

Selaginella austrotibetica (Selaginellaceae), a new spikemoss from Xizang, China

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Selaginella austrotibetica, a new spikemoss species from China, is described. The species is epipetric and was found on a moss-covered rocky cliff by a riverbank. Morphologically, it is unlike any anisosporophyllous species in the genus *Selaginella*. However, it resembles species of the *S. sanguinolenta* group, especially similar to *S. sajanensis* in gross morphology, except for the characters of strobilus. The molecular phylogenetic analysis clearly showed that *S. austrotibetica* was nested within the anisosporophyllous species clade, forming a sister relationship with the clade of *S. vaginata* and *S. xipholepis*, and distinct from the *S. sanguinolenta* group, including *S. sajanensis*. Although *S. austrotibetica* is sister to the clade of *S. vaginata* and *S. xipholepis*, the species can be clearly distinguished by its sparse leaves on branches, dorsal leaves that are broad-ovate, base obtuse to rounded, margin denticulate or very shortly sparsely ciliolate, and leaves that are slightly thick and shiny. Therefore, we described *Selaginella austrotibetica* as a new species and discuss its phylogenetic relationships within the genus *Selaginella*.

Keywords: new species, *rbcL*, *Selaginella austrotibetica*, spikemoss, taxonomy

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INTRODUCTION

The cosmopolitan lycophyte genus *Selaginella* P. Beauv. includes ca. 750 species and is mainly distributed in humid tropical and subtropical regions (Jermy, 1986; 1990; Zhang, 2004; Zhang *et al.*, 2013). Recent molecular phylogenetic studies supported the latest infrageneric classification of the genus *Selaginella*, dividing it into seven subgenera (Weststrand and Korall, 2016; Zhang *et al.*, 2020a; Tang *et al.*, 2023). Most species in *Selaginella* have uniform, tetragonal strobili formed by isomorphic sporophylls (isosporophylls); the sporophylls are morphologically similar in shape and size, but some 60 species have dimorphic sporophylls (anisosporophylls) (Jermy,

1990; Zhang, 2004; Zhang *et al.*, 2013). The anisosporophyllous species were previously classified into an independent subgenus *Heterostachys* Baker based on the dimorphic-sporophyll morphology (Baker, 1883; Jermy, 1990); however, molecular phylogenetic studies resolved that the anisosporophyllous *Selaginella* species belong to subg. *Stachygynandrum* (P. Beauv. ex Mirb.) Baker (Weststrand and Korall, 2016; Zhang *et al.*, 2020a; Tang *et al.*, 2023).

China is the center of species diversity for anisosporophyllous *Selaginella*, with more than 40 species (Zhang, 2004; Zhang *et al.*, 2013; Sun *et al.*, 2015; Wu *et al.*, 2017; Shalimov *et al.*, 2019; Zhang *et al.*, 2020b; Huang *et al.*, 2022; Shalimov and Zhang, 2022a; 2022b; Wei *et*



Fig. 1. Habitats of *Selaginella austrotibetica* and *S. vaginata*. A: *S. austrotibetica* collected from Xizang; B: *S. vaginata* collected from Yunnan.

al., 2023). During our recent field studies, an unknown anisosporophyllous *Selaginella* species was collected from Xizang (Tibet). It is morphologically unlike any other anisosporophyllous species, such as the arrangement of leaves on stems and branches, and the shape of ventral and dorsal leaves. In contrast, the plant exhibits morphol-

ogy similar to that of an isosporophyllous *Selaginella* group, the *S. sanguinolenta* group, with its short creeping habitat and dark green, thick, and glossy leaves. To examine whether the plant is a new species or not, we performed a morphological comparison and phylogenetic analysis of *Selaginella*, including both anisosporophyl-

lous and isosporophyllous species. Our results demonstrated that the plant is a distinct species, unlike any other *Selaginella* species.

