

Selaginella orientali-chinensis, a new resurrection spikemoss species from southeastern China based on morphological and molecular evidences*

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Abstract: *Selaginella tamariscina* (Beauv.) Spring and *S. pulvinata* (Hook. et Grev.) Maxim. are two closely related rosette forming and resurrection spikemoss species widespread in Asia. In this paper, *Selaginella orientali-chinensis* Ching et C. F. Zhang ex H. W. Wang et W. B. Liao was described and illustrated. This species was closely related to *S. tamariscina* and *S. pulvinata* and previously misidentified as *S. tamariscina*. By the gross morphological and molecular evidences, we found that this species is easily distinguished from *S. tamariscina* and *S. pulvinata* by the absolutely dichotomously branched main stems. Our field works confirmed that this species is widely distributed in southeastern China and mainly inhabits Danxia landforms areas. In some cases, this species shares the same habitats with *S. tamariscina*. Illustrating the morphological, phylogenetic and distribution characters of this new species would provide new data for the taxonomic studies on the genus *Selaginella* in the future.

Key words: *Selaginella*; Danxia landforms; morphology; phylogeny; distribution

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中国东南部复苏卷柏一新种: 东方卷柏

——基于形态学和分子生物学证据

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摘要: 卷柏和垫状卷柏是两种近缘的莲座状复苏卷柏, 两者在亚洲皆有广阔的分布区域。本研究描述并修订了东方卷柏这一新种; 该种是卷柏和垫状卷柏的近缘种, 此前常被错误鉴定为卷柏。东方卷柏与卷柏和垫状卷柏最大的形态差异在于东方卷柏的主茎为二叉分枝, 而卷柏和垫状卷柏的主茎为羽状分支。形态学和分子系统发育数据分析强烈支持卷柏、垫状卷柏和东方卷柏各自独立的物种地位。通过查阅以往被错误鉴定的东方卷柏

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标本, 可知该种广泛地分布于华东和华南地区。此外, 野外调查表明该新种主要生长于丹霞地貌区, 有时会和卷柏混生。本文对东方卷柏新种的形态、系统发育和地理分布进行了描述, 这将有助于未来的植物学和生态学野外工作者开展针对性的相关研究。东方卷柏学名的种加词 *orientali-chinensis* 是我国蕨类分类学家秦仁昌及张朝芳 1982 年在标本台纸上对该种作出的鉴定, 使用的是裸名, 没有描述, 本文在此正式合格发表继续采用了该名, 以资纪念。

关键词: 卷柏属; 丹霞地貌; 形态学; 系统发育; 地理分布

Among the three extant lycophyte families, the largest one (Selaginellaceae, or spikemoss) contains only one genus *Selaginella* but more than 700 living species, which occupy a variety of habitats in every continent except Antarctic^[1-4]. Some spikemoss species are well-known for their capability of desiccation tolerance (DT) and resurrection. For example, members of the *Rosulatae* and *S. lepidophylla* clades that newly validated by Zhou et al^[1] can survive under complete dehydration for as long as a decade and have therefore gained attention by researchers who focus on the mechanism of plant DT^[5-7]. In Asia, the extensive seasonally arid environments are ideal habitats of resurrection spike mosses, such as *S. tamariscina* and *S. pulvinata*^[8-9]. These two species resemble each other in morphology and are widely distributed across Siberia, Mongolia, East Asia, Qinghai-Tibet Plateau, Indian Subcontinent, Indo-China Peninsula, and Philippines, but occurred in different habitats. *S. pulvinata* is usually in alpine areas, while *S. tamariscina* prefers lower elevation and warmer environment^[10].

