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An Extraordinary Rosette and Resurrection New Spikemoss, *Selaginella iridescens* (Selaginellaceae) from Hainan Island, China †

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Abstract: *Selaginella iridescens* (Selaginellaceae), a new species of spikemoss from Hainan Island, China, is described based on morphological and molecular evidence. The new species morphologically resembles *S. pseudotamariscina* from Vietnam which was recently recognized and segregated from *S. tamariscina* and *S. pulvinata*. Some characters of *S. iridescens*, including the iridescent leaves and long arista (0.35–1.20 mm long), the widely membranaceous margin of axillary leaves (ca. 2/3), dorsal leaves sulcate extending to the top, and slender main stem, dorsoventrally prostrate, distinguish it from *S. pseudotamariscina*. Phylogenetic results based on *rbcL* of 28 *Selaginella* species indicate that *S. iridescens* is sister to *S. pseudotamariscina*, and distant from the *S. tamariscina*-*S. pulvinata* clade. The ancestral character reconstruction result reveals that the rosette is apomorphic and has evolved independently at least six times in *Selaginella*.

Keywords: lycophytes; new species; rosette-forming; taxonomy

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1. Introduction

Selaginella P. Beauv., the single genus in Selaginellaceae, is the largest extant lycophyte genus that comprises approximately 700–800 taxonomically difficult species [1–4]. The monophyly of *Selaginella* was well-supported by recent phylogenetic studies, however, the infrageneric classification is still uncertain [1,5–7]. Based on the morphological characters, Jermy [1] divided the genus *Selaginella* into five subgenera, whose classification was the most widely accepted before molecular phylogenetic studies. Recently, Weststrand and Korall [3,4] divided the genus *Selaginella* into seven subgenera based on DNA sequences (*rbcL*, *pgiC*, and *SQD1*) and morphological data. This classification system was supported by the phylogenomic study based on plastome datasets, which further resolved the uncertainty of the *S. sanguinolenta* group as the most basal clade of *S.* subg. *Stachygynandrum* [7].

Species of *Selaginella* have various growth forms, including rosette, creeping, climbing, prostrate, erect, and suberect [2,8,9]. The rosette is a special morphological feature in *Selaginella*, but only few species are known to be rosette-forming [4,6,10]. In China, *S. tamariscina* (P. Beauv.) Spring and *S. pulvinata* (Hook. et Grev.) Maxim are two famous rosette-forming species of *Selaginella* [2,9]. In Hainan Island, about 14 *Selaginella* species are recorded in Flora of China (FOC), including two endemic species (*S. hainanensis* X. C. Zhang and Nooteboom and *S. scarbrifolia* Ching and Chu H. Wang) and one rosette-forming species (*S. tamariscina*) [2,11]. During a field trip in Hainan Island of China, a new rosette-forming *Selaginella* species was collected. A detailed examination on morphology of our newly collected materials and potential similar species revealed that these specimens were morphologically distinct from the two famous rosette-forming species in China, *S. tamariscina* and *S. pulvinata*, but morphologically similar to *S. pseudotamariscina* X.C. Zhang and C.W. Chen, a new species recently described from Vietnam [12]. Then, we conducted phylogenetic analyses using *rbcL* sequences, including this new species and other rosette species within *Selaginella*. In this paper, we present evidence from morphology and molecular phylogenetic analysis to support the new species and conduct ancestral character reconstruction to reveal the evolutionary history of rosette character in *Selaginella*.

2. Materials and Methods

2.1. Morphological Observations

Morphology of the newly collected specimens was examined and photographed under a Leica S9D stereo microscope, and compared with *S. pseudotamariscina*, *S. pulvinata* and *S. tamariscina*. The sterile leaves, strobili, and sporophylls were observed and measured.

2.2. Taxon Sampling and Sequencing

We sampled three individuals of the new species from one site on Hainan Island (Figure 1). Total genomic DNA was extracted from silica gel dried materials using the Plant Genomic DNA Kit DP305 (TianGen Biotech, Beijing, China) following the manufacturer's protocol. Libraries for pair-end 150 bp sequencing with a 350 bp insert size were conducted using an Illumina NovaSeq 6000 platform at BioMarker Co (Beijing, China). Raw reads were filtered based on the following criteria: pair-end reads with >10% 'N' bases; reads, on which more than 50% of the bases have a quality score less than 10 (Phred-like score). Finally, approximate 6 Gb high-quality sequences were obtained for each sample (only *rbcL* sequences were extracted for this study, this high-throughput data will be used for further study).