MATERIALS AND METHODS

Morphology

Field observation was conducted by the collectors of the type specimens in Xizang, China. Morphological observations were conducted using a Leica S9D stereoscopic microscope (Leica Microsystems). The morphology of the newly collected specimens was examined and compared to specimens from the herbarium of the Institute of Botany, CAS (PE) of *S. sajanensis* Stepanov & Sonnikova, *S. vaginata* Spring, and *S. xipholepis* Baker.

Phylogenetic analysis

A total of 76 individuals, representing seven subgenera of *Selaginella*, were used for phylogenetic analysis, with *Isoetes flaccida* Shuttlew. ex A. Braun as the outgroup. Detailed information of voucher specimens and NCBI GenBank accession numbers is listed in Appendix 1. Phylogenetic reconstruction was performed using plastid *rbcL* sequences.

For the new species, we isolated total genomic DNA from silica gel-dried materials using the Plant Genomic DNA Kit DP305 (TianGen Biotech, Beijing, China), following the manufacturer's protocol. Library construction and sequencing were performed at Biomarker Technologies Corporation (Beijing, China). Approximately 6 GB sequencing reads of the new species were obtained using the Illumina NovaSeq 6000 platform. The plastid genome was initially assembled by GetOrganelle v. 1.5.2a (Jin *et al.*, 2020) with the plastid genome sequence of *S. sajanensis* (OL813819). Gene annotation for the assembled contigs of the plastome was done by comparing the plastid genome of *S. sajanensis* in Geneious v. 11.1.4 (Kearse *et al.*, 2012), and the *rbcL* gene sequence was extracted for phylogenetic analysis.

The *rbcL* sequences of 76 accessions were aligned using MUSCLE (Edgar, 2004), followed by manual adjustment in PhyDE v0.9971 (Müller *et al.*, 2010). A maximum likelihood (ML) analysis was performed using RAxML 7.2.6 (Stamatakis, 2006), with 1000 bootstrap replicates under the GTR + G model. The best-fit substitution model was determined for the datasets using jModelTest v.0.1 (Posada, 2008). The ML tree and the branch support values were visualized using FigTree v. 1.4.2 (Rambaut, 2014).