Among specimens of *S. tamariscina* collected from Danxiashan National Geological Park (Guangdong Province, China) in 2016, several distinct specimens were selected out for further analysis, and confirmed to be an unrecognized species closely related to *S. tamariscina* and *S. pulvinata* based on morphological and molecular phylogenetic evidences. To obtain more distribution information of this new species and its two closely related species, we attempted to examine specimens of *S. tamariscina* and *S. pulvinata* deposited in several herbaria (CSFI, CSH, CZH, GXMG, FJFC, IBSC, JIU, LBG, PE, SYS). To our surprise, dozens of specimens of this new species which were ever misidentified as *S. tamariscina*, were screened out. Interestingly, two specimens in PE labeled with “typus” indicated that this

species had ever been informally denominated as “*Selaginella orientali-chinensis*” by R. C. Ching and C. F. Zhang in 1982 (H. H. Chung 979 (PE)), but was treated as *S. tamariscina* again in 1991 by X. C. Zhang. Here, we describe and illustrate this new species, *Selaginella orientali-chinensis* Ching et C. F. Zhang ex H. W. Wang et W. B. Liao.

1 Method

1.1 Samples collection and morphological analysis

A total 34 samples, including 14 of *S. orientali-chinensis*, 14 of *S. tamariscina* and 6 of *S. pulvinata*, were collected from six locations for molecular and morphology analysis (Table 1). Morphological features were examined from fresh samples and dried specimens. Voucher specimens were deposited at Sun Yat-sen University Herbarium (SYS).

1.2 DNA extraction, sequencing and sequence treatment

A green portion of each individual was cleaned with tap water and sterilized with commercial bleach. Genomic DNA was extracted with the CTAB protocol^[11]. ITS and *rbcL* fragments were amplified with primer pairs ITS 1 and ITS 4^[12] and *rbcL* 192 F and *rbcL* 1324 R^[3], respectively, in the 20 μ L reactions containing 0.2 mmol/L of each dNTP, 1.0 μ mol/L of each primer, 1 \times EasyTaq Buffer, 1 U EasyTaq and 1 μ L of DNA extract. PCR conditions are as described by Wu et al^[3]. Amplicons were resolved by 1% agarose gel and those with the expected sizes (ca. 650 bp for ITS and ca. 1 kb for *rbcL*) were purified and sequenced with ABI 3730XL analyzer (Applied Biosystems, Foster City, California, USA). Sequences of *rbcL* and ITS were deposited in GenBank (accession numbers and voucher see Table 1).

1.3 Phylogenetic analysis

Using the ITS and *rbcL* sequences of the new

species as query, BLASTn searches were performed on the NCBI website, the top hits were obtained from *S. tamariscina* and *S. pulvinata*. Previous research on the phylogeny of *Selaginella*^[1,3] showed that in the clade Rosulatae, *S. tamariscina* clustered first with *S. pulvinata*, then *S. stauntoniana*, and then a monogroup including *S. imbricata*, *S. pilifera*, *S. helioclada* and *S. digitata*). So ITS and *rbcL* sequences of the above mentioned species were down-

loaded from NCBI website (accession number see Table 1). Together with those obtained in this study, the sequences were aligned with SeqMan, DNA-Star^[13] and checked manually. Phylogeny trees based on ITS and *rbcL* sequences were constructed with the maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. MP analysis was performed in PAUP* v. 4.0b10^[14] and all characters were weighted equally. The most parsim-

Table 1 Materials and the GenBank accession number of ITS and *rbcL* sequences obtained in this study

