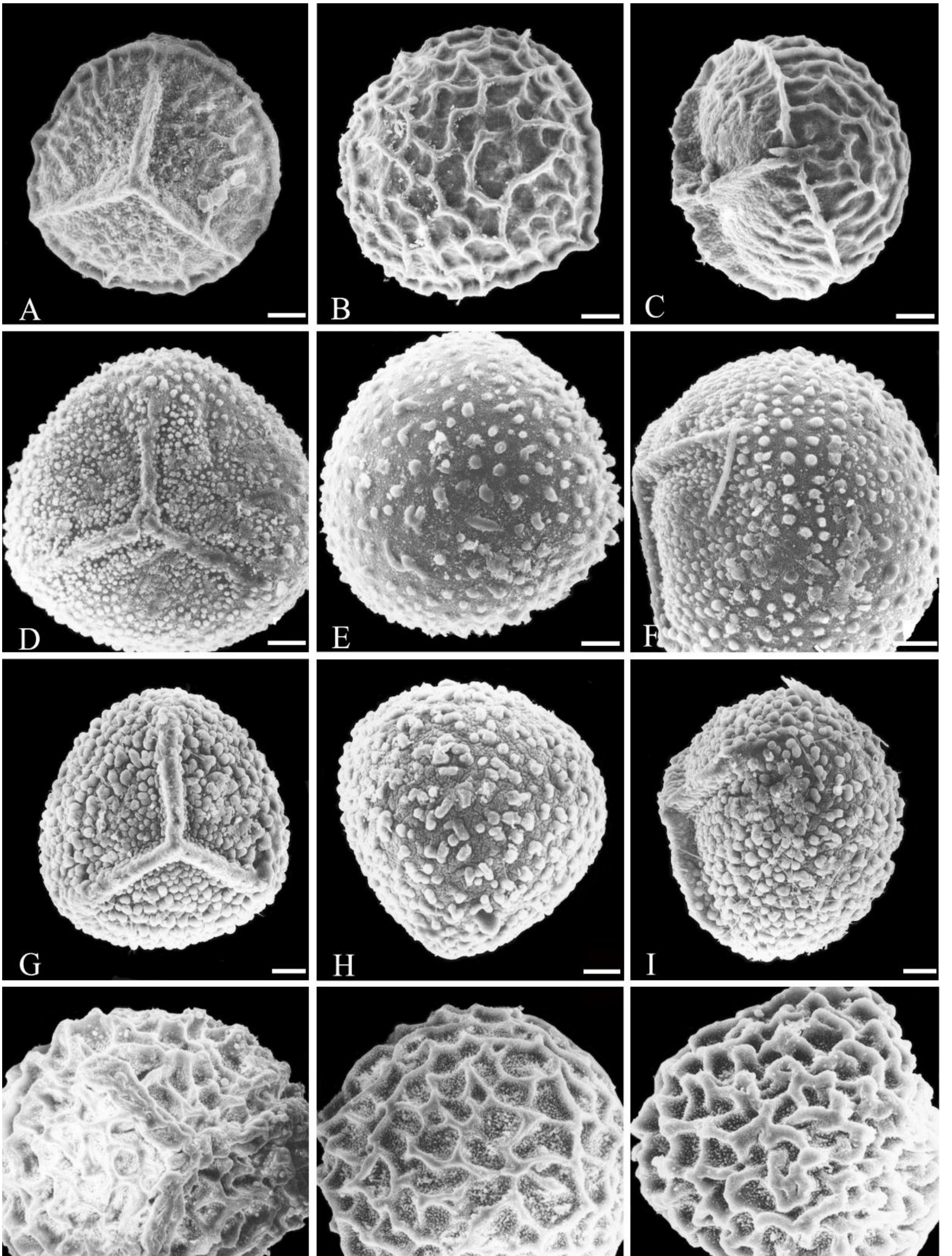

Fig. 4. Scanning electron microscope (SEM) micrographs of *Selaginella* megaspores. A-C. *Selaginella rolandi-principis*, polar view (A, B), equatorial view (C). D-F. *Selaginella scabrifolia*, polar view (D, E), equatorial view (F). G-I. *Selaginella siamensis*, polar view (G, H), equatorial view (I). J-L. *Selaginella vardei*, polar view (J, K), equatorial view (L). Scale bars: 50 μm (A-L).

In this study, the majority of megaspores have the same type of ornamentation at proximal and distal polar surface with few exceptions. These exceptional megaspores have slightly different ornamentation types which are often fine and dense at the proximal polar surface, but sparse and coarse near the equator and at the distal polar surface as typically seen in *S. amblyphylla* (Fig. 1A-C), *S. megaphylla* (Fig. 3A-C), *S. ornata* (Fig. 3G-I) and *S. scabrifolia* (Fig. 4D-F). The study suggests that the surface ornamentation of megaspores is less diverse than that of microspores in Selaginellaceae and could be divided into five types, in agreement with the view of Xia *et al.*, (2013).

The terminologies used to describe the surface ornamentation of megaspores were not consistent in the previous studies, even for those of the same species (Table 2). Therefore, it is necessary to normalize the palynological terminology. For example, Liu *et al.*, (1989) found the surface ornamentation of *S. remotifolia* megaspores has armor-like lamellas while Xia *et al.*, (2013) thought that *S. remotifolia* megaspores had reticulate surface ornamentation, consistent with our observations. Compared with previous observations, these five types of ornamentation are also applicable to other megaspores of Selaginellaceae (Table 2).