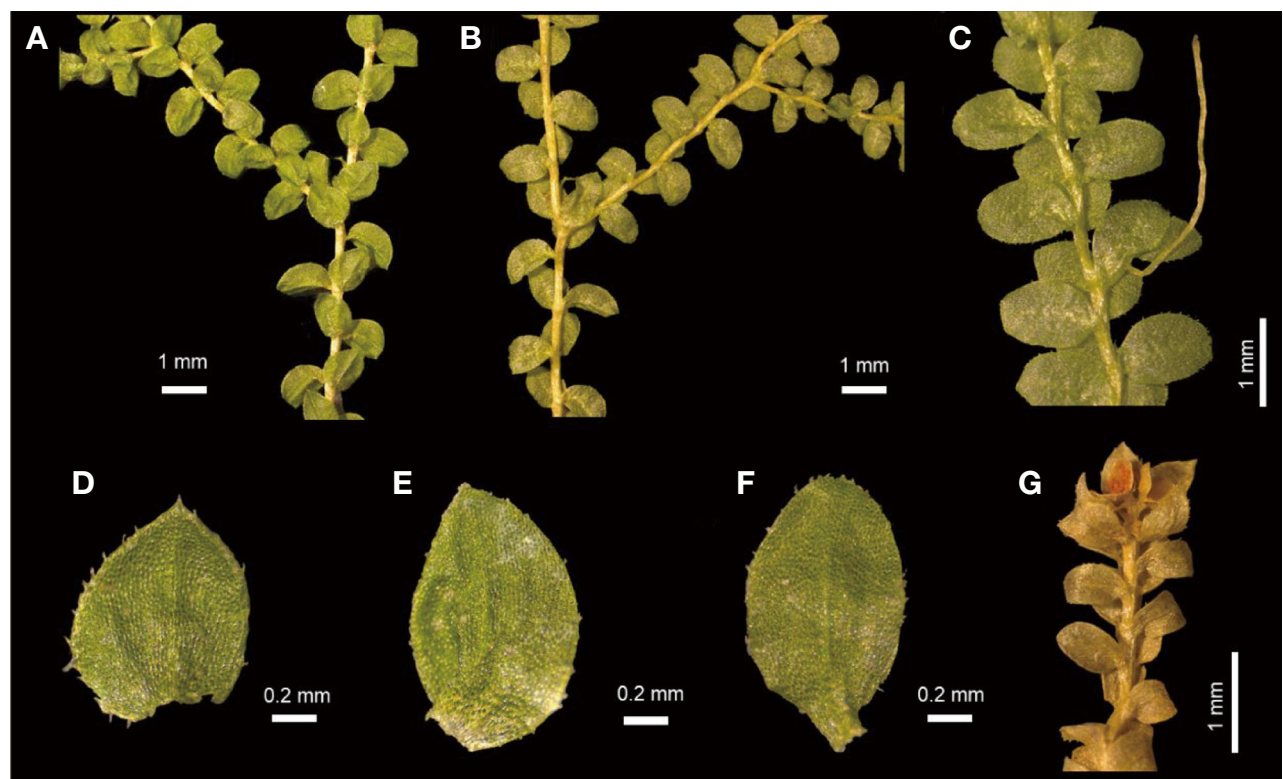


Fig. 2. Morphology of *Selaginella austrotibetica* X. C. Zhang. A: Adaxial view of branchlet; B: Abaxial view of branchlet; C: Rhizophore on ventral side; D: Dorsal leaf; E: Ventral leaf; F: Axillary leaf; G: Strobilus (a portion).