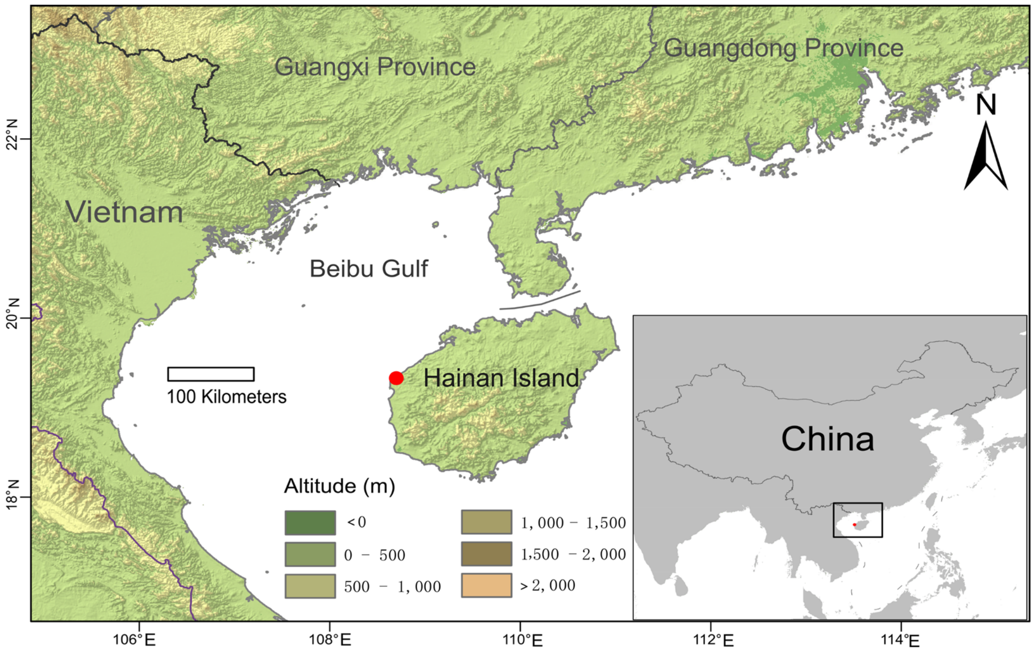

Figure 1. Distribution map of *Selaginella iridescens*. The red dot represents the location of *S. iridescens*. The color scale indicates the elevation in the meters above sea level as recorded in the raster layers of 30 s resolution of the NASA Shuttle Radar topographic Mission (SRTM) elevation database deposited in WorldClim (https://www.worldclim.org, accessed on 24 October 2021). The basic map of China was downloaded from National Catalogue Service for Geographic Information (https://www.webmap.cn, accessed on 25 October 2021).

2.3. Phylogenetic Analysis

The chloroplast *rbcL* gene was the most commonly used marker in *Selaginella* [3,5,6,10]. Therefore, *rbcL* was selected to reconstruct a phylogeny with an extensive species sampling to clarify the relationships between the new *Selaginella* species and other rosette species. In total, 32 individuals of 28 ingroup species representing all the seven subgenera of *Selaginella* according to Weststrand and Korall [3,4] were included, while *Isoetes histrix* Bory and Durand and *I. sinensis* Palmer were selected as outgroups. The *rbcL* of new species were obtained by Geneious mapping using Illumina short reads in Geneious v. 11.1.4 [13] and *rbcL* sequences for other species were downloaded from GenBank (voucher information and GenBank accession numbers listed in the Table 1).

Table 1. Species names and GenBank accession numbers of *rbcL* sequences used in this study.

No.	Species	Collector and Voucher No.	Locality	Herbarium	Accession No.
1	*Selaginella bryopteris* (L.) Baker	C. R. Fraser-Jenkins 4370	Nepal	L	KY022983
2	*S. convoluta* (Arn.) Spring	R. M. Harley 16181	Brazil: Bahia	U	KY023003
3	*S. deflexa* Brack.	D. Pamer 2651	USA: Hawaii	KANU	AF093253
4	*S. digitata* Spring	N. Wikström et al. 110319-2	Madagascar	S	KY023013
5	*S. digitata* Spring	P. Phillipson 1826	Madagascar	L	KY023012
6	*S. exaltata* (Kunze) Spring	Korall 1996-1	Sweden	S	AJ010849
7	*S. helicoclada* Alston	Rakotondrainibe 3262	Madagascar	P	AJ295896
8	*S. heterostachys* Baker	X. C. Zhang 7088	China: Guizhou	PE	MH814896
9	*S. imbricata* (Forssk.) Spring ex Decne.	Rothfels et al. 4275	Oman	DUKE	KT161486
10	*S. iridescens* X. C. Zhang and Y. R. Wang	Y. R. Wang and L. X. Yuan wyr20200110-1	China: Hainan	PE	OK181884
11	*S. iridescens* X. C. Zhang and Y. R. Wang	Y. R. Wang and L. X. Yuan wyr20200110-2	China: Hainan	PE	OK181885
12	*S. iridescens* X. C. Zhang and Y. R. Wang	Y. R. Wang and L. X. Yuan wyr20200110-3	China: Hainan	PE	OK181886
13	*S. kraussiana* A. Braun	M. Mokoso 3098	Democratic Republic of the Congo	BR	KY023057
14	*S. lepidophylla* (Hook. and Grev.) Spring	Worthington s.n.	USA: Texas	US	AF419051
15	*S. moellendorffii* Hieron.	Ju and Deng HGX12295	China: Sichuan	CDBI	KT161531
16	*S. nipponica* Franch. and Sav.	X. C. Zhang et al. 7066	China: Guizhou	PE	MW407367
17	*S. nothohybrida* Valdespino	Rothfels et al. 3069	Mexico: San Luis Potosí	DUKE	KT161545
18	*S. novoleonensis* Hieron.	F. Drouet and D. Richards 3942	Mexico: Sonora	S	KY023097
19	*S. nubigena* J.P.Roux	A. Larsson AL810	South Africa	UPS	KY023098
20	*S. pallescens* (C. Presl) Spring	Beck 1120	Mexico: Hidalgo	DUKE	KT161555
21	*S. pilifera* A. Braun	Pringle 13959	N/A [1]	S	AJ295862
22	*S. pseudotamariscina* X.C. Zhang and C.W. Chen	C.W. Chen Wade 5314	Vietnam: Khanh Hoa Province	PE	MZ159980
23	*S. pulvinata* (Hook. et Grev.) Maxim	D. E. Boufford et al. 37879	China: Sichuan	A	KY023124
24	*S. pulvinata*	D. E. Boufford et al. 35254	China: Yunnan	A	KY023125
25	*S. remotifolia* Spring	Gaoligong Shan Biodiversity Survey; 21081	China: Yunnan	GH	KY023130
26	*S. selaginoides* (L.) P. Beauv. ex Mart. and Schrank	S. Weststrand 104	Sweden	UPS	KY023148
27	*S. sibirica* (Milde) Hieron.	L. A. Viereck and K. Jones 5667	USA: Alaska	S	KY023153
28	*S. stauntoniana* Spring	Zhao 169	China: Beijing	CDBI	KT161614
29	*S. tamariscina* (P.Beauv.) Spring	N/A TNS759348	Japan: Okinawa	TNS	AB574655
30	*S. uliginosa* Spring	Holmgren and Wanntorp 253	Sweden	S	AJ010843
31	*S. uncinata* (Desv. ex Poir.) Spring	Zhang and Zhou DJY04101	China: Sichuan	CDBI	KT161626
32	*S. vardei* H. Lév.	D. E. Boufford et al. 32425	China: Tibet	A	KY023169
33	*Isoetes sinensis* Palmer	N/A 743727	Japan	TNS	AB574660
34	*I. histrix* Bory and Durand	Wanntorp N/A	Sweden	S	AF404497

