Journal of Species Research 9(3):221-232, 2020

Selaginella subvaginata (Selaginellaceae), a new spikemoss from China

Xian-Chun Zhang[1,*], Aleksandr Petrovich Shalimov[1,2], Jong-Soo Kang[1,2] and Meng-Hua Zhang[1,2]

[1]*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences (CAS), Beijing 100093, China*
[2]*University of Chinese Academy of Science, Beijing, 100049, China*

**Correspondent: zhangxc@ibcas.ac.cn*

Selaginella vaginata is a common montane species with broad distribution in China and the Himalaya region, and several species that are morphologically similar to *S. vaginata* are distributed in Asia. The taxonomic revision of *S. vaginata* and related species was performed by morphological comparison of leaves, strobili, and spores, and phylogenetic analysis. Based on these results, a new species, *S. subvaginata*, sp. nov., has been identified. Morphologically, *S. subvaginata* has intermediate form between *S. vaginata* and *S. repanda*, which differs mainly in its main stem being erect, dorsal leaves long-ciliolate on inner margin and outer margin denticulate or with 2−4 cilia at base (long-ciliolate on both inner and outer margins in *S. vaginata*, denticulate on both inner and outer margins in *S. repanda*), and acroscopic base of ventral leaves long ciliolate (sparsely long ciliolate in *S. vaginata*, short ciliolate to denticulate in *S. repanda*). Moreover, phylogenetic analysis using three chloroplast markers (*rbcL*, *atpI*, and *psbA*) revealed that *S. subvaginata* is a distinct species among the anisosporophyllous species clade in Selaginellaceae.

Keywords: new species, *Selaginella subvaginata*, taxonomy, *rbcL*, *atpI*, *psbA*

© 2020 National Institute of Biological Resources
DOI:10.12651/JSR.2020.9.3.221

Introduction

Selaginellaceae is an ancient group of lycopods comprising some 700−750 living species in one genus, *Selaginella* P. Beauv. (Jermy, 1990). The most often referred infrageneric classification was proposed by Jermy (1986), with five subgenera: Subg. *Selaginella* (2 species), Subg. *Tetragonostachys* Jermy (ca. 50 species), Subg. *Ericetorum* Jermy (3 species), Subg. *Heterostachys* Baker (ca. 60 species), and Subg. *Stachygynandrum* (P. Beauv. ex Mirb.) Baker (ca. 600 species). More recently, Weststrand and Korall (2016) proposed a new infrageneric classification of *Selaginella*, which classified *Selaginella* into seven subgenera: Subg. *Selaginella* (2 species), Subg. *Rupestrae* Weststrand & Korall (ca. 50 species), Subg. *Lepidophyllae* (Li Bing Zhang & X.M.Zhou) Weststrand & Korall (2 species), Subg. *Gymnogynum* (P.Beauv.) Weststrand & Korall (ca. 40 species), Subg. *Exaltatae* Weststrand & Korall (3 species), Subg. *Ericetorum* Jermy (4 species), and Subg. *Stachygynandrum* (P.Beauv. ex Mirb.) Baker (ca. 600 species).

The majority of *Selaginella* species have uniform, monomorphic sporophylls (isosporophyllous), approximately 10% species have dimorphic sporophylls (anisosporophylls). South China to Southeast Asia is the diversity center of anisosporphyllous *Selaginella*, with more than 36 species distributed in China alone (Zhang, 2004; Zhang *et al*., 2013). Recently, a few anisosporophyllous species have been newly reported from China (Sun *et al*., 2015; Shalimov *et al*., 2019). Taxonomy of the anisosporophyllous species is often difficult because most species are small plants with minute and obscure characters, even the status of dimorphism of sporophylls is obscure and sometimes only slightly different from isosporophylls. Molecular phylogeny has resolved the isosporophyll species and the anisosporophyll species being separate lineages (Korall *et al*., 1999; 2000; 2004) at least for the majority of Old World species (except *S. bisulcata* Spring and *S. pennata* (D. Don) Spring).

Selaginella vaginata Spring (synonym: *Selaginella compta* Hand.-Mazz.) belongs to Subg. *Heterostachys* Baker in Jermy's classification (1986) and Subg. *Stachygynandrum* (P.Beauv. ex Mirb.) Baker in Weststrand and Korall (2016). This species is widely distributed from

the Northeastern Himalaya (the type locality of *S. vaginata*) to Mt. Qinglin, also was recently discovered from Mt. Yanshan in the vicinity of Beijing. Distinguishing between *S. vaginata* and its closely related species is complicated. Although *S. vaginata* is well-known from the Himalayas (Alston, 1934; 1945), a synonym that is *S. compta* Hand.-Mazz., has often been used (Zhou *et al*., 2015; Zhou and Zhang, 2015). During the field investigation of *Selaginella* in China, we collected an uncertain species twice from Sichuan Province that is morphologically similar to *S. vaginata*. To examine whether or not the uncertain species is a new species, we performed the morphological comparison with closely related species, such as *S. vaginata* Spring, *S. repanda* (Desv. ex Poir.) Spring, and *S. kurzii* Baker, and phylogenetic analysis of 27 anisosporophyllous *Selaginella* species using three chloroplast markers (*rbcL*, *atpI*, and *psbA*). Both of morphological and phylogenetic results demonstrated that the uncertain species is a new species among the anisosporophyllous *Selaginella* species.

Materials and Methods

Morphology

More than 100 specimens of the *S. vaginata* group were examined, as well as field observation was conducted in Sichuan, Yunnan, Guizhou, and Guangxi Provinces on several occasions. The morphological photographs of the species were taken with a Nikon DXM 1200F connected to a stereomicroscope (Nikon SMZ 1000) and computer and measurement were done by D 3.10 (http://www.nikoninstruments.com). To measure the morphological characteristics including axillary, dorsal, ventral leaves, stems, and strobili, ImageJ (https://imagej.nih.gov/ij/) was used. Descriptions of the new species follow the format of Zhang *et al*. (2013). Scanning Electron Microscopy (SEM) was used for the visualization of leaves, strobili, and spores. Leaves and strobili were taken from the type collection (*X.C. Zhang, R.H. Jiang & E.F. Huang* 8838, PE), and spores from the paratype (*Nanshuibeidiao Team 4374*, PE). These materials were fixed on double line tape, and then covered with gold-palladium mixture. Materials were examined and photographed at different magnifications using a Hitachi S-4800 at 10−20 kV.

Phylogenetic analyses

A total of 38 individuals, which represents 27 anisosporophyllous *Selaginella* species with resupinate strobilus including the putative new species, were used for the phylogenetic analyses (Appendix 1). According to the phylogenetic relationship that has been reported previously (Weststand and Korall, 2016; Zhou and Zhang, 2015), *S. laxistrobila*, *S. nipponica*, *S. braunii*, and *S. delicatula* were included as outgroup (Appendix 1). Detailed voucher information and GenBank accession numbers are listed in Appendix 1.

Total genomic DNA was isolated from silica-dried material using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol, and the primers and PCR condition of three chloroplast genes (*rbcL*, *atpI* and *psbA*) as those described in Shalimov *et al*. (2019). Obtained sequences were assembled with ContigExpress. Multiple-sequence alignment was performed in Clustal X v.1.83 (Thompson *et al*., 1997), followed by manual adjustment in BioEdit v.7.1.11 (Hall, 1999), and gaps were treated as missing data. All newly obtained sequences for the present study were deposited in GenBank (Appendix 1).