Taxon name	Locality	Collection number	ITS	<i>rbcL</i>
<i>Selaginella pulvinata</i>	KY1 - KY6 Kunming, Yunnan	<i>Q. Fan 17124</i> (SYS)	MW035052- MW035057	MW119324- MW119329
	Sichuan, China	<i>Zhou 066</i> (CDBI)	—	KT161576*
	Xizang, China	<i>Zhou 501</i> (PYU, CDBI)	—	KT161577*
<i>Selaginella orientali-chinensis</i>	RG1 - RG5 Renhua, Guangdong	<i>H-W. Wang and Y-S. Huang 18001</i> (SYS)	MW035058- MW035062	MW119330- MW119334
	LZ1 - LZ3 Lishui, Zhejiang	<i>H-W. Wang and Y-S. Huang 18036</i> (SYS)	MW035063- MW035065	MW119335- MW119337
	GF1 - GF3 Guanzhaishan, Fujian	<i>H-W. Wang and Y-S. Huang 19046</i> (SYS)	MW035066- MW035068	MW119338- MW119340
	YJ1 - YJ3 Yanshan, Jiangxi	<i>H-W. Wang and Y-S. Huang 19050</i> (SYS)	MW035069- MW035071	MW119341- MW119343
<i>Selaginella tamariscina</i>	GF4 - GF6 Guanzhaishan, Fujian	<i>H-W. Wang and Y-S. Huang 19037</i> (SYS)	MW035072- MW035074	MW119344- MW119346
	RG6 - RG10 Renhua, Guangdong	<i>Q. Fan et al. 16984</i> (SYS)	MW035075- MW035078	MW119347- MW119350
		<i>H-W. Wang and Y-S. Huang 18006</i> (SYS)	MW035079	MW119351
	ZG1 - ZG3 Zijing, Guangdong	<i>H-W. Wang 18091</i> (SYS)	MW035080- MW035082	MW119352- MW119354
	FZ1 - FZ3 Fanyan, Zhejinag	<i>H-W. Wang and Y-S. Huang 19031</i> (SYS)	MW035083- MW035085	MW119355- MW119357
Okinawa, Japan		<i>TNS759348</i> (TNS)	—	AB574655*
<i>Selaginella stauntoniana</i>	—	<i>Kenrick s. n.</i> (S)	—	AJ295869*
	Henan, China	<i>Quan 1</i> (PYU)	—	KT161615*
	Beijing, China	<i>Zhao 169</i> (CDBI)	—	KT161614*
	—	<i>TKMVP00000949</i>	KY218783*	—
<i>Selaginella imbricata</i>	Dhofar, Governorate, Oman	<i>Rothfels et al. 4275</i> (DUKE)	—	KT161486*
<i>Selaginella helioclada</i>	d'Andohahela, Madagascar	<i>Rakotondrainibe 3262</i> (P)	—	AJ295896*
<i>Selaginella digitata</i>	d'Andohahela, Madagascar	<i>Rakotondrainibe 3255</i> (P)	—	AJ295895*
<i>Selaginella pilifera</i>	—	<i>Pringle 13959</i> (S)	—	AJ295862*

A: dash (—) indicates missing data; B: asterisk (*) indicates that the data is from the NCBI database.

monious trees were obtained with heuristic searches of 1 000 replicates random stepwise sequence addition (RAS) replicates, and MP bootstrap values (BS) were calculated with 1 000 replicates. ML analysis was performed in IQ-TREE^[15], and the appropriate nucleotides substitution model was searched by ModleFinder implemented in it^[15]. ML Bootstrap percentages (BP) were calculated based on 1 000 fast bootstrap replicates (bb=1 000). BI analyses was carried out in MrBayes v 3. 2. 7^[16]. Among the three supported models, F81 was selected for its best perform, and substitution rate was set as Gamma, while other parameters were set as default, and sampled the tree every 1 000 generations of 1 100 000 generations. The first 25% of samples were discarded as burn in, and the remaining trees were used to calculate a 50% majority-rule consensus topology and posterior probability (PP) values.

Further, an ILD test^[17] (incongruence length difference) was performed on ITS and *rbcL* databases and no conflict was detected between them. So ITS and *rbcL* sequences were concatenated to perform phylogenetic analysis to discriminate *S. tamariscina*, *S. pulvinata*, *S. orientali-chinensis*, using *S. stautoniana* as outgroup.