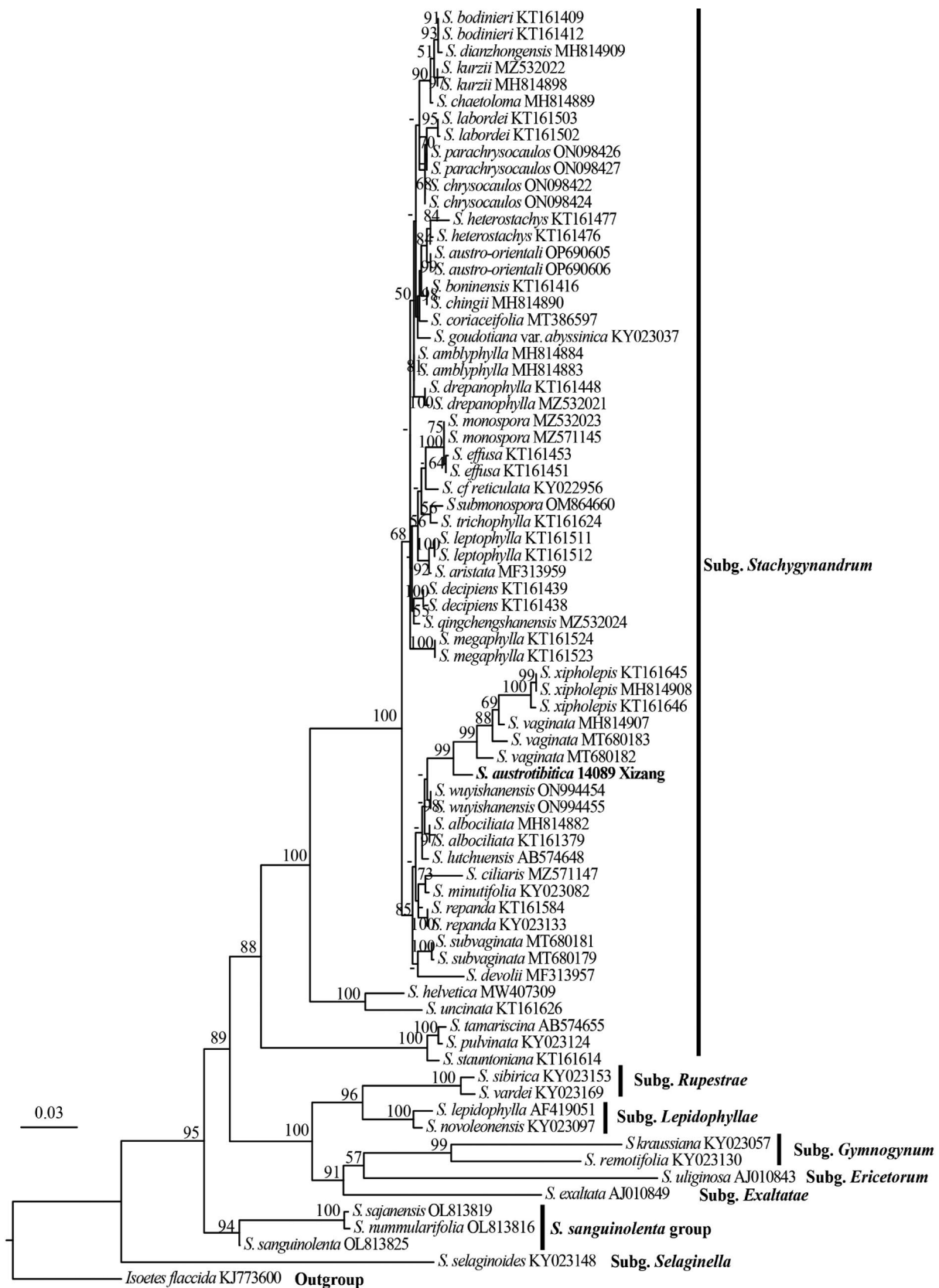


Fig. 3. The maximum likelihood (ML) tree of the new species and related species. Maximum likelihood bootstrap values ($\geq 50\%$) are shown on the branches. The new species is shown in bold.

RESULTS

Taxonomic treatment

Selaginella austrotibetica X.C. Zhang, sp. nov.
(Figs. 1A and 2) 藏南卷柏 (Zangnan Juanbai)

Type. CHINA, Xizang, Mêdog, on moss-covered rocky slope, 95.50°E, 29.66°N, altitude 2067 m, 22 December 2022, X.C. Zhang *et al.*, 14089 (Holotype, PE!).

Diagnosis. This new species is similar to *S. sajanensis*, such as creeping and slightly thick and shiny leaves, but distinct from it by having anisosporephylls, ventral and dorsal leaves that are ovate or broad-ovate, and an acute apex.

Description. Plant epilithic, seasonally green, shortly creeping. Rhizophores restricted to lower part of stem, borne on ventral side in axils of branches, 0.1 mm in diam., 2.5–3.0 mm in length. Main stems branched throughout, terete, not sulcate, glabrous, branchlets sparse. Axillary leaves on branches symmetrical or slightly asymmetrical, ovate, 1.1–1.5 × 0.6–0.7 mm, slightly carinate, base exauriculate, margin entire in basal half, upward slightly ciliolate. Dorsal leaves slightly ascending, asymmetrical, broad-ovate, 0.7–1.0 × 0.6–0.8 mm, slightly carinate, base obtuse to rounded, margin denticulate or very shortly sparsely ciliolate, not membranous, apex acute, the distance between upper and lower two adjacent dorsal leaves are about 2.0 mm. Ventral leaves slightly ascending, asymmetrical, ovate, 1.0–1.3 × 0.6–0.8 mm, margin almost entire or denticulate, apex acute, and the distance between the upper and lower two adjacent ventral leaves is about

1.0 mm. Strobili solitary, terminal; sporophylls slightly dimorphic, dorsal sporophylls asymmetrical, broad-ovate, 0.8–1.3 × 0.5–0.7 mm; ventral sporophylls ovate, 0.8–1.3 × 0.5–0.7 mm. Sporangia only borne on the ventral sporophylls in the few already drying and incomplete strobili, only microsporangia are found, microspores yellowish orange.

Distribution and habitat. *Selaginella austrotibetica* is known so far only from its type location in Mêdog County, Xizang, China, where it creeps on moss-covered rocky cliffs.