Maximum likelihood (ML) and Bayesian inference (BI) trees were constructed. To select the appropriate substitution model for both analyses, jModelTest 0.1.1 (Posada, 2008) was used. The ML analysis was performed using RAxML 7.2.6 (Stamatakis, 2006), with 1000 bootstrap replicates under the GTRGAMMA model, and the BI analysis was performed using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001), with GTR + I + G model (four gamma categories). BI analysis employed random starting trees and four Markov chain Monte Carlo (MCMC) simulations were run simultaneously and sampled every 1000 generations for 10 million generations. The average standard deviation of split frequencies (<0.01) was used to assess the convergence of the two runs. Bayesian posterior probabilities (PP) were calculated as the majority consensus of all sampled trees with the first 25% discarded as burn-in.

Results

Taxonomic treatment

Selaginella subvaginata X.C. Zhang & Shalimov, sp. nov. (Figs. 1, 2)

The new species is morphologically closer to *S. vaginata* and *S. repanda*, but differs by the following characters: main stems are erect, axillary leaves are ovate or ovate-triangular, the dorsal leaves are oblique at base, ovate-lanceolate, inner margin long ciliolate, outer margin entire or with 2−4 cilia, ventral leaves are broadly oblong-falcate, acuminate at apex.

Type. CHINA. Sichuan, Jiulong County, hydropower station, 18 Aug 2017, *X.C. Zhang, R.H. Jiang & E.F. Huang 8838* (Holotype, PE; isotype PE).

Description. *Plants* terrestrial, main stems erect, basal part short creeping, plants 8−13 (−25) cm long, 0.5−1.0

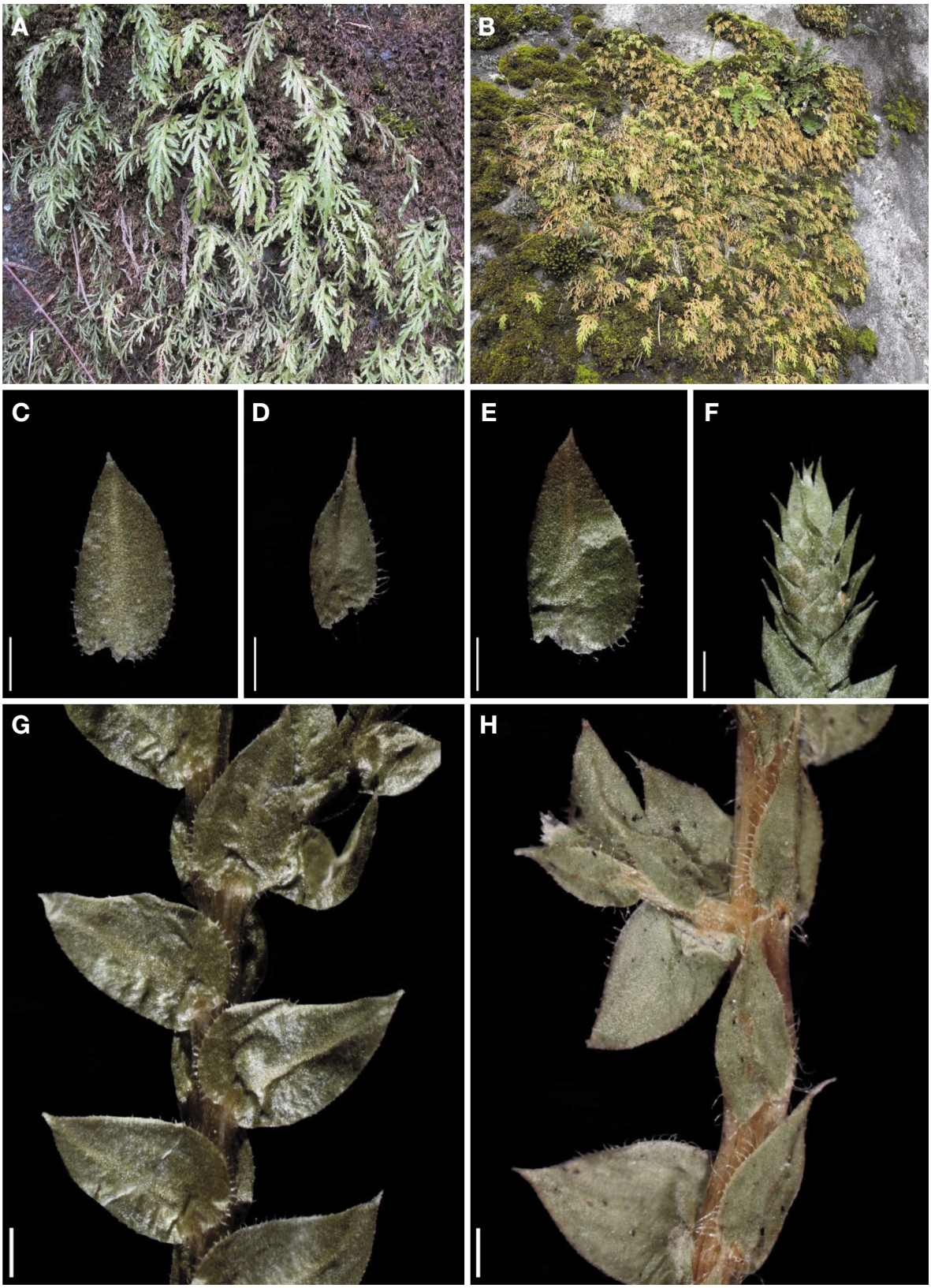


Fig. 1. *Selaginella subvaginata* X.C. Zhang & Shalimov. A, B. Habitat. C. Axillary leaf, D. Dorsal leaf, E. Ventral leaf, F. Strobilus, G. Ventral side of main stem, H. Dorsal side of main stem, C−H. from the holotype, *X.C. Zhang, R.H. Jiang & E.F. Huang 8838* (PE). Scale bar = 0.5 mm.