[1] N/A = not available.

All the sequences were aligned using MAFFTT v7.313 [14,15]. Both maximum likelihood (ML) analysis and Bayesian inference (BI) were carried out in this study. ML analysis was performed using RAxML 7.2.6 [16], with 1000 bootstrap replicates under the GTRGAMMA model selected according to the Bayesian Information Criterion (BIC) by ModelFinder [17]. BI analysis was performed using MrBayes v. 3.2.6. [18], under the SYM + G4 model selected according to the BIC by ModelFinder [17]. For each Bayesian analysis, four MCMC chains were run simultaneously for 1 million generations and sampled every 1000 generations. The average standard deviation of split frequencies (<0.01) was used to assess the convergence. ML and BI trees and the branch support values were visualized using FigTree v.1.4.2 [19].

2.4. Character Evolution Analysis

We used Mesquite v.3.61 [20] to infer the ancestral states of rosette character. The ancestral character reconstruction was performed under ML models “Mk1” [21], with the phylograms of 1000 RAxML bootstrap trees based on *rbcL* sequences as input phylogenies in order to consider any phylogenetic uncertainties. The results were finally summarized as percentage of changes of character states on a given branch across all 1000 trees using the “Average-frequencies-across-trees” option.

3. Results

3.1. Taxonomy Treatment

Selaginella iridescens X. C. Zhang and Y. R. Wang, sp. nov.

Type. CHINA, Hainan, Changjiang, Changhua, at foot of Mt. Daling, on quartzite rocky slope, in open grass, 108.700° E, 19.328° N, altitude 31 m, 10 January 2020, Y. R. Wang and L. X. Yuan wyr20200110 (holotype, PE!); ibid. 11 June 2021, X. C. Zhang and E. F. Huang 11,594 (paratype, PE!).

Diagnosis. *Selaginella iridescens* differs from *S. pseudotamariscina* by its iridescent leaves and apex with long arista (0.35–1.2 mm), dorsal leaves adaxially sulcate, margins of axillary leaves widely membranaceous (ca. 2/3), and main stem slender, dorsiventrally prostrate (Figures 2–4; Table 2).

Description. Rosettes, xerophytic. Rhizophores restricted to base of stem; rhizophores much forked, forming thick massive rootstock. Stems and roots entangled forming prostrate dorsiventral trunks; main stems branched near or above the base, pinnately or anisotomously branched, stramineous or brown; primary leafy 2–3 pinnately branched, branchlets compact and regular. Leaves thick and surface smooth, fresh leaves iridescent. Axillary leaves on branches symmetrical, ovate-oblong, ca. 2.02 × 0.87 mm, margin widely membranaceous (about 2/3 of the entire leaf), lacerate or subentire, base obtuse, ciliolate or denticulate, apex arista (ca. 0.52 mm long). Dorsal leaves strongly ascending, imbricate, symmetrical, lanceolate, 2.27 × 0.70 mm, lower side sulcate extends to the top, upper side carinate, base obtuse, margin ciliolate or denticulate, not membranaceous, apex arista (ca. 0.76 mm long). Ventral leaves slightly ascending, overlapping, asymmetrical, ovate-lanceolate to ovate-triangular, ca. 2.6 × 1.5 mm, adaxially sulcate extends to the top, apex arista (ca. 0.35–1.20 mm long); basiscopic margin ciliolate or denticulate; acroscopic base enlarged, broader, widely membranaceous, lacerate or subentire, ciliolate or lacerate at base. Strobili solitary, terminal, compact, slightly dorsoventrally complanate, very short (ca. 1–5 mm long); sporophylls anisophyllous; dorsal sporophylls smaller than the ventral ones, symmetrical, lanceolate, ca. 2.0 × 0.8 mm, margin slightly membranaceous, shortly ciliolate, apex arista; ventral sporophylls triangular, ca. 2.21 × 1.30 mm, margin widely membranaceous, denticulate, ciliolate or lacerate, apex arista; sporangia borne only on the ventral sporophylls; microspores yellowish orange, megaspores pale yellow to white (Figures 2 and 3).