However, there are controversies. For example, Liu *et al.*, (1989) found that the megaspores of in *S. doederleinii* had rugulate and reticulate ornamentation while Xiao *et al.*, (2001) found that it was verrucate or granulate ornamentation while Xia *et al.*, (2013) thought that the spores verrucate-rugulate ornamentation. The ornamentations are not consistent even with the ornamentation types in our study. We speculate that these discrepancies might be due to misidentification of species. Plants of Selaginellaceae are generally small and share similar morphological characteristics and the same habitats (Zhang *et al.*, 2013a). All these factors often make the identification and classification of their species more difficult (Wan *et al.*, 2008; Schulz *et al.*, 2013).

Liu *et al.*, (1989) found that most megaspores of Selaginellaceae were trilete and only about 5% megaspores (*S. uncinata*) were monolete. Our observations shows that all megaspores of Selaginellaceae are trilete, most of which have *obvious* profiles. The blurry profile of trilete shapes of *S. arbuscula* may be due to that it is covered by the successive ornamentation at the proximal polar surface, while that of *S. mairei* and *S. megaphylla* may be related to the common presence of projections similar to the ornamentation on the proximal polar surface. Liu *et al.*, (1989) observed that the length of the sutura of Selaginellaceae megaspores is generally less than 2/3, even less than 1/3 of spore radius. Our study showed that the length of the sutura is about 2/3 or 3/4 of spore radius, or almost reaches the equator.

Tryon & Lugardon (1991) proposed that Selaginellaceae megaspores and microspores often have cingulum, while Yan *et al.*, (2016) reported that only microspores of *S. nummularifolia* have cingulum. Our observations showed that only the megaspores of *S. commutata* and *S. rolandi-principis* have cingulum.

Subgenus classification of Selaginella: According to the classification of Hieronymus (1901), of the species of *Selaginella* in the study (Table 2), three species belong to the subgenus *Homoeophyllum* and their megaspore surfaces are reticulate. Fifty five species and one subspecies belong to the subgenus *Heterophyllum* and have 5 ornamentations on megaspore surfaces. Among the latter 55 species and one subspecies, the proximal surfaces of megaspores are mainly granulate, verrucate and reticulate and distal surfaces of megaspores are mainly verrucate-rugulate, reticulate and granulate. The megaspores of the subgenus *Homoeophyllum* only have reticulate surface ornamentation, on which many spherical particles distribute, and their reticula are wider, higher and more abundant than those of the subgenus *Heterophyllum*.

According to the classification of Weststrand & Korall (2016a, 2016b), of the species of *Selaginella* in the study (Table 2), three species belong to the subgenus *Rupestrae* and have reticulate ornamentation on megaspore surfaces. One species belongs to the subgenus *Gymnogynum* and has reticulate ornamentation on megaspore. 54 species and one subspecies belong to the subgenus *Stachygynandrum* and have all five ornamentations on megaspore surfaces. Their proximal surfaces are mainly granulate, verrucate, reticulate and their distal surfaces are mainly verrucate-rugulate and reticulate.

According to the classification of Zhou & Zhang (2015) and Zhou *et al.*, (2015, 2016), of the species of *Selaginella* in the study (Table 2), four species belong to the subgenus *Ericetorum* and have reticulate ornamentation on megaspore surfaces. Four species belong to the subgenus *Boreoselaginella* and have granulate ornamentation on their megaspore surfaces and granules denser on distal surfaces of megaspores than other subgenera. 38 species belong to the subgenus *Heterostachys* and mainly have granulate, verrucate and tuberculate ornamentations on the proximal surfaces of their megaspores and the verrucate-rugulate, reticulate and tuberculate ornamentations on the distal surfaces of megaspores. Three species belong to the subgenus *Pulviniella* and have different ornamentations on megaspore surfaces. The proximal surfaces of megaspores are verrucate-rugulate in *S. pulvinata*, verrucate in *S. stauntoniana* and granulate in *S. tamariscina*. Nine species and one subspecies belong to the subgenus *Stachygynandrum* and mainly have reticulate ornamentation on megaspore surfaces.

The palynological characteristics of *S. arbuscula* and *S. drepanophylla* are reported for the first time. Zhou and Zhang (2015) classified *S. arbuscula* and *S. drepanophylla* into the subgenus *Heterostachys*. The megaspore surfaces of *S. arbuscula* and *S. drepanophylla* meet the features of the subgenus *Heterostachys* summarized in our study.

Hieronymus (1901) and Weststrand & Korall (2016a, 2016b) classified *S. indica*, *S. sibirica* and *S. vardei* into one subgenus (the subgenus *Homoeophyllum* in the former and the subgenus *Rupestrae* in the latter). However in the classification of Zhou & Zhang (2015) and Zhou *et al.*, (2015, 2016), *S. remotifolia* along with the former three species is classified in the subgenus *Ericetorum*. The megaspores of *S. indica*, *S. sibirica*, *S. vardei* and *S. remotifolia* all have reticulate surface ornamentation while the former three species have many spherical particles on the surface. In addition, *S. remotifolia* has narrower and shorter reticula than the former three species.

Conclusions

In our study, we find that the megaspore surface ornamentation is a sufficient characteristic with a certain reference value. The terminologies used to describe the surface ornamentation of megaspores were not consistent in the previous studies. So we classified the megaspore surface ornamentations into five major types: granulate, tuberculate, verrucate, verrucate-rugulate and reticulate. These five types of ornamentation are also applicable to other megaspores of Selaginellaceae. For the infrageneric classification, our megaspore data are not in agreement with the three classifications reported in the literature. Therefore, the subgeneric relationships of the genus *Selaginella* need to be further studied.

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