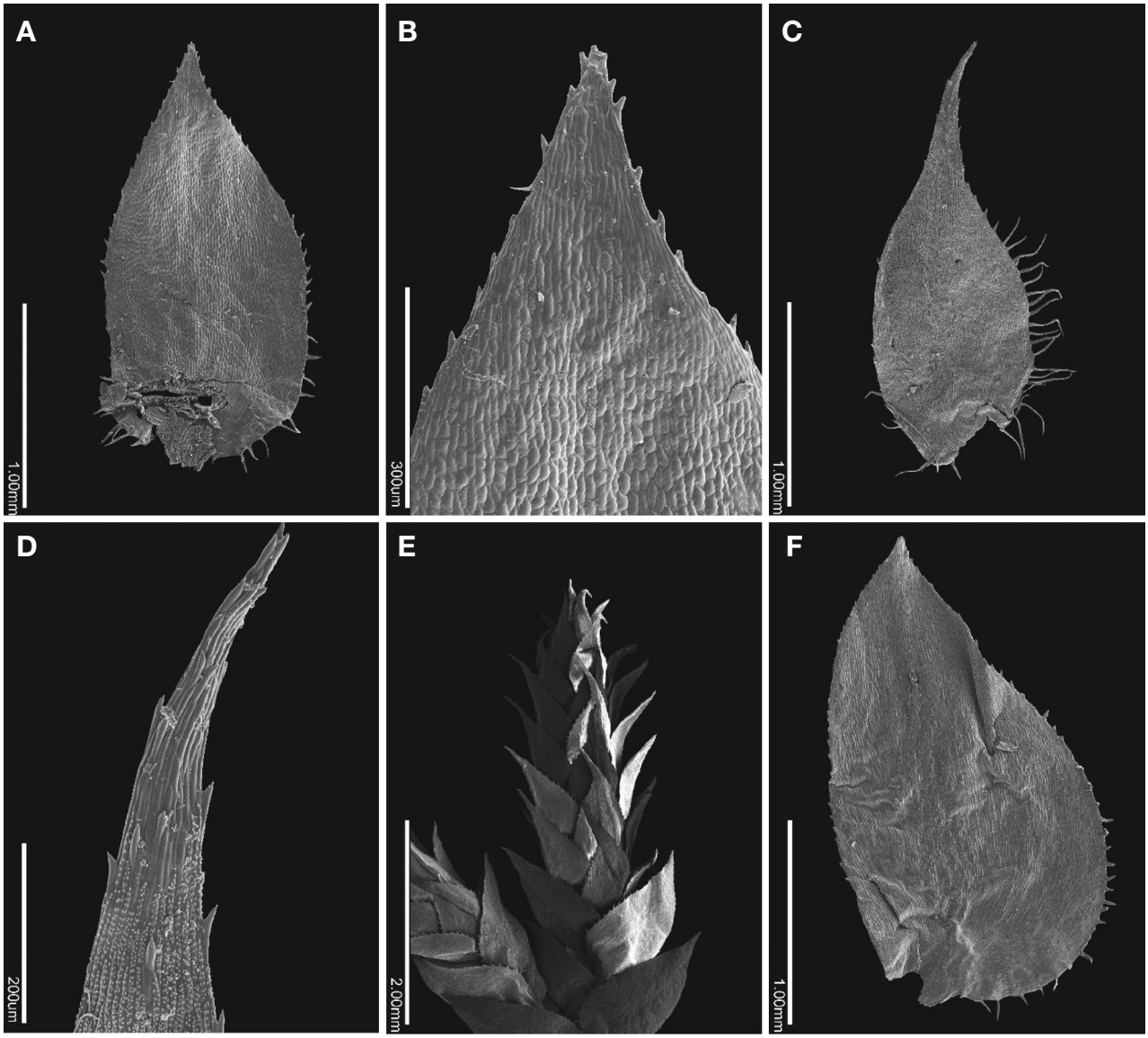


Fig. 2. *Selaginella subvaginata* X.C. Zhang & Shalimov. A. Lower surface of axillary leaf, B. Close-up of apex and upper portion of axillary leaf, C. Upper surface of dorsal leaf, D. Close-up of apex of dorsal leaf, E. Strobilus, lower surface, F. Lower surface of lateral leaf, A–F. taken from the holotype, *X.C. Zhang, R.H. Jiang & E.F. Huang 8838* (PE).

mm diam. at base. *Rhizophores* restricted in basal part of the unbranched main stems. Main stems branched from middle part, basal part slightly reddish, upwards stramineous; unbranched main stem 2.5–5.0 cm tall; primary leafy branches 10–14 pairs, once or twice pinnately branched, adjacent primary branches on main stem 0.5–1.5 cm apart, leafy portion of main stem including leaves 2.5–4.0 mm wide at middle, ultimate branches 1.5–2.3 mm wide including leaves. *Axillary leaves* on main stems larger than those on branches, ovate to ovate-triangular, 1.3–2.4 × 0.5–1.4 mm, base obtuse, margin ciliolate at basal and middle, denticulate to the apex, apex acuminate (Fig. 1C). *Dorsal leaves* ± symmetrical, ovate-lanceolate, 1.5–2.3 × 0.5–1.1 mm, carinate, base oblique, inner margin long ciliolate, outer margin denticulate or with 2–4 cilia at base, aristate at apex (Fig. 1D, H). *Ventral leaves* asymmetrical, those on main stem larger than those on branches; broadly oblong-falcate, 1.2–2.4 × 0.6–1.5 mm; basiscopic base decurrent, margin with few cilia at base and subentire to apex; acroscopic base enlarged, broader, overlapping stem and branches, margin densely long ciliolate at base, subentire to upward, acuminate at apex (Fig. 1E, G). *Strobili* compact, dorsiventrally complanate, 1.4–4.0 × 0.6–1.7 mm (Fig. 1F). *Sporophylls* anisophyllous, resupinate, slightly white-margined, dorsal sporophylls not obviously longer

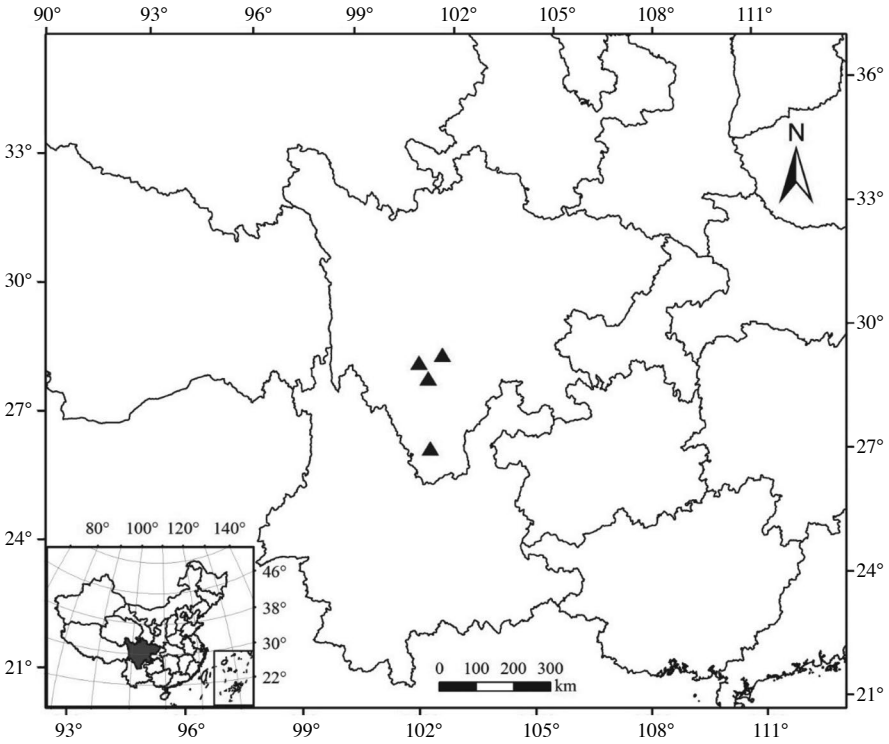


Fig. 3. Distribution of *Selaginella subvaginata* X.C. Zhang & Shalimov.

than the ventral sporophylls; *dorsal sporophylls* ovate, carinate, margin slightly ciliolate to denticulate, apex acuminate; *ventral sporophylls* ovate, carinate, margin slightly ciliolate to denticulate, apex acuminate. Megaspores yellowish, 187.84−326.00 × 157.92−186.53 μm, proximal and distal surface covered with reticulate and verrucate ornamentation (Fig. 6E). Microspores orange, 37.44−52.74 × 23.39−34.37 μm, proximal and distal surface smooth (Fig. 6F).