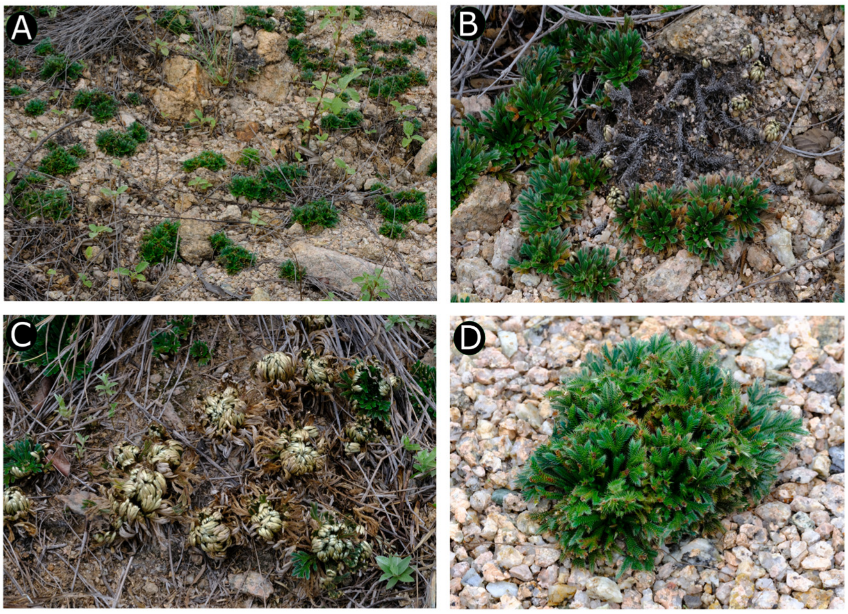

Figure 2. Habitat of *Selaginella iridescens* X. C. Zhang and Y. R. Wang sp. nov. (**A**) Habitat; (**B**) prostrate stems; (**C**) semi-dry individuals; (**D**) fresh individuals.

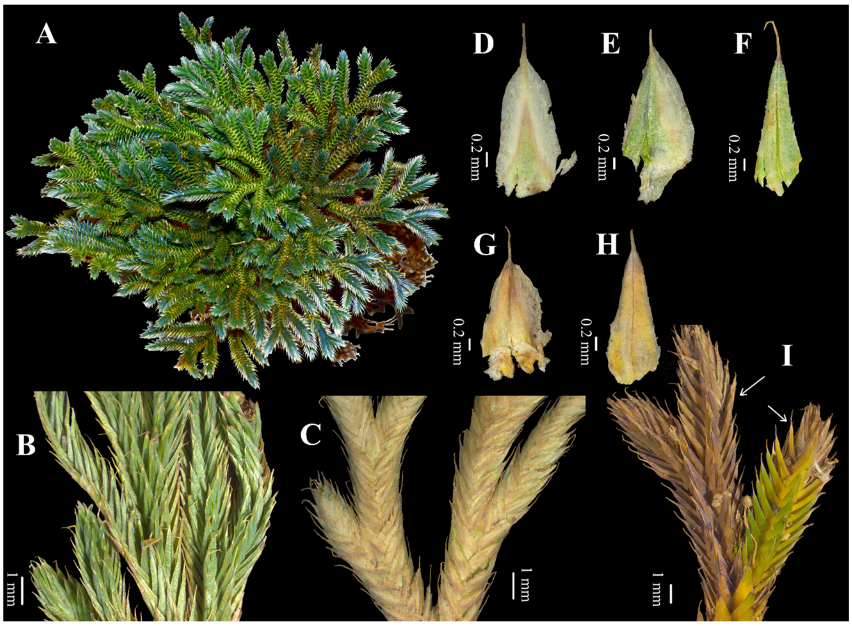

Figure 3. Morphology of *Selaginella iridescens* X. C. Zhang and Y. R. Wang sp. nov. (**A**) Individual; (**B**) adaxial view of branchlets; (**C**) abaxial view of branchlets; (**D**) axillary leaf; (**E**) ventral leaf; (**F**) dorsal leaf; (**G**) ventral sporophyll; (**H**) dorsal sporophyll; (**I**) adaxial view of strobili (arrows).