2 Results and discussion

The aligned ITS and *rbcL* sequences were 531 bp and 1 018 bp in length, and no heterozygous sites were detected. The phylogenetic tree constructed from ITS or *rbcL* sequences showed that *S. orientali-chinensis*, *S. tamariscina* and *S. pulvinata* formed a monogroup as expected, though species relationship among the three species was not strongly supported (Fig. 1). As ITS and *rbcL* sequences were concatenated, the phylogenetic tree showed that individuals of each species formed monogroups with high supports, *S. orientali-chinensis* clustered first with *S. tamariscina*, and then *S. pulvinata*, though with low supports (Fig. 2).

Although the plant form, sporangium and spore morphology are similar in the three species, *S. orientali-chinensis* can be distinguished from *S. tamariscina* and *S. pulvinata* easily. The branches

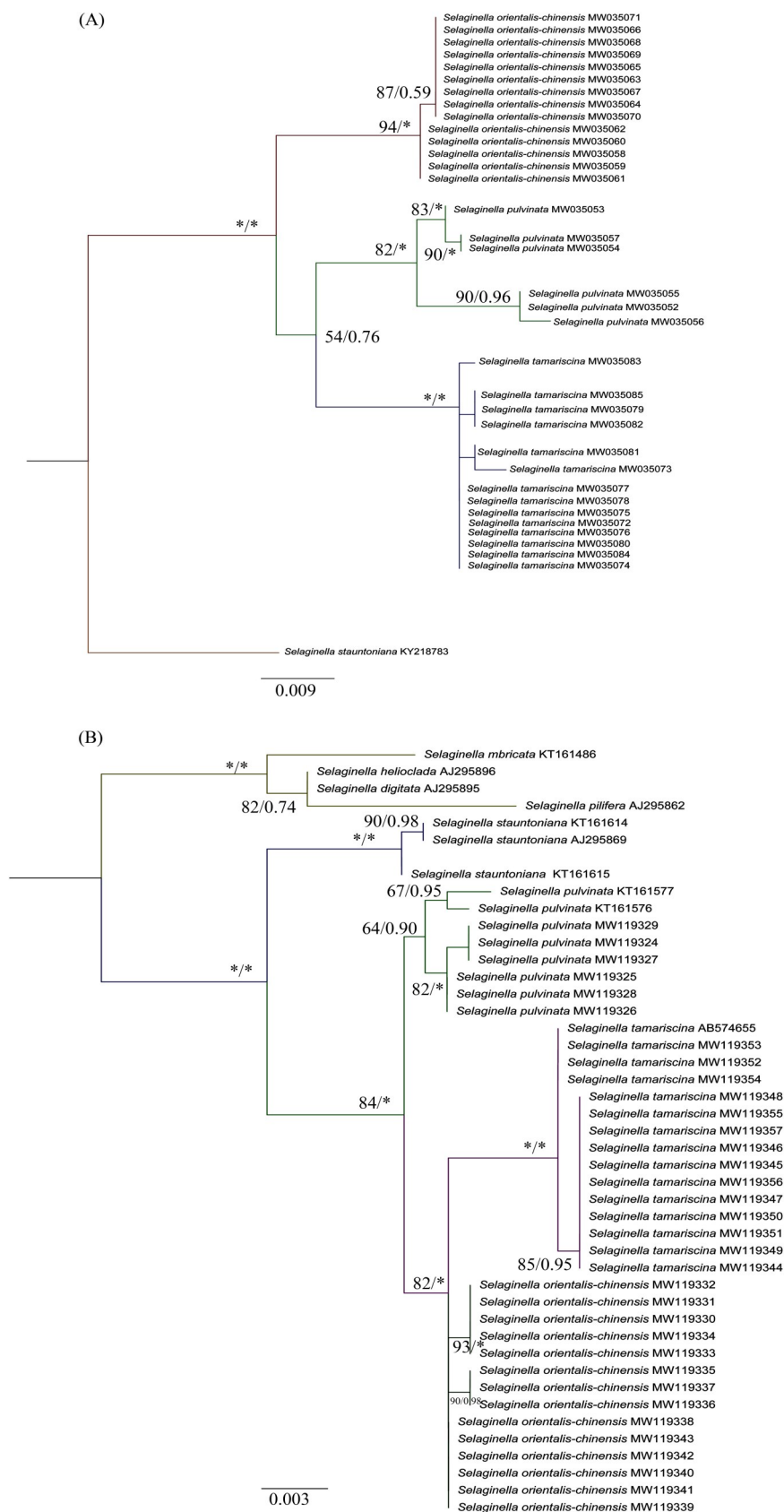
of *S. orientali-chinensis* were absolutely dichotomously branched, while the branches of *S. tamariscina* and *S. pulvinata* were pinnately branched (Figs. 3–5). Additionally, the differences among *S. orientali-chinensis*, *S. tamariscina* and *S. pulvinata* are also obvious. The former two species usually form a slender tree-like trunk mainly composed of rhizophores and roots, while *S. pulvinata* always forms compact cushion due to the persistent withered branches. According to our own field investigations and other specimens and photography records, *S. orientali-chinensis* is not as widespread as *S. tamariscina*, but is still widely distributed in Guangdong, Guangxi, Fujian, Hunan, Jiangxi, and Zhejiang provinces of China. *S. pulvinata* mainly inhabits cold and alpine environment, including the Himalayan, the Yunnan-Guizhou Plateau, West Sichuan, Qinling Mountains, Taihang Mountains, Northeast China and Siberia. While, *Selaginella orientali-chinensis* and *S. tamariscina* commonly prefer seasonally arid habitats, and in some cases, co-occurs (Fig. 5). However, *S. orientali-chinensis* is mainly distributed in Danxia landforms, while *S. tamariscina* can be observed in multiple landscapes.