Etymology. The specific epithet ‘austrotibetica’ refers to its location at South Xizang.

Conservation status. *Selaginella austrotibetica* was found only in a small area with few individuals in Mêdog County, Xizang. Therefore, this species should be considered as Critical Endangered (CR), according to the IUCN Red List guidelines.

Phylogenetic analysis

The aligned length of the plastid *rbcL* gene sequences was 969 bp. Our molecular phylogenetic result showed that each subgenus was monophyletic (Fig. 3), and the topology was consistent with previous studies in which plastid *rbcL* sequences were used for phylogenetic reconstruction (Zhou and Zhang, 2015; Weststrand and Korall, 2016). In the phylogeny, *S. austrotibetica* was located within subg. *Stachygynandrum*, and sister to the clade of *S. vaginata* and *S. xipholepis*, with strong branch support (BS = 99%) (Fig. 3).

Table 1. Character comparison of *S. austrotibetica*, *S. sajanensis*, and *S. vaginata*.

Characters/Species	<i>S. austrotibetica</i>	<i>S. sajanensis</i>	<i>S. vaginata</i>
Habit	stem creeping	stem creeping	stem creeping, fertile stems erect
Axillary leaves	ovate, 1.1–1.5 × 0.6–0.7 mm, margin entire in basal half, upward slightly ciliolate	obovate to flabellate, ca. 1.29 × 0.8 mm, margin entire	ovate-triangular, 1.2–2.5 × 0.5–1.5 mm, margin long ciliolate at base
Dorsal leaves	broad-ovate, 0.7–1.0 × 0.6–0.8 mm, pex acute, base obtuse to rounded, margin denticulate or very shortly sparsely ciliolate	obovate to flabellate, ca. 1.0 × 0.58 mm, apex round and 2–5 cuspidate, base peltate and slightly reflexed, margin entire or sparsely ciliolate	ovate-lanceolate or triangular, 0.8–2.4 × 0.4–1.2 mm, apex acuminate or aristate, base subcordate, cuneate, or obtuse, both inner and outer margin long-ciliolate
Ventral leaves	ovate, 1.0–1.3 × 0.6–0.8 mm, apex acute, margin almost entire or denticulate	obovate to flabellate, ca. 1.28–1.6 × 0.6–1 mm, apex round, margin entire or sparsely ciliolate	ovate-lanceolate or oblong-falcate, 1.6–3.2 × 0.8–1.5 mm, apex acute, margin sparsely long ciliolate at base
Sporophylls	slightly anisophyllous	isophyllous	slightly anisophyllous

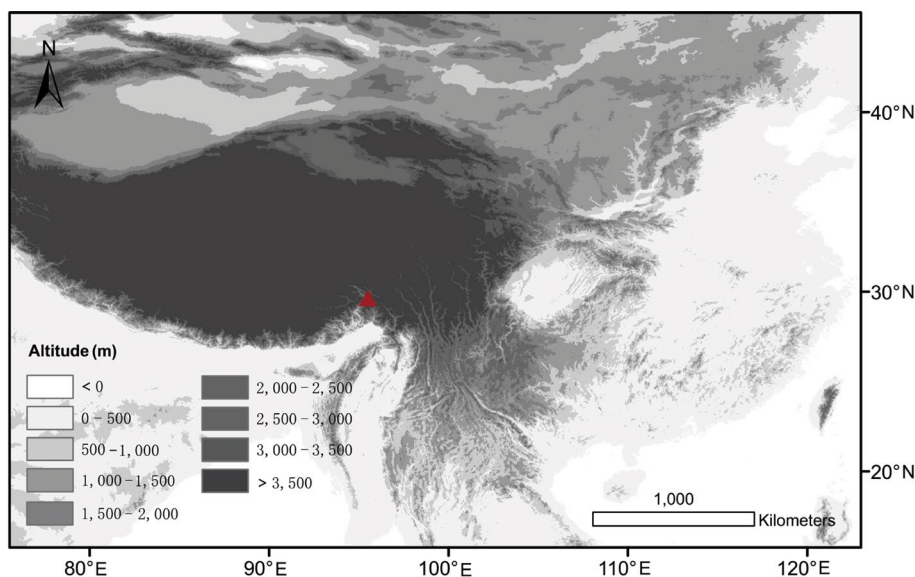


Fig. 4. Distribution of *Selaginella austrotibetica* X. C. Zhang, sp. nov.