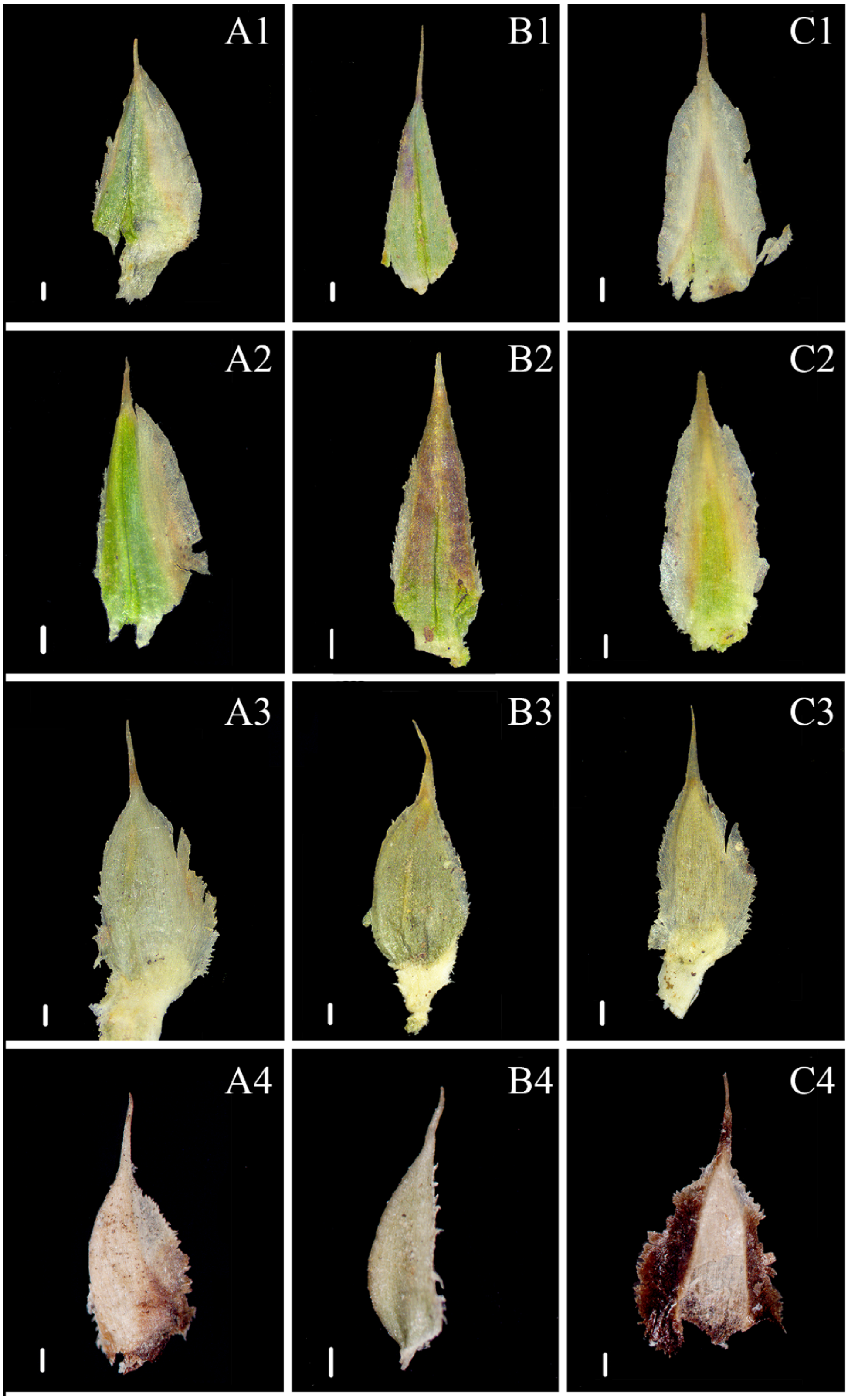

Figure 4. Comparison of leaf morphology of *Selaginella iridescens*, *S. pseudotamariscina*, *S. tamariscina*, and *S. pulvinata*. (**A1**–**C1**): *S. iridescens*, Hainan, Y. R. Wang, wyr20200110 (PE); (**A2**–**C2**): *S. pseudotamariscina*, Vietnam, C. W. Chen, Wade 5314 (PE); (**A3**–**C3**): *S. tamariscina*, Hainan, H. Y. Liang 63,716 (PE); (**A4**–**C4**): *S. pulvinata*, Yunnan, X. C. Zhang 088 (PE). A. ventral leaves; B. dorsal leaves; C. axillary leaves. Scale bars = 0.2 mm.

Table 2. Character comparison of *S. iridescens*, *S. pseudotamariscina*, *S. pulvinata*, and *S. tamariscina*.

Characters/Species	***S. iridescens***	***S. pseudotamariscina***	***S. pulvinata***	***S. tamariscina***
Distribution	Hainan of China	Khanh Hoa and Ninh Thuan of Vietnam	Southwestern and central China, India, Thailand, Vietnam	Eastern and Southeastern China (including Hainan), Japan, Korean Peninsula, Philippines, Thailand
Habit	Stems and roots entangled forming slender, dorsiventrally prostrate trunk	Stems and roots entangled forming thick, radial erect treelike trunk	Stems and roots entangled not forming treelike trunk	Stems and roots entangled forming treelike trunk
Leaves	Iridescent	Not iridescent	Not iridescent	Not iridescent
Axillary leaves	Ovate-oblong, margin membranaceous (about 2/3 of the entire leaf), lacerate or subentire, base ciliolate or denticulate	Lanceate, or ovate-lanceate, margin membranaceous (about 1/2 of the entire leaf), acerate-ciliolate	Ovate to triangular, margin membranaceous (about 1/2 of the entire leaf), lacerate-ciliolate, base ciliolate or lacerate	Ovate, ovate-triangular, or elliptic, margin membranaceous (about 1/3 of the entire leaf), denticulate-lacerate, base ciliolate or denticulate
Dorsal leaves	Symmetrical, lanceate, adaxially sulcate extends to the top, apex with long arista (ca. 0.76 mm long), margin ciliolate or denticulate	Symmetrical, lanceate, adaxially sulcate extends to half of the leaves, apex with short arista (ca. 0.2 mm long), margin ciliolate or denticulate	Asymmetrical, obliquely ovate or triangular, adaxially not sulcate, margin lacerate or entire	Asymmetrical, elliptic, adaxially not sulcate, margin denticulate (shortly ciliolate at base)
Ventral leaves	Ovate-lanceate to ovate-triangular, adaxially sulcate, apex with long arista (ca. 0.35–1.20 mm long)	Ovate-lanceate to ovate-triangular, adaxially sulcate, apex with short arista (0.02–0.40 mm long)	Oblong, adaxially not sulcate; apex with long arista (ca. 0.35–1.20 mm long)	Ovate to triangular or oblong-ovate, adaxially not sulcate; apex arista
Strobili	Slightly dorsoventrally-complanate	Slightly dorsoventrally complanate	Tetragonal	Tetragonal
Sporophylls	Slightly anisophyllous; sporangia borne only on the ventral sporophylls	Slightly anisophyllous; sporangia borne only on the ventral sporophylls	Isophyllous; sporangia borne on both dorsal and ventral sporophylls	Isophyllous; sporangia borne on both dorsal and ventral sporophylls

Distribution and habitat. *Selaginella iridescens* is only known from Western Hainan, Changjiang County, on quartzite rocky slope, open area (Figure 1).

Etymology. The specific epithet 'iridescens' refers to its iridescent leaves.

Conservation status. *Selaginella iridescens* is known only from one population with about 300 individuals in the type locality. No other localities were discovered during our investigation conducted both in 2020 and 2021. Moreover, detailed examination of relevant specimens collected from Hainan and adjacent regions of rosette *Selaginella* species in virtual herbaria (CVH and GBIF) revealed no additional specimens of this species. Even if the assignment of a conservation status of this new species could be premature, it is most likely an endemic to Hainan Island and could be temporarily considered as Vulnerable (VU) according to the IUCN Red List guidelines criterion D1D2 based on current data [22].