Habitat and Distribution. *Selaginella subvaginata* is currently distributed in Sichuan province (Jiulong, Shimian, Mianning and Miyi Counties), growing on mossy rocks, altitude 1400−2440 m (Fig. 3).

Preliminary conservation assessment. On the basis of available information and according to the IUCN (2018) criteria, this new species can be classified into Data Deficient (DD).

Etymology. The specific epithet '*subvaginata*' refers to its close relation and morphological similarity with *S. vaginata*.

Additional specimens examined. ***Selaginella subvaginata***: **CHINA. Sichuan:** Jiulong to Mianning, 28°39′N, 01″38′E, alt. 2006 m, 3 Oct 2013, *X.C. Zhang et Q.P. Xiang 6947* (PE); Shimian County, Gongga Mountain Nature Reserve, alt. 2300 m, 17 Apr 2019, *X.C. Zhang et al. 9450* (PE); Miyi County, Nanchang village, alt. 2437 m, 22 Oct 2015, *H. Liu 182* (PE), ibid., Wangouchang, alt. 1977 m, 30 Oct 2015, *H. Liu 183* (PE); Mianning County, Jinkuang, 23 Sep 1960, *Nanshuibeidiao Team 4374* (PE); ***Selaginella repanda***: **NEPAL. Makawanpur:** Suntari, W of Hetauda, Makawanpur, alt. c. 200 m, 8 Nov 1988, *T. Nakaike 3719* (PE); **CHINA. Yunnan:** Cangyuan County, Nangunhe Nature Reserve. *J.C. Zhao. 2000-4* (PE); Mengla County, Mengpeng, alt. 900 m, 16 Jun 2009, *X.C. Zhang et B.G. Li 5655* (PE); Pingbian County, Daweishan, alt. 1600 m, 21 Dec 1990, *Y.M. Shui 1390-35* (PE); Mengla County, alt. 650 m, 29 Dec 1989, *K.H. Shing et al. 06942* (PE), ibid., Caipingfeng, alt. c. 700−850 m, 1 Oct. 1993, *T. Nakaike 387* (PE), ibid., Menglun, *Chu W.M. 2115, 8 Aug 1975* (PE); ***Selaginella vaginata***: **NEPAL. Kaski**: Mahendra Cave, Pokhara, alt. c. 700 m, 11 Nov 1988, *T. Nakaike 3829* (PE); **Makawanpur:** [Pisulin] Fishling, near Mugling, Gorkha, alt. c. 300 m, 12 Nov 1988, *T. Nakaike 3830* (PE); **Kathmandu:** near Tribhuwan Airport, alt. c. 1300 m, 15 Sep 1986, *T. Nakaike 1506* (PE); **BHUTAN. Trimo:** Nyam Jang Chu. 27°55′N, 91°53′E, alt. 10500 ft., on rocky cliff, 1 Nov 1938, *F. Ludlow*, *G. Sherriff*, *G. Taylor 6458* (PE); Shamgong Area, 1985, *I. Broad B46* (E); Punakha district: 2 km below Chuzomsa, Tang Chu, 27°30′N, 89°58′E, Warm broad-leaved forest on river bank, S facing, on mossy rocks, 1410 m, 20 Apr 1982, *A.J.C. Grierson et D.G. Long 4517* (E); CHINA. Sichuan: Danba County, Mozigou, 2350 m, 27 Aug 1958,

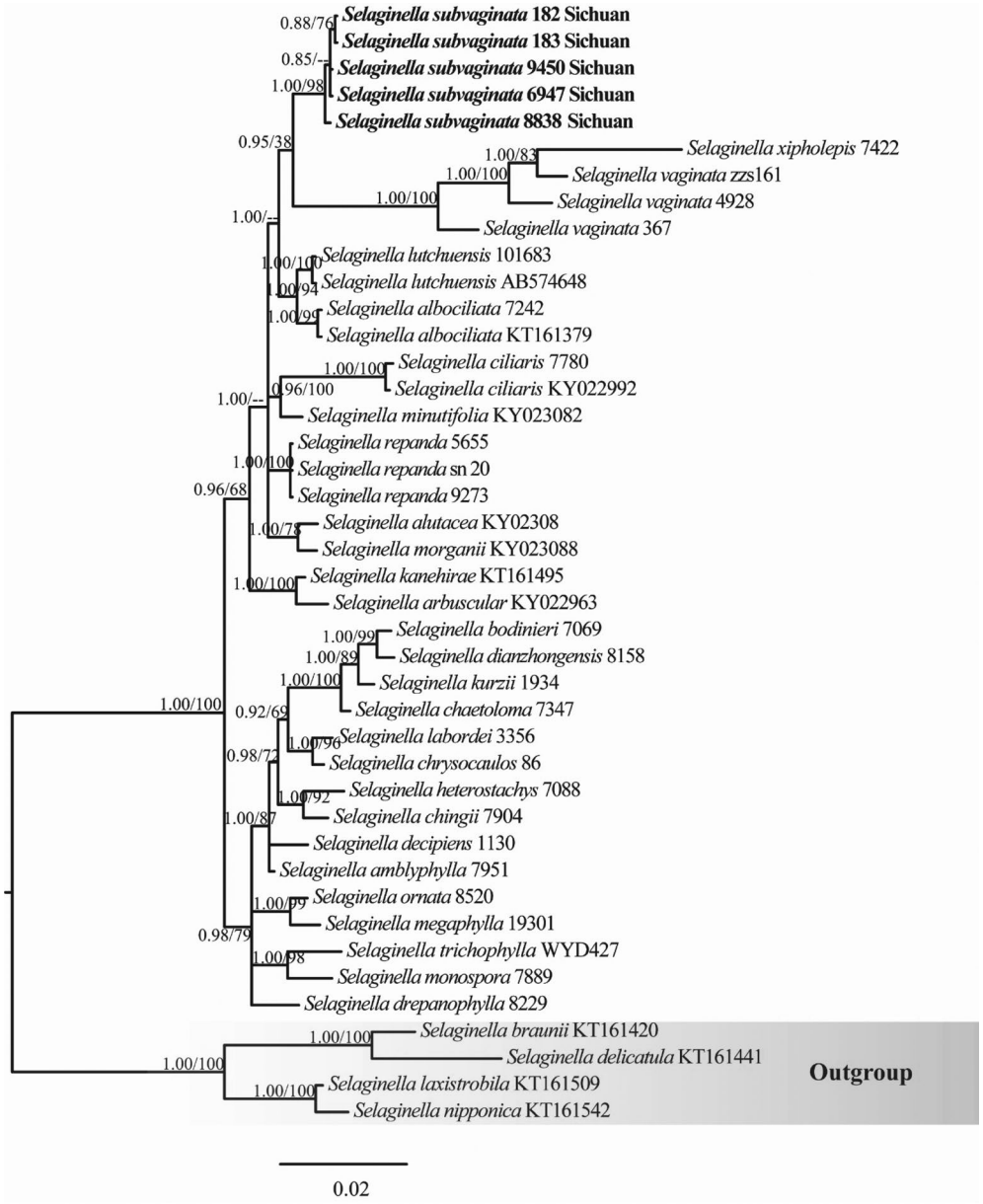


Fig. 4. The Bayesian inference (BI) tree of the new species and related species. Bayesian posterior probabilities and bootstrap supporting values calculated by maximum likelihood (ML) analysis are shown on the branches, respectively. The dash (--) indicates no values, and the new species is shown in bold.

s.c. s.n. 5493 (PE); Dujiangyan City, Longchi, 1900 m, 2 May 1994, *X.C. Zhang 0726* (PE); Yunnan: Dali City, Cangshan, c. 500 m, 9 Aug 1982, *Y.L. Ma et Q. Xia 0149* (PE); Heqing County, under the pine-oak forest, in grass, 2200 m, 14 Aug 1963, *Northwest Yunnan Jinshajiang Team 6475* (KUN); Luquan County, 11 May 1964, *W.M. Chu et Y.M. Feng 1699* (PE).