3 Taxonomic treatment

Selaginella orientali-chinensis Ching et C. F. Zhang ex H. W. Wang et W. B. Liao, sp. nov.

1) **Type.** China. Guangdong: Renhua County, Danxiashan National Nature Reserve, 24°56'N, 113°45'E, 290 m a. s. l., 23 May 2020, *Q. Fan 17870* (holotype, SYS! isotypes IBSC! PE! SYS!) (Fig. 3–5).

2) **Diagnosis.** The new species is similar to *S. tamariscina* and *S. pulvinata* in plant form and the morphology of dorsal leaves, ventral leaves, and sporophylls. However, the main stems of *S. orientali-chinensis* are absolutely dichotomously branched, while those of *S. tamariscina* and *S. pulvinata* are pinnately branched. Additionally, *S. pulvinata* usually inhabits alpine, plateau, and temperate region, and the tree-like trunks usually form compact cushions, but *S. orientali-chinensis* and *S. tamariscina* are distributed in the mountains or lowland of subtropical



Maximum likelihood ultrafast bootstrap support values (BS) and Bayesian posterior probabilities (PP) are shown.

"*": BS \geq 95% or PP \geq 0.99.

Fig. 1 The ML phylogenetic tree constructed from ITS sequences (A) and *rbcL* sequences (B) of *Selaginella orientalis-chinensis* and other closely related species