DISCUSSION

Morphologically, *S. austrotibetica* is superficially similar to species in the *S. sanguinolenta* group, particularly a Siberian species, *S. sajanensis*, having slightly thick and shiny leaves, but their sporophylls differ (Figs. 1 and 2, Table 1). The sporophylls of *S. austrotibetica* are slightly dimorphic (anisosporophylls), which is the typical character of the *Heterostachys* group in Subg. *Stachygynandrum*, whereas *S. sajanensis* has isomorphic sporophylls. These two species can be distinguished by their axillary and dorsal leaves as well. *Selaginella austrotibetica* has ovate axillary leaves and a margin with a few ciliolate, but *S. sajanensis* has obovate to flabellate axillary leaves and an entire margin. The dorsal leaves of *S. austrotibetica* are broad-ovate, slightly carinate, margin denticulate or very shortly sparsely ciliolate, and apex acute, whereas those of *S. sajanensis* are obovate to flabellate (not carinate), margin entire or sparsely ciliolate, and apex round with 2–5 cuspidate (Table 1). Geographically, *S. austrotibetica* is only known to be distributed in Xizang, China (Fig. 4), while *S. sajanensis* is endemic to south Siberia (Stepanov *et al.*, 2005).

Our phylogenetic result based on the plastid *rbcL* gene revealed a sister relationship between *S. austrotibetica* and the clade of *S. vaginata* and *S. xipholepis*, and the *S. sanguinolenta* group was phylogenetically distinct from the anisosporophyllous *Selaginella* species (Fig. 3). Species delimitation and relationships between *S. vaginata* Spring (synonym: *S. compta* Hand.-Mazz.) and *S. xipholepis* Baker are still unresolved, but these two species are distinguished from *S. austrotibetica* by densely set leaves on

stems and branches, long ciliolate leaf margins, and ovate-triangular axillary leaves (Fig. 1B). Therefore, by considering the morphological characters and phylogenetic evidence, *S. austrotibetica* should be treated as an independent species in the genus *Selaginella*.

CONFLICTS OF INTEREST

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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