Phylogenetic analyses

We used 27 anisosporophyllous species with resupinate stobilus for phylogenetic reconstruction. Total alignment length was 2,020 bp (976 bp of *rbcL*, 304 bp of *atpI*, and 740 bp of *psbA*), and 384 of them were parsimony informative sites (19.0%). The topologies obtained from both ML and BI analyses are identical, hence the BI tree is shown in Fig. 3. Although the phylogenetic relationship between *S. vaginata* and *S. xipholepis* is still unresolved, our results indicated that all five individuals of the new species formed a well-supported clade (PP = 1.0, BP = 98), and is sister to the clade of *S. vaginata* and *S. xipholepis* (PP = 0.95, BP = 38) (Fig. 4).

Table 1. Morphological characters of *S. kurzii*, *S. repanda*, *S. subvaginata* and *S. vaginata*.

Characters/Species	*S. kurzii*	*S. repanda*	*S. subvaginata*	*S. vaginata*
Habit	Stem creeping 10−20 cm long	Stem ascending from a decumbent base, 8−30 cm long	Stem erect, 8−13(−25) cm long	Stem creeping, 5−10 cm long
Axillary leaves	Ovate to ovate-lanceolate, 1−2.5 × 0.6−1.6 mm, margin rather long ciliolate at base	Ovate to ovate-lanceolate, 2−3 × 1−1.4 mm, margin short ciliolate at base	Ovate to ovate-triangular, 1.3−2.4 × 0.5−1.4 mm, margin short ciliolate at base	Ovate-triangular, 1.2−2.5 × 0.5−1.5 mm, margin long ciliolate at base
Dorsal leaves	Ovate to ovate-elliptic, 1−1.2 × 0.4−0.8 mm, base subcordate or obtuse, margin ciliolate at base	Obliquely ovate, 0.7−1.6 × 0.4−0.9 mm, base obliquely subcordate, margin denticulate	Ovate-lanceolate, 1.5−2.3 × 0.5−1.1 mm, base obliquely subcordate, inner margin long-ciliolate, outer margin sparsely ciliolate at base	Ovate-lanceolate or triangular, 0.8−2.4 × 0.4−1.2 mm, base subcordate, cuneate, or obtuse, both inner and outer margin long-ciliolate
Ventral leaves	Ovate-triangular, 1.6−3.8 × 0.6−1.6 mm	Oblong−falcate, 2.5−3 × 1−1.5 mm	Broadly oblong-falcate, 1.2−2.4 × 0.6−1.5 mm	Ovate-lanceolate or oblong-falcate, 1.6−3.2 × 0.8−1.5 mm
Acroscopic margin of ventral leaves	Long ciliolate at base, subentire to the apex	Short ciliolate at base	Long ciliolate at base	Sparsely long ciliolate at base
Strobili	6−8 × 2−3mm	3−7 × 1.5−3 mm	1.4−4.0 × 0.6−1.7 mm	10−15(−45) × 2−3.5 mm
Sporophylls	Strongly anisophyllous	Slightly anisophyllous	Slightly anisophyllous	Slightly anisophyllous
Dorsal sporophylls	Ovate-lanceolate, sharply carinate, margin ciliolate, apex acute or acuminate	Ovate, sharply carinate, margin ciliolate	Ovate, carinate, margin ciliolate to denticulate	Ovate-lanceolate, sharply carinate, margin ciliolate or denticulate
Ventral sporophylls	Ovate, margin ciliolate	Ovate, margin ciliolate	Ovate, margin ciliolate to denticulate	Ovate-lanceolate, margin denticulate or ciliolate

Spore morphology

Megaspores of *S. subvaginata* are reticulate with verrucate ornamentation (Fig. 6E), which is different from the megaspores of *S. kurzii* and *S. vaginata*. The megaspores of *S. kurzii* in Liu *et al*. (2003) are verrucate with spinules (Fig. 6A), but it has shown the verrucate and slightly vermiculate in Zhou *et al*. (2015). Megaspores of *S. vaginata* are verrucate with reticulate ornamentation in Liu *et al*. (2006) (Fig. 6G) and Xia *et al*. (2013), but it has shown to be verrucate and smooth in Zhou *et al*. (2015). The megaspore morphology of *S. repanda* was very variable. The megaspore of *S. repanda* showed verrucate surface in most studies (Huang, 1981; Minaki, 1984; Chang *et al*., 2002; Liu *et al*., 2003) (Fig. 6C). However, they were rugulate or reticulate in Singh *et al*. (2014) and granulate in Wang *et al*. (2018). Moreover, those were verrucate but with dense spinules in Zhou *et al*. (2015).

However, the microspores of *S. kurzii*, *S. repanda*, and *S. vaginata* were all verrucate ornamentation (Liu *et al*., 2003; Liu *et al*., 2006; Xia *et al*., 2013; Zhou *et al*., 2015; Yan *et al*., 2016; Wang *et al*., 2018) (Fig. 6B, D, H), whereas the microspores of *S. subvaginata* are almost smooth, or sometimes with blunt particles (Fig. 6F).

Discussion

Our results demonstrate that the new species, *S. subvaginata*, is clearly distinguished from *S. vaginata* and its related species. Morphological comparison indicate that the new species is most similar to *S. vaginata*, *S. repanda* and *S. kurzii*, but differs mainly on the shape and margin of ventral and dorsal leaves (Table 1 & Fig. 5). Using three chloroplast markers, phylogenetic construction show that all five individuals of *S. subvaginata* formed their own subclade (Fig. 4). Moreover, *S. subvaginata* showed distinct spore morphology, compared with *S. vaginata*, *S. repanda*, and *S. kurzii*. Consequently, those results support that *S. subvaginata* should be treated as an independent species, and *S. subvaginata* is described as a new species. In subg. *Heterostachys* with anisosporophylls, the species identification and delimitation have often emerged as a problem. The species can be distinguished by morphology and/or molecular phylogeny, but sometimes cannot be distinguished, like the phylogenetic relationship of *S. vaginata* and *S. xipholepis* (Fig. 4). The unresolved relationship between two species was raised in a previous study (Zhou and Zhang, 2015). To solve such a problem, large-scale sampling and additional molecular markers