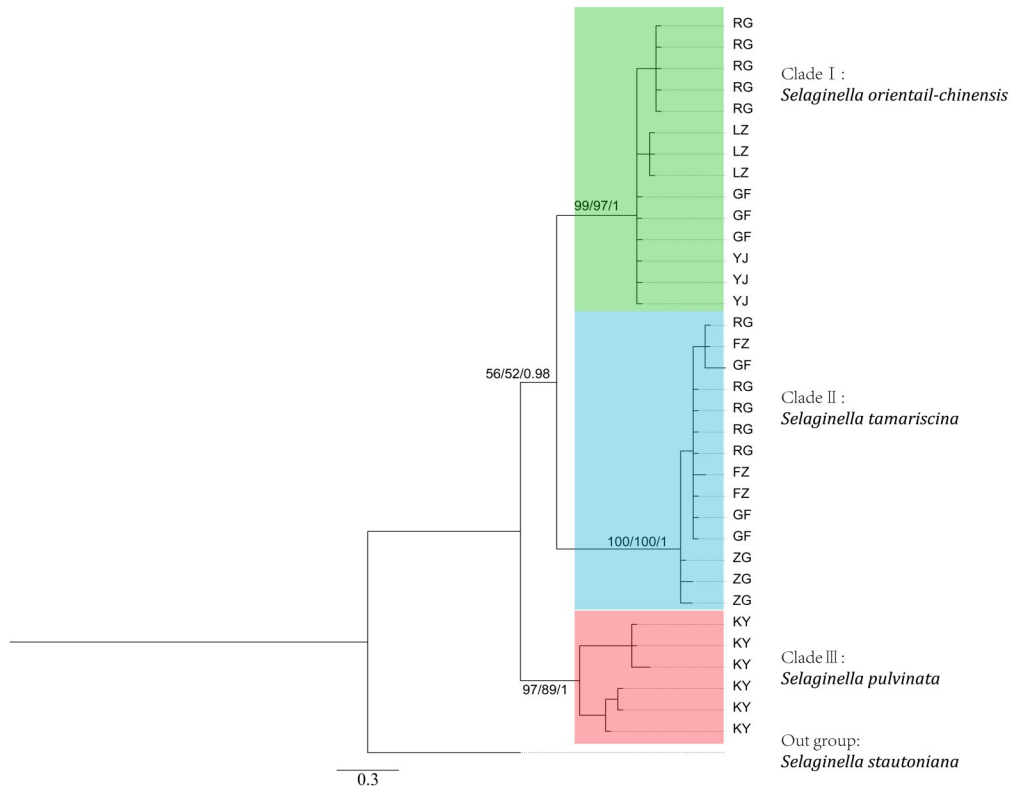


Fig. 2 The 50% majority rule consensus tree derived from Bayes inference presenting the relationship between *Selaginella orientali-chinensis*, *S. tamariscina* and *S. pulvinata*

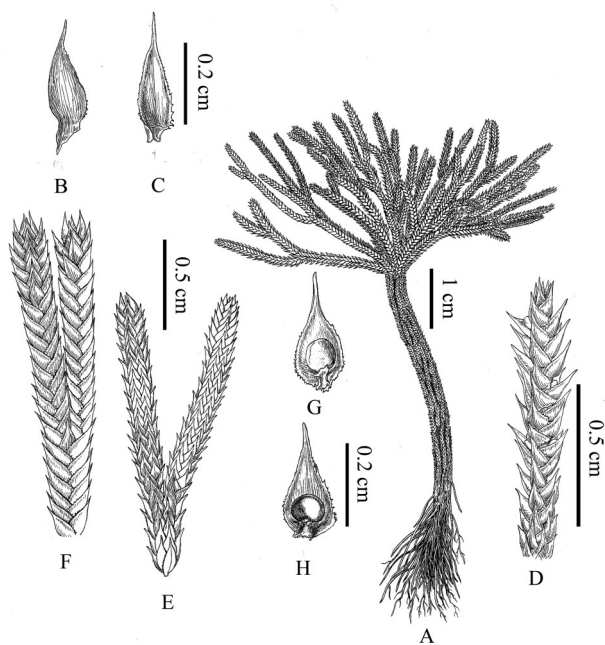
regions, and have tree-like trunks that are slender and do not form compact cushions.