Taxon	Voucher	Country	<i>rbcL</i>
<i>Isoetes flaccida</i>	Abbott 20265 (FLAS)	–	KJ773600
<i>S. albociliata</i>	Zhang L.B. 5302 (CDBI)	China, Guangxi	KT161379
<i>S. albociliata</i>	Zhang X.C. 7242 (PE)	China, Guizhou	MH814882
<i>S. amblyphylla</i>	Zhang X.C. 2924 (PE)	China, Yunnan	MH814883
<i>S. amblyphylla</i>	Zhang X.C. 7951 (PE)	China, Yunnan	MH814884
<i>S. aristata</i>	Chang H.M. <i>et al.</i> 7119 (TAIE)	China, Taiwan	MF313959
<i>S. austro-orientali</i>	Jin <i>et al.</i> 7965 (CSH, IBK, PE, PYU)	China, Jiangxi	OP690605
<i>S. austro-orientali</i>	Jin <i>et al.</i> 7982 (CSH),	China, Jiangxi	OP690606
<i>S. austrotibetica</i>	Zhang X.C. <i>et al.</i> 14089 (PE)	China, Xizang	OR474089*
<i>S. bodinieri</i>	Zhang 5193 (CDBI)	China, Guizhou	KT161409
<i>S. bodinieri</i>	Zhou <i>et al.</i> HGX11073 (CDBI)	China, Sichuan	KT161412
<i>S. boninensis</i>	Zhang <i>et al.</i> 6594 (CDBI, MO, VNMN, PYU)	Vietnam, Lang Son	KT161416
<i>S. cf. reticulata</i>	Fraser-Jenkins C.R. 1653 (L)	Indonesia, Java	KY022956
<i>S. chaetoloma</i>	Zhang X.C. 7347 (PE)	China, Guizhou	MH814889
<i>S. chingii</i>	Zhang X.C. 7904 (PE)	China, Guangxi	MH814890
<i>S. chrysocaulos</i>	Zhang S.R. 133 (PE)	Nepal	ON098422
<i>S. chrysocaulos</i>	Zhang S.R. 5 (PE)	Nepal	ON098424
<i>S. ciliaris</i>	Knapp 2351 (P)	China, Taiwan	MZ571147
<i>S. coriaceifolia</i>	Zhang L.B. <i>et al.</i> 7347 (CDBI, MO)	Vietnam, Quang Binh	MT386597
<i>S. decipiens</i>	Zhang <i>et al.</i> 6764 (CDBI, MO, VNMN, PYU)	Vietnam, Bac Kan	KT161438
<i>S. decipiens</i>	Zhang <i>et al.</i> 6761 (CDBI, MO, VNMN, PYU)	Vietnam, Bac Kan	KT161439
<i>S. devolii</i>	Chang H.M. <i>et al.</i> 7116 (TAIE)	China, Taiwan	MF313957
<i>S. dianzhongensis</i>	Zhu Y.M. 8158 (PE)	China, Yunnan	MH814909
<i>S. drepanophylla</i>	Zhang 5862 (CDBI)	China, Guizhou	KT161448
<i>S. drepanophylla</i>	Zhang L.B. <i>et al.</i> 5458 (CDBI)	China, Guizhou	MZ532021
<i>S. effusa</i>	Zhang 5442 (CDBI)	China, Guangxi	KT161451
<i>S. effusa</i>	Dong 2470 (PYU)	China, Guangdong	KT161453
<i>S. exaltata</i>	Korall 1996-1 (S)	Ecuador	AJ010849
<i>S. goudotiana</i> var. <i>abyssinica</i>	Kornas J. 1172A (BR)	Tanzania	KY023037
<i>S. helvetica</i>	Xiang Q.P. s.n. (PE)	China, Heilongjiang	MW407309
<i>S. heterostachys</i>	Zhang 5180 (CDBI)	China, Guangxi	KT161476
<i>S. heterostachys</i>	Gao <i>et al.</i> DJY05340 (CDBI)	China, Sichuan	KT161477
<i>S. kraussiana</i>	Mokoso M. 3098 (BR)	Congo, South Kivu	KY023057
<i>S. kurzii</i>	Zhang X.C. 1934 (PE)	China, Yunnan	MH814898
<i>S. kurzii</i>	PYUS2105 (PYU)	China, Yunnan	MZ532022
<i>S. labordei</i>	Gao <i>et al.</i> DJY04311 (CDBI)	China, Sichuan	KT161502
<i>S. labordei</i>	Li C.H. 9667 (PE)	China, Sichuan	KT161503
<i>S. lepidophylla</i>	–	–	AF419051
<i>S. leptophylla</i>	Zhang 5199 (CDBI)	China, Guangxi	KT161511
<i>S. leptophylla</i>	Zhou 011 (CDBI)	China, Sichuan	KT161512
<i>S. lutchuensis</i>	TNS:759343	Japan	AB574648
<i>S. megaphylla</i>	Zhang 5185 (CDBI)	China, Guangxi	KT161523
<i>S. megaphylla</i>	Zhang 5200 (CDBI)	China, Guangxi	KT161524
<i>S. minutifolia</i>	Larsen K. <i>et al.</i> 1389 (S)	Thailand	KY023082
<i>S. monospora</i>	Zhang L. 1296	China, Yunnan	MZ532023
<i>S. monospora</i>	Zhang L.B. <i>et al.</i> 7425 (CDBI)	Vietnam, Quang Binh	MZ571145
<i>S. novoleonensis</i>	Drouet F. & Richards D. 3942 (S)	Mexico, Sonora	KY023097
<i>S. nummularifolia</i>	Zhang X.C. & Wang L. 4642 (PE)	China, Xizang	OL813816
<i>S. parachrysocaulos</i>	Zhang X.C. <i>et al.</i> 2154 (PE)	China, Yunnan	ON098426

Appendix 1. Continued.