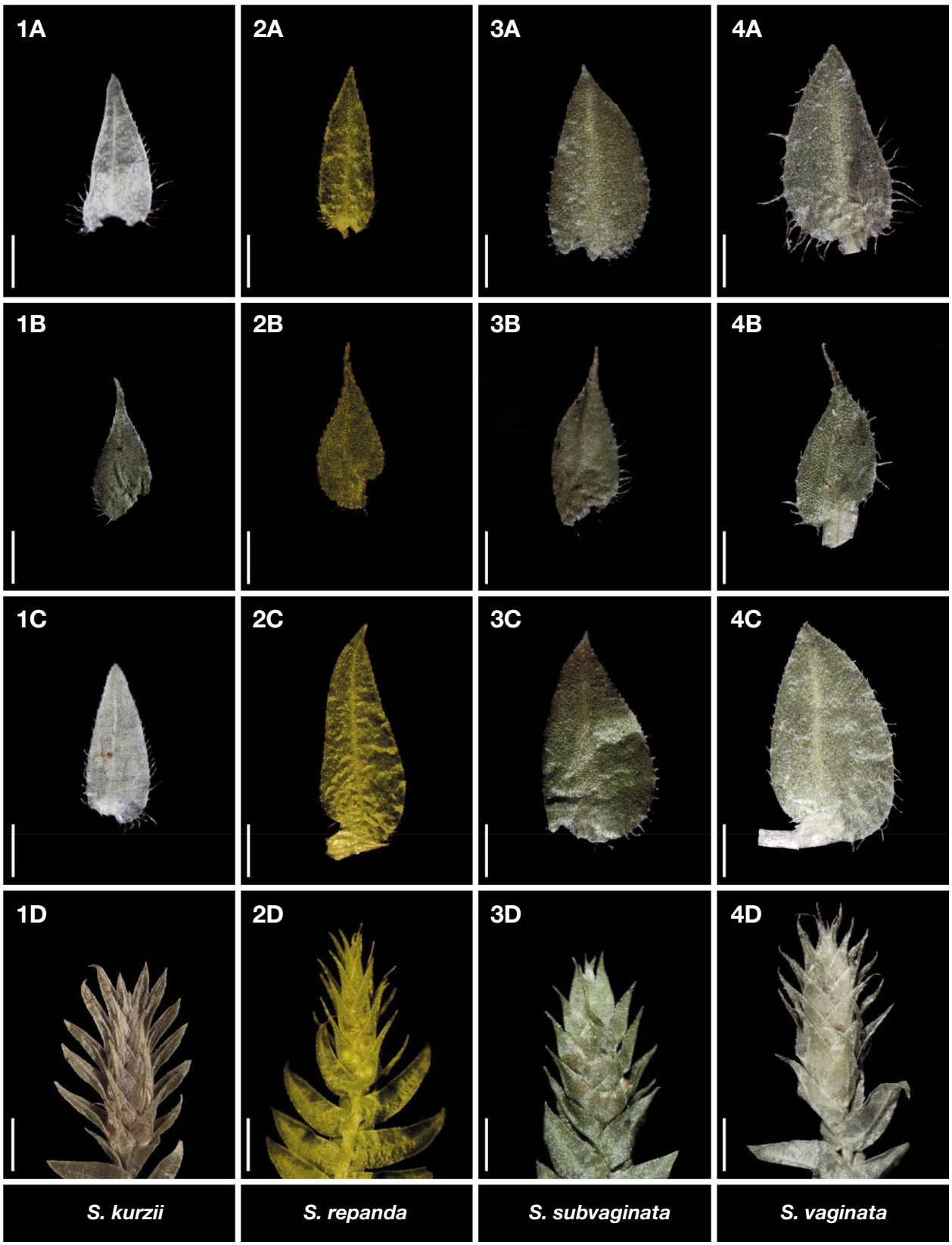


Fig. 5. Leaves and strobilus: 1A−1D. *S. kurzii* (*Y.M. Shui 1390-35*, PE00452047), 2A−2D. *S. repanda* (*X.C. Zhang & B.G. Li 5655*, PE01857722), 3A−3D. *S. subvaginata* (*X.C. Zhang, R.H. Jiang & E.F. Huang 8838*, PE), 4A−4D. *S. vaginata* (*X.C. Zhang 4928*, PE01792549). A. Axillary leaves, B. Dorsal leaves, C. Ventral leaves, D. Strobili. Scale bars = 0.5 mm (A−C) and 1 mm (D).