3) Description. Plants terrestrial or epilithic, resurrectional; stems forming rosette, evergreen or seasonally green; stems, rhizophores and roots entangled forming treelike trunk, 1–20 cm in length and ca. 0.5 cm in diam. Rhizophores restricted to base of stem, 0.5–20 cm, slender and adhering to the treelike trunk, forked and forming rootstock near the ground. Stems stramineous or brown, terete, not sulcate, dichotomously branched, not crooked under dehydration. Branches sparse and regular, dorsiventrally flattened, 2–3 mm wide including leaves. Axillary leaves symmetrical, ovate, ovate-triangular, or elliptic, (1.5–2.5) mm × (0.5–1.0) mm, base exauriculate, margins denticulate and obviously white-margined. Dorsal leaves imbricate, asymmetrical, elliptic, (1.5–3.0) mm × (0.5–1.5) mm, not carinate, base obtuse, not peltate, margin denticulate, obviously white-margined, apex aristate, spreading or parallel to axis. Ventral leaves slightly ascending,

overlapping, asymmetrical, ovate to triangular or oblong-ovate, (1.5–2.5) mm × (0.5–1.2) mm, apex aristate; basiscopic margin subentire, serrate or ciliolate (at base), revolute; acroscopic base enlarged, broader, overlapping stem and branches, margin lacerate or denticulate. Strobili solitary, terminal, compact, tetragonal, (12–15) mm × (1.2–2.6) mm; sporophylls uniform, white-margined and hyaline, ovate-triangular, margin denticulate, membranous, apex acuminate or aristate; megasporophylls ± randomly distributed on both sides of strobilus; microsporangia transversely elliptic, relatively thick; microspores yellowish orange, megaspores pale yellow.

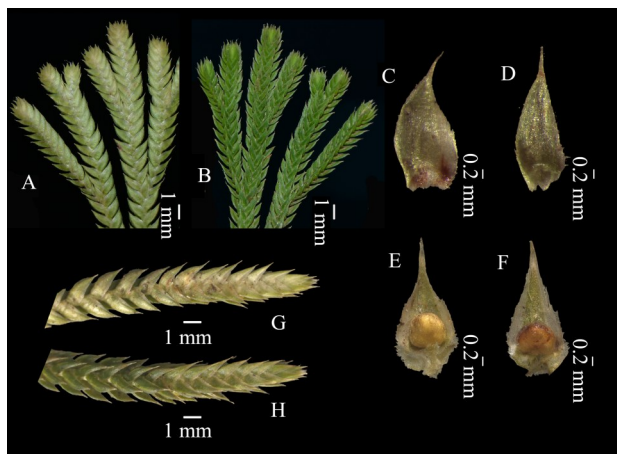
4) Distribution and ecology. *Selaginella orientali-chinensis* is widely distributed in southern and eastern China (Guangdong, Guangxi, Fujian, Hunan, Jiangxi and Zhejiang provinces), growing on bare rock and grassy slope at 100 to 1 000 m a.s.l., and mainly in Danxia landforms.

5) Conservation status. Due to its wide distri-



A: Habit; B: Dorsal leaf; C: Ventral leaf; D: Strobilus; E: Part of main stem showing ventral leaves, axillary leaves; F: Part of main stem showing dorsal leaves; G: Megasporophyll; H: Microsporophyll.

Fig. 3 *Selaginella orientali-chinensis* Ching et C. F. Zhang ex H. W. Wang et W. B. Liao (Drawn by Yun-Xiao Liu)

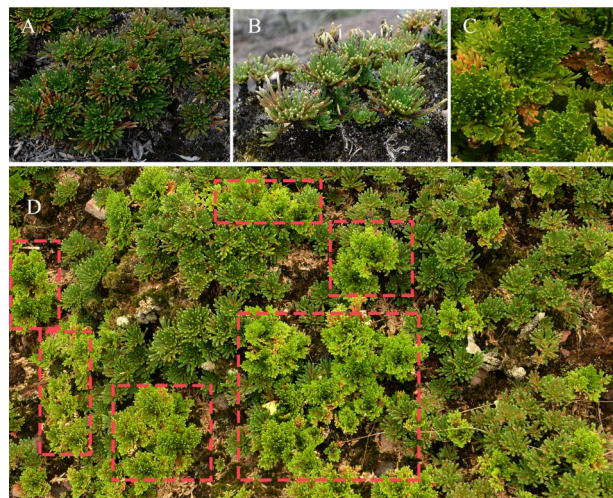


A: Lower view of branchlets; B: Upper view of branchlets; C: Dorsal leaf; D: Ventral leaf; E: Megasporophyll; F: Microsporophyll; G: Lower view of strobilus; H: Upper view of strobilus.