3.2. Phylogenetic Analysis

The total length of *rbcL* alignment is 1428 bp, with 475 parsimony informative sites. The sequences of three *Sealginella iridescens* individuals are identical and have 14 nucleotide variations when compared with *S. pseudotamariscina*, the most related species to *S. iridescens*.

Our phylogenetic analyses of 28 species of *Selaginella* based on *rbcL* gene are generally similar to the former phylogeny studies and consistent with the seven subgenera classification [3–5]. Three individuals of *S. iridescens* are clustered together and sister to *S.*

pseudotamariscina, and formed a monophyletic clade belonging to the Subg. *Stachygynandrum* (Figure 5). This clade is sister to the *S. helicoclada-S. imbricata* clade with strong support (BS = 100/PP = 100). The clade grouping of these two latter clades (*S. iridescens-S. pseudotamariscina* clade and *S. helicoclada-S. imbricata* clade) is sister to the *S. pulvinata-S. stauntoniana* clade (BS = 99/PP = 100).

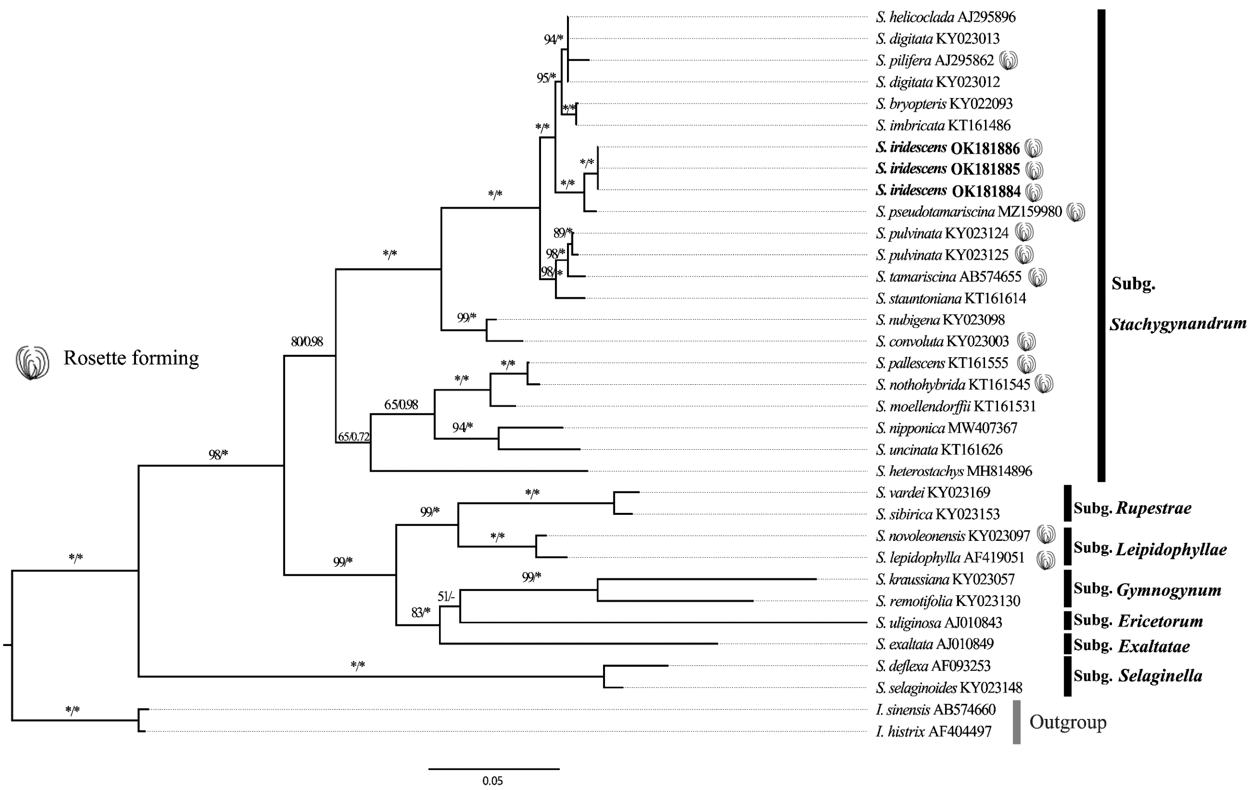

Figure 5. Maximum likelihood tree based on the *rbcL* gene. Maximum likelihood bootstrap (BS) and Bayesian inference posterior probability (PP) are noted above the branch (PP/BS, * = 1 or 100%).

3.3. Ancestral Reconstruction of Rosette Character

Ancestral character reconstruction results reveal that rosette is apomorphic in *Selaginella*. This character has experienced a complicated evolutionary history and has evolved independently at least six times in *Selaginella*. There were five times in subg. *Stachygynandrum* and once in subg. *Lepidophyllae*. The five times in subg. *Stachygynandrum* appeared in *S. nothohybrida-S. pallescens* clade, *S. convoluta-S. nubigena* clade, *S. pulvinata-S. tamariscina* clade, *S. irisdescens-S. pseudotamariscina* clade, and *S. pilifera* clade, respectively (Figure 6).