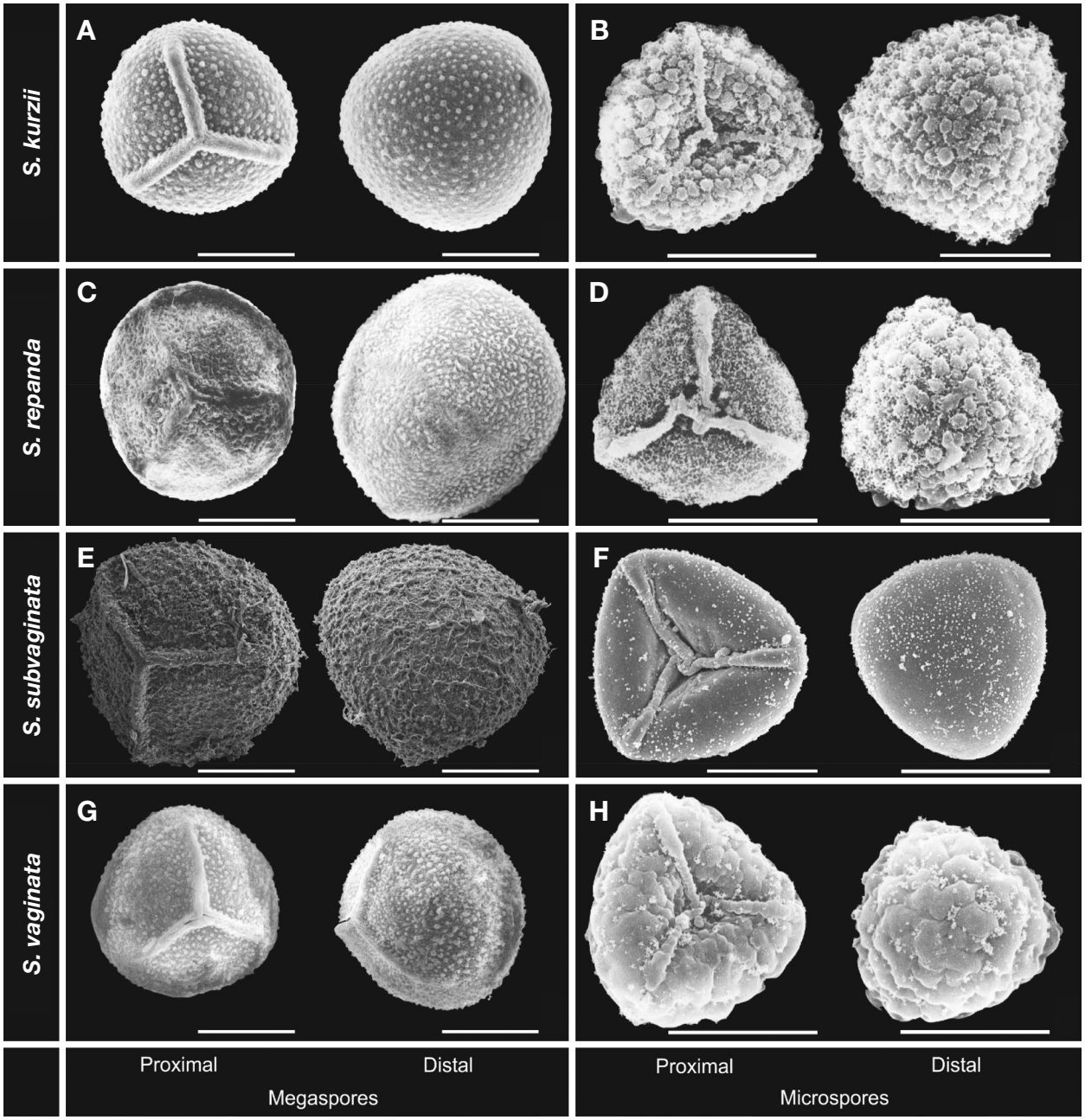


Fig. 6. Spore morphology. A. *S. kurzii* megaspores from *W.M. Chu et al. 15682* (PE), B. *S. kurzii* microspores from *W.M. Chu et al. 15682* (PE), C. *S. repanda* megaspores from *K.H. Shing 06679* (PE01563529), D. *S. repanda* microspores from *Y.M. Shui 1390-35* (PE00452047), E. *S. subvaginata* megaspores from *Nanshuibeidiao Team 4374* (PE), F. *S. repanda* microspores from *Nanshuibeidiao Team 4374* (PE), G. *S. vaginata* megaspores from *X.C. Zhang. 0777* (PE00452208), H. *S. vaginata* microspores (30/30 μm) from *X.C. Zhang 2250* (PE00452230). Scale bars = 200 μm (A and G), 100 μm (C and E), 30 μm (proximal in B, D, H, and distal in F), and 20 μm (distal in B, and proximal in F).

are needed, and we believe that it is worthy to study for understanding the species of subg. *Heterostachys*.

Key to *S. subvaginata* and related species

1. Main stems creeping, fertile branch erect ····· 2
1. Main stems erect, suberect or ascending from decumbent base····· 3
2. Basiscopic base of ventral leaves denticulate or rarely ciliolate, sporophylls more or less anisophyllous; megaspores surfaces verrucate with reticulate ornamentation or verrucate and smooth ornamentation, microspores surfaces with verrucate ornamentation ····· ····· *S. vaginata*

2. Basiscopic base of ventral leaves with few cilia at base, sporophylls strongly anisophyllous; megaspore surfaces with verrucate and/or slightly vermiculate, with spinules micro-sculptures, microspores surfaces verrucate ····· *S. kurzii*
3. Plants 8−30 cm, main stems ascending from decumbent base, ventral leaves oblong-falcate, acute at apex, acroscopic base short ciliolate to denticulate; megaspore surfaces with verrucate, rugulate, reticulate or granulate ornamentation, microspores surfaces verrucate ····· *S. repanda*
3. Plants 8−13(−25) cm, main stems subetect, ventral leaves broadly oblong-falcate, acuminate at apex, acroscopic base densely long ciliolate; megaspores are reticulate with verrucate ornamentation, microspores are smooth, or sometimes with blunt particles ····· *S. subvaginata*

Acknowledgements

This study was supported by the National Natural Science Foundation of China (NSFC, No. 31670205).

References

Alston, A.H.G. 1934. An enumeration of the Chinese species *Selaginella*. Bulletin of the Fan Memorial Institute of Biology (Botany) 5(6):261-304.

Alston, A.H.G. 1945. An enumeration of the Indian species of *Selaginella*. Proceedings of the National Institute of Sciences of India 11:211-235.

Chang, C.Y., Q.R. Liu and X.D. Chen. 2002. Megaspore morphology of eight species of *Selaginella*. Journal of Chinese Electron Microscopy Society 21:566-567.

Huang, T.C. 1981. Spore flora of Taiwan. Botany Department Press, National Taiwan University, Taipei, 105-111.

Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755. https://doi.org/10.1093/bioinformatics/17.8.754

IUCN. 2018. The IUCN Red List of Threatened Species. Version 2018-1. http://www.iucnredlist.org [Downloaded on 11 January 2018]

Jermy, A.C. 1986. Subgeneric names in *Selaginella*. Fern Gaz. 13:117-118.

Jermy, A.C. 1990. Selaginellaceae. In: K. Kubitzki, K.U. Kramer, P.S. Green (eds.), The Families and Genera of Vascular Plants, Pteridophytes and Gymnosperms. 1st edn, Vol. 1. Springer, Berlin.

Korall, P., P. Kenrick and J.P. Therrien. 1999. Phylogeny of Selaginellaceae: Evaluation of generic/subgeneric relationships based on *rbcL* gene sequences. International Journal of Plant Sciences 160:585-594.

Korall, P. and P. Kenrick. 2002. Phylogenetic relationships in Selaginellaceae based on *rbcL* sequences. Amer. J. Bot. 89:506-517. https://doi.org/10.3732/ajb.89.3.506

Korall, P. and P. Kenrick. 2004. The phylogenetic history of Selaginellaceae based on DNA sequences from the plastid and nucleus: extreme substitution rates and rate heterogeneity. Molec. Phylogen. Evol. 31:852-864. https://doi.org/10.1016/j.ympev.2003.10.014

Liu, B.D., W.M. Bao and C.W. Aur. 1989. Studies on the spores of morphology of the family Selaginellaceae from China. Bulltin of Botanical Research 9:113-131.

Liu, J.X., X.H. Sun, Y. Jin, L. Lu, X.X. Yu and X.C. Zhang. 2003. Spore morphology of eight species of Selaginellaceae from Yunnan. Acta Botanica Yunnanica 25:303-312.

Minaki, M. 1984. Macrospore morphology and taxonomy of *Selaginella* (Selaginellaceae). Pollen et spores 26:421-480.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25(7):1253-1256. https://doi.org/10.1093/molbev/msn083

Shalimov, A.P., Y.M. Zhu, M.H. Zhang and X.C. Zhang. 2019. *Selaginella dianzhongensis* (Selaginellaceae), a new spikemoss from China. PhytoKeys 118:75-87. https://doi.org/10.3897/phytokeys.118.30375

Singh, S.K., B.B. Yadav, M. Srivastava, P.K. Shukla and G.K. Srivastava. 2014. Micro-morphology of *Selaginella megaspores* from India. Grana 53(3):197-220.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688-2690. https://doi.org/10.1093/bioinformatics/btl446

Sun, Q.W., J.H. Zhao and L.B. Zhang. 2015. *Selaginella daozhenensis* (Selaginellaceae), a new lycophyte from a limestone cave in northern Guizhou, China. Phytotaxa 207(2):187-192. https://doi.org/10.11646/phytotaxa.207.2.5

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25(24): 4876-4882. https://doi.org/10.1093/nar/25.24.4876

Wang, L.J., X.C. Zhang and J.X. Liu. 2018. Studies on the complementary relationship of surface ornamentations between megaspores and microspores of *Selaginella* P. Beauv. (Selaginellaceae). Microscopy Research & Technique: 1-15. https://doi.org/10.1002/jemt.23148

Weststrand, S. and P. Korall. 2016. A subgeneric classification of *Selaginella* (Selaginellaceae). American Journal of Botany 103(12):2160-2169. https://doi.org/10.3732/ajb.1600288

Xia, Y., X.L. Dai, Y.H. Yan, J.G. Cao and Q.X. Wang. 2013. Spore morphology of pteridophytes from China XIV. Selaginellaceae. Acta Botanica Boreali-Occidentalia Sinica 33:1595-1604.