Taxon	Voucher	Country	<i>rbcL</i>
<i>S. parachrysocaulos</i>	Zhang X.C. 2427 (PE)	China, Sichuan	ON098427
<i>S. pulvinata</i>	Boufford D.E. <i>et al.</i> 37879 (A)	China, Sichuan	KY023124
<i>S. qingchengshanensis</i>	Liang Z.L. & Pu X. 097 (CDBI, PYU)	China, Sichuan	MZ532024
<i>S. remotifolia</i>	Gaoligong Shan Biodiversity Survey 21081 (GH)	China, Yunnan	KY023130
<i>S. repanda</i>	He & Jiang 405-1 (CDBI)	China, Yunnan	KT161584
<i>S. repanda</i>	Leeuwenberg A.J.M. & v. Meer P.P.C. 13009 (L)	Indonesia, Java	KY023133
<i>S. sajanensis</i>	Shaulo D.N. s.n. (ABGI)	Russia	OL813819
<i>S. sanguinolenta</i>	Zhang X.C. & Xiang Q.P. 9345 (PE)	China, Heilongjiang	OL813825
<i>S. selaginoides</i>	S. Weststrand 104 (UPS)	Sweden	KY023148
<i>S. sibirica</i>	Viereck L.A. & Jones K. 5667 (S)	Alaska	KY023153
<i>S. stauntoniana</i>	Zhao 169 (CDBI)	China, Beijing	KT161614
<i>S. submonospora</i>	Zhang X.C. <i>et al.</i> 8111 (PE)	China, Yunnan	OM864660
<i>S. subvaginata</i>	Zhang X.C. <i>et al.</i> 8838 (PE)	China, Sichuan	MT680179
<i>S. subvaginata</i>	Zhang X.C. <i>et al.</i> 9450 (PE)	China, Sichuan	MT680181
<i>S. tamariscina</i>	TNS:759348	Japan	AB574655
<i>S. trichophylla</i>	Zhang <i>et al.</i> 6784 (CDBI, MO, VNMN, PYU)	Vietnam, Cao Bang	KT161624
<i>S. uliginosa</i>	Holmgren & Wanntorp 253 (S)	–	AJ010843
<i>S. uncinata</i>	Zhang & Zhou DJY04101 (CDBI)	China, Sichuan	KT161626
<i>S. vaginata</i>	Zhang Z.S. 161 (PE)	China, Shaanxi	MH814907
<i>S. vaginata</i>	Bartholomew B. <i>et al.</i> 367 (PE)	China, Guizhou	MT680182
<i>S. vaginata</i>	Zhang X.C. 4928 (PE)	China, Xizang	MT680183
<i>S. vardei</i>	Boufford D.E. <i>et al.</i> 32425 (A)	China, Xizang	KY023169
<i>S. wuyishanensis</i>	Xu K.W. WY598 (PYU)	China, Fujian	ON994454
<i>S. wuyishanensis</i>	Wei H.J. JSL7744A (CSH)	China, Fujian	ON994455
<i>S. xipholepis</i>	Dong 2377 (PYU)	China, Guangdong	KT161645
<i>S. xipholepis</i>	Zhang <i>et al.</i> 6668 (CDBI, MO, VNMN, PYU)	Vietnam, Bac Kan	KT161646
<i>S. xipholepis</i>	Zhang X.C. 7422 (PE)	China, Guizhou	MH814908

Notes. ‘–’ indicates missing data, and ‘**’ indicates newly obtained sequences in this study.