Fig. 4 *Selaginella orientali-chinensis* Ching et C. F. Zhang ex H. W. Wang et W. B. Liao

bution and numerous individuals in each investigation site, *Selaginella orientali-chinensis* should be classified as the Least Concern (LC) category according to IUCN Categories [18].

6) **Etymology.** The specific epithet "*orientali-*



A-B: *S. orientali-chinensis*; C: *S. tamariscina*; D: A co-occurred population of *S. orientali-chinensis* and *S. tamariscina* (*S. tamariscina* is showed in the red dotted boxes).

Fig. 5 Morphology comparison between *Selaginella orientali-chinensis* and *S. tamariscina*

chinensis" was designated by the famous fern botanists Qin Renchang and Zhang Chaofang.

7) **Key to *Selaginella orientali-chinensis* and related species.**

- 1 Main stems dichotomously branched; stems, rhizophores and roots form tree-like trunk..... *S. orientali-chinensis*
- 1 Main stems pinnately branched; stems, rhizophores and roots form tree-like trunk or compact cushion 2
- 2 Stems, rhizophores and roots form tree-like trunk
..... *S. tamariscina*
- 2 Stems, rhizophores and roots form compact cushions, in stead of tree-like trunk *S. pulvinata*

8) **Paratypes. China. Fujian:** Putian, 17 Feb 1923, *H. H. Chung* 979 (PE); **Hunan:** Youxian, 22 Sept 2015, *G. X. Chen et al.* LXP-06-5369 (JIU).

9) **Additional specimens examined. China. Fujian:** *s. n.*, 11 Oct 1974, *Anonymous* 0284 (PE); Zhanghu, 31 July 1984, *II Nan* 84434 (FJFC); Dehua, 1 Aug 1958, *Nanjing University* 23387 (FJFC); Cong'an, 1 Aug 1958, *East China Normal University* 48230 (FJFC); Ningde, 1 Sept 1958, *Fujian Agriculture and Forestry University* 30180 (FJFC); **Guangdong:** Fengshun, 6 Aug 2009, *C. F. Zeng and Q. Y. Zeng* ZXF 7379 (CZH); *s. n.*, 3 Aug 2014, *X. L. Zhou et al.* ZXL09943 (CSH); **Guangxi:** Tengxian, 31 Oct 1960, *Z. M. Liu* 13282 (PE); Guiping, 28 Oct 2017, *Guiping survey team*

450881171028062LY (GXMG); **Hunan**: Liuyang, 3 Aug 1959, *J. Y. Xiao 00018* (CSFI); **Jiangxi**: Guixi, 4 Nov 1979, *S. J. Sheng and D. F. Huang 399* (LBG); Zixi, 4 Oct 1985, *S. S. Lai and D. F. Huang 160* (LBG); Zixi, 26 July 2015, *B. C. Guan NCU201507 MTS0085* (JXU); Suichuan, 1 June 1984, *J. L.*

Wang and D. F. Huang 19 (LBG); Jinggangshan, 16 Nov 1982, *J. L. Wang et S. C. Zhang 8210131*; **Zhejiang**: Suichang, 26 June 1980, *P. X. Qiu and G. H. Yao 5674* (PE); Qinyuan, 10 Oct 1977, *P. X. Qiu 4300* (PE); Qinyuan, 24 Feb 1991, *C. Z. Zheng and C. F. Zhang 7926* (PE).

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