Yan, D., L.J. Wang, Y.Y. Song, L. Wang, T.T. Du and J.X. Liu. 2016. Microspore morphology of Selaginellaceae

in China and its systematic significance. Plant Syst Evol 302:561-574. https://doi.org/10.1007/s00606-016-1284-8

Zhang, X.C. 2004. Selaginellaceae. In: X.C. Zhang (ed.), Flora Republica Popularis Sinicae. Science Press, Beijing, Vol. 6:86-219.

Zhang, X.C., H.P. Nooteboom and M. Kato. 2013. Selaginellaceae. In: Z.Y. Wu, P.H. Raven and D.Y. Hong (eds.), Flora of China. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, Vols 2-3, 37-66.

Zhou, X.M., L.J. Jiang, L. Zhang, X.F. Gao, Z.R. He and L.B. Zhang. 2015. Spore morphology of *Selaginella* (Selaginellaceae) from China and its systematic significance. Phytotaxa 237(1):001-067. https://doi.org/10.11646/phytotaxa.237.1.1

Zhou, X.M. and L.B. Zhang. 2015. A classification of *Selaginella* (Selaginellaceae) based on molecular (chloroplast and nuclear), macromorphological, and spore features. Taxon 64(6):1117-1140. https://doi.org/10.12705/646.2

Submitted: April 28, 2020
Revised: June 3, 2020
Accepted: June 9, 2020

Appendix 1. Voucher information and GenBank accession numbers for material used.

Taxon	Voucher	Country	*rbcL*	*atpI*	*psbA*
S. albociliata P.S. Wang	Zhang X.-C. 7242 (PE)	China, Guizhou	MH814882	MH814826	MH814854
S. albociliata P.S. Wang	Zhang L. B. 5302 (CDBI)	China, Guangxi	KT161379	–	–
S. alutacea Spring	P. Korall 2006-29 (S)	Malaysia	KY023088	–	–
S. amblyphylla Alston	Zhang X.-C. 7951 (PE)	China, Yunnan	MH814884	MH814828	MH814856
S. arbuscula (Kaulf.) Spring	H. H. Iltis et al. 96 (U)	USA, Hawaii	KY022963	–	–
S. bodinieri Hieron.	Zhang X.-C. 7069 (PE)	China, Guizhou	MH814887	MH814831	MH814859
S. braunii Baker	Zhang 1332 (PYU, CDBI)	China, Hainan	KT161420	–	–
S. chaetoloma Alston	Zhang X.-C. 7347 (PE)	China, Guizhou	MH814889	MH814833	MH814861
S. chingii Alston	Zhang X.-C. 7904 (PE)	China, Guangxi	MH814890	MH814834	MH814862
S. chrysocaulos (Hook. & Grev.) Spring	Zhang X.-C. 86 (PE)	China, Sichuan	MH814891	MH814835	MH814863
S. ciliaris (Retz.) Spring	Zhang X.-C., Liu B.-D. 7780 (PE)	China, Yunnan	MH814892*	MH814836*	MH814864*
S. ciliaris (Retz.) Spring	P. Korall 2006-2 (S)	Malaysia	KY022992	–	–
S. decipiens Warburg	Zhang X.-C. 1130 (PE)	China, Guangxi	MT680175*	MT680166*	MT680157*
S. delicatula (Desv. ex Poir.) Alston	Gao X.F. et al. HGX10734 (CDBI)	China, Sichuan	KT161441	–	–
S. dianzhongensis X.C. Zhang	Zhu Y.M. 8158 (PE)	China, Yunnan	MH814909	MH814853	MH814881
S. drepanophylla Alston	Zhang X.-C. 8229 (PE)	China, Yunnan	MH814894	MH814838	MH814866
S. heterostachys Baker	Zhang X.-C. 7088 (PE)	China, Guizhou	MH814896	MH814840	MH814868
S. kanehirae Alston	Wood 13568 (PTBG)	Micronesia, Caroline Is., Pohnpei	KT161495	–	–
S. kurzii Baker	Zhang X.-C. 1934 (PE)	China, Yunnan	MH814898	MH814842	MH814870
S. labordei Hieron. ex Christ	Zhang X.-C. 3356 (PE)	China, Hubei	MH814899	MH814843	MH814871
S. laxistrobila K.H. Shing	Chu W.-M. et al. 24449 (PYU)	China, Yunnan	KT161509	–	–
S. lutchuensis Koidzumi	TNS: 101683 (TNS)	Japan	MT680176*	MT680167*	MT680158*
S. lutchuensis Koidz.	TNS:759343	Japan	AB574648	–	–
S. megaphylla Baker	Jin X.-H. 19301 (PE)	China, Tibet	MH814901	MH814845	MH814873
S. minutifolia Spring	K. Larsen et al. 1389 (S)	Thailand	KY023082	–	–
S. monospora Spring	Zhang X.-C. 7889 (PE)	China, Guangxi	MH814902	MH814846	MH814874
S. morganii Zeiller	P. Korall 2006-29 (S)	Malaysia	KY023088	–	–
S. nipponica Franch. & Sav.	Zhou X.M. et al. DJY07479 (CDBI)	China, Sichuan	KT161542	–	–
S. ornata (Hook. & Grev.) Spring	Zhang X.-C. 8520 (PE)	China, Yunnan	MH814903	MH814847	MH814875
S. subvaginata Shalimov et X.C. Zhang	Liu H. 182 (PE)	China, Sichuan	MT680177*	MT680168*	MT680159*
S. subvaginata Shalimov et X.C. Zhang	Liu H. 183 (PE)	China, Sichuan	MT680178*	MT680169*	MT680160*
S. subvaginata Shalimov et X.C. Zhang	Zhang X.-C. et al. 8838 (PE)	China, Sichuan	MT680179*	MT680170*	MT680161*
S. subvaginata Shalimov et X.C. Zhang	Zhang X.-C., Xiang Q.-P. 6947 (PE)	China, Sichuan	MT680180*	MT680171*	MT680162*
S. subvaginata Shalimov et X.C. Zhang	Zhang X.-C. et al. 9450 (PE)	China, Sichuan	MT680181*	MT680172*	MT680163*
S. repanda (Desv. ex Poir.) Spring	Zhang X.-C. 5655 (PE)	China, Yunnan	MH814904	MH814848	MH814876
S. repanda (Desv. ex Poir.) Spring	Zhang X.-C. 9273 (PE)	China, Yunnan	MH814905	MH814849	MH814877
S. repanda (Desv. ex Poir.) Spring	Li B.G. sn_20 (PE)	China, Yunnan	MH814906	MH814850	MH814878
S. trichophylla K.H. Shing	Wu Y.D. 427 (PE)	China, Guizhou	MH814895	MH814839	MH814867
S. vaginata Spring	B. Bartholomew et al. 367 (PE)	China, Guizhou	MT680182*	MT680173*	MT680164*
S. vaginata Spring	Zhang X.-C. 4928 (PE)	China, Xizang	MT680183*	MT680174*	MT680165*
S. vaginata Spring	Zhang Z.S. 161 (PE)	China, Shaanxi	MH814907	MH814851	MH814879
S. xipholepis Baker	Zhang X.-C. 7422.(PE)	China, Guizhou	MH814908	MH814852	MH814880

Herbarium acronyms follow index Herbariorum. '–' indicates missing data, and '*' indicates newly obtained sequences in this study.