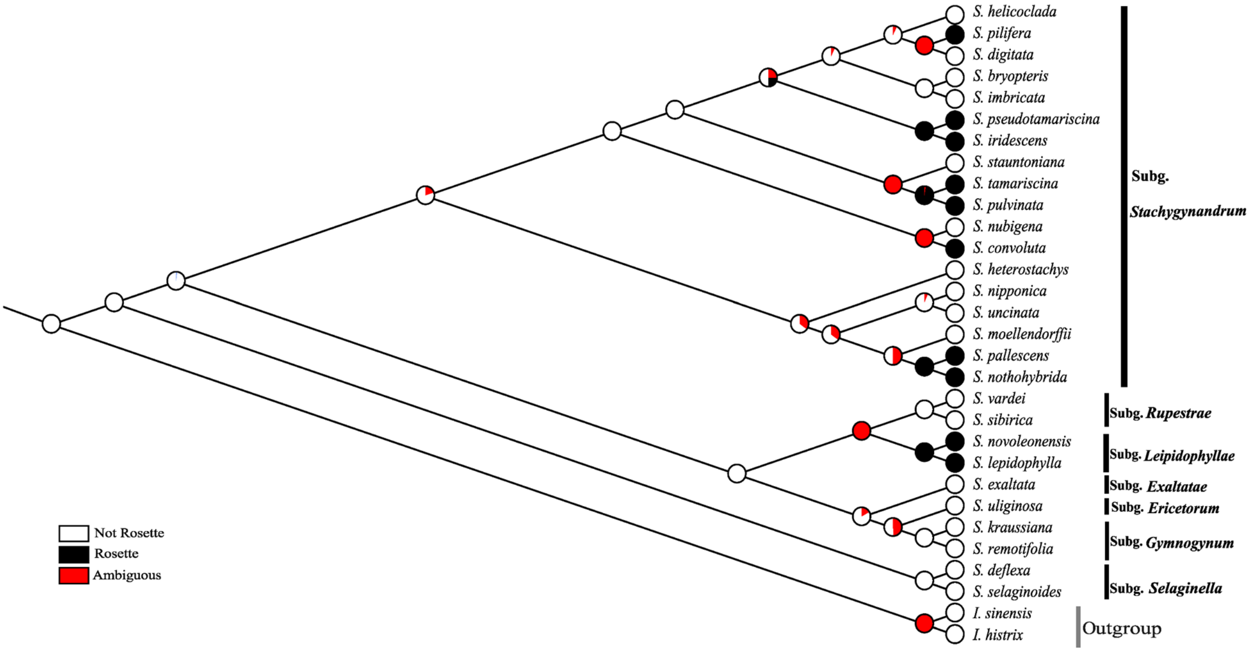

Figure 6. The inferred ancestral states for rosette habit based on the Mk1 model. Black and white in the pie charts on each branch indicate the probabilities of ancestral states inferred by the analysis based on the 1000 RAxML bootstrap trees.

4. Discussion

4.1. Morphological Comparison with Three Related Rosette Species

Morphological comparison with *S. pulvinata* and *S. tamariscina* shows that *S. iridescens* is distinguished by its fresh leaves iridescent; dorsal leaves symmetrical, lanceate, adaxially sulcate, margin ciliolate or denticulate; ventral leaves adaxially shallow sulcate; strobili dorsoventrally complanate, similar shape of dorsal sporophylls and dorsal sterile leaves; dorsal sporophylls smaller than the ventral ones, and sporangia borne only on the ventral sporophylls (Figure 3 and Table 2). The leaves of *S. pulvinata* and *S. tamariscina* are not iridescent, dorsal leaves asymmetrical, ventral leaves adaxially not sulcate (Figure 4). The strobili of *S. pulvinata* and *S. tamariscina* are tetragonal, sporophylls isophyllous, margin denticulate, sporangia borne on both dorsal and ventral sporophylls (Table 2).

Morphological comparison with *S. pseudotamariscina* shows that *S. iridescens* is similar to *S. pseudotamariscina* by its rosette-forming habit and lanceate symmetrical dorsal leaves. However, *S. iridescens* is further characterized by the following unique features: the leaves iridescent with long arista apex (0.35–1.20 mm), dorsal leaves sulcate extending to the top, margins of axillary leaves widely membranaceous (ca. 2/3), and main stem slender, dorsiventrally prostrate (Figures 2–4 and Table 2). The leaves of *S. pseudotamariscina* are not iridescent and the apex is short arista (0.02–0.40 mm), dorsal leaves sulcate to the middle of leaves, margins of axillary leaves widely membranaceous (ca. 1/2) (Figure 4 and Table 2). The main stem of *S. pseudotamariscina* is thick, radial, and erect. It is obvious that these two similar species are very distinct in detail.

4.2. How Many Rosette Species in *Selaginella*?

Rosette is a special morphological feature, which has evolved independently at least six times in *Selaginella* (Figure 6). However, there are only about 12 *Selaginella* species known to be rosette-forming according to our new finding and former studies [3,6,12]. These 12 rosette species only disperse in two subgenera of *Selaginella*, S. subg. *Lepidophyllae*, and subg. *Stachygynandrum.* Subg. *Lepidophyllae* contains four rosette species mainly distributed in North America, but only two species (*S. lepidophylla* and *S. novoleonensis*) have been sequenced and obtained a confirmed position [3,4]. There are still two rosette-forming resurrection species, *S. gypsophila* A. R. Sm. and T. Reeves and *S. ribae* Valdespino with unknown systematic position, that may still be members of subg. *Lepidophyllae* [4,23,24]. There are eight rosette-forming species in subg. *Stachygynandrum*, mainly distributed in America (*S. nothohybrida*, *S. pallescens*, *S. convoluta*, and *S. pilifera*) and East Asia (*S. iridescens*, *S. pseudotamariscina*, *S. pulvinata*, and *S. tamariscina*) [3,10,12]. These eight rosette-forming species are not monophyletic, they fell into four small groups and occupied different positions both in present and previous phylogenetic analyses [3,10,12].

4.3. Key to *S. iridescens*, *S. pseudotamariscina*, *S. pulvinata*, and *S. tamariscina*

1. Strobili dorsoventrally complanate; sporangia borne only on the ventral sporophylls; dorsal leaves symmetrical, lanceolate, adaxially sulcate; ventral leaves abaxially shallowly sulcate . . . . 2.

2. Main stem slender, dorsiventrally prostrate; leaves iridescent, apex long arista (0.35–1.20 mm long); margins of axillary leaves widely membranaceous (ca. 2/3); dorsal leaves adaxially sulcate to the apex . . . . *S. iridescens*.

2. Main stem thick, radially erect; leaves not iridescent, apex shortly arista (0.02–0.40 mm long); margins of axillary leaves membranaceous (ca. 1/2); dorsal leaves adaxially sulcate not to the apex . . . . *S. pseudotamariscina*.

1. Strobili tetragonal; sporangia borne on both ventral and dorsal sporophylls; dorsal leaves asymmetrical, obliquely ovate, triangular, or elliptic, not sulcate . . . . 3.

3. Stems and roots entangled not forming treelike trunk; inner margins of dorsal leaves lacerate or entire, outer margins revolute and entire . . . . *S. pulvinata*.

3. Stems and roots entangled often forming treelike trunk; inner margins of dorsal leaves denticulate, outer margins denticulate or shortly ciliolate . . . . *S. tamariscina*.

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References

1. Jermy, A.C. Subgeneric names in *Selaginella*. *Fern Gaz.* **1986**, *13*, 117–118.
2. Zhang, X.C.; Nooteboom, H.P.; Kato, M. Selaginellaceae. In *Flora of China*; Wu, Z.Y., Raven, P.H., Hong, D.Y., Eds.; Science Press: Beijing, China, 2013; Volumes 2–3, pp. 37–66.
3. Weststrand, S.; Korall, P. Phylogeny of Selaginellaceae: There is value in morphology after all! *Am. J. Bot.* **2016**, *103*, 2136–2159. [CrossRef]
4. Weststrand, S.; Korall, P. A subgeneric classification of *Selaginella* (Selaginellaceae). *Am. J. Bot.* **2016**, *103*, 2160–2169. [CrossRef] [PubMed]
5. Korall, P.; Kenrick, P. Phylogenetic relationships in Selaginellaceae based on *rbcL* sequences. *Am. J. Bot.* **2002**, *89*, 506–517. [CrossRef]
6. Zhou, X.M.; Zhang, L.B. A classification of *Selaginella* (Selaginellaceae) based on molecular (chloroplast and nuclear), macromorphological, and spore features. *Taxon* **2015**, *64*, 1117–1140. [CrossRef]
7. Zhang, H.R.; Wei, R.; Xiang, Q.P.; Zhang, X.C. Plastome-based phylogenomics resolves the placement of the sanguinolenta group in the spikemoss of lycophyte (Selaginellaceae). *Mol. Phylogenet. Evol.* **2020**, *147*, 106788. [CrossRef] [PubMed]
8. Jermy, A.C. Selaginellaceae. In *The Families and Genera of Vascular Plants, Pteridophytes and Gymnosperms*; Kramer, K.U., Green, P.S., Eds.; Springer: Berlin, Germany, 1990; Volume 1, pp. 39–45.
9. Zhang, X.C. Selaginellaceae. In *Flora Reipublica Popularis Sinicae*; Zhang, X.C., Ed.; Science Press: Beijing, China, 2004; Volume 6, pp. 86–219.

10. Zhou, X.M.; Rothfels, C.J.; Zhang, L.; He, Z.R.; Le Péchon, T.; He, H.; Lu, N.T.; Knapp, R.; Lorence, D.; He, X.J.; et al. A large-scale phylogeny of the lycophyte genus *Selaginella* (Selaginellaceae: Lycopodiopsida) based on plastid and nuclear loci. *Cladistics* **2015**, *32*, 360–389. [CrossRef]
11. Zhang, X.C.; Xiang, Q.P.; Nooteboom, H.P. A new species of *Selaginella* from Hainan Island, China, with a key to the Hainan species. *Bot. J. Linnean Soc.* **2005**, *148*, 323–327. [CrossRef]
12. Zhang, M.H.; Yang, J.; Luu, H.T.; Tran, G.; Zhang, X. *Selaginella pseudotamariscina* (Selaginellaceae), an overlooked rosette-forming resurrection spikemoss from Vietnam. *Guihaia* **2021**. [CrossRef]
13. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [CrossRef] [PubMed]
14. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef] [PubMed]
15. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef] [PubMed]
16. Stamatakis, A. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **2006**, *22*, 2688–2690. [CrossRef] [PubMed]
17. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.; Von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 491–587. [CrossRef] [PubMed]
18. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef] [PubMed]
19. Rambaut, A. FigTree, Version 1.4.2. Computer Program and Documentation Distributed by the Author. 2014. Available online: https://tree.bio.ed.ac.uk/software/figtree/ (accessed on 10 September 2021).
20. Maddison, W.P.; Maddison, D.R. Mesquite: A Modular System for Evolutionary Analysis. Version 3.61. 2019. Available online: https://www.mesquiteproject.org (accessed on 14 September 2021).
21. Lewis, P.O. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* **2001**, *50*, 913–925. [CrossRef] [PubMed]
22. IUCN. Guidelines for Using the IUCN Red List Categories and Criteria, Version 14. Prepared by the Standards and Petitions Committee. 2019. Available online: https://www.iucnredlist.org/documents/RedListGuidelines.pdf (accessed on 24 December 2020).
23. Smith, A.R.; Reeves, T. *Selaginella gypsophila* (Selaginellaceae), yet another new edaphic endemic from northern Mexico. *SIDA Contrib. Bot.* **1984**, *10*, 211–215.
24. Mickel, J.T.; Smith, A.R.; Valdespino, I.A. *Selaginella*. In *The Pteridophytes of Mexico*; Mickel, J.T., Smith, A.R., Eds.; Parts I and II Memoirs of the New York Botanical Garden; New York Botanical Garden Press: New York, NY, USA, 2004; pp. 88, 550–602, 968–989.