NOTE

Rediscovery of an 'Extinct' species *Scleria sumatrensis* Retz. in Taiwan using both morphological and molecular authentications

Shih-Hui LIU[1], Chau-Ching HUANG[2,*], Chun-Kuei LIAO[3,*]

1. Department of Biological Sciences, National Sun Yat-sen University, No. 70, Lien-Hai Road, Kaohsiung City 804201, Taiwan.
2. Botany Division, Endemic Species Research Institute, No. 1, Ming-Sheng East Road, Ji-Ji Township, Nantou County 552203, Taiwan.
3. Biotope Environment Planning Co., No. 453-2, Min-zu Road, Lin-Luo Township, Pingtung County 909143, Taiwan.
*Corresponding authors' C.C. Huang: E-mail: hcch0725@tesri.gov.tw; C.K. Liao: E-mail: chunkuei.liao@gmail.com

ABSTRACT: Determining the correct conservation status of a species provides fundamental and critical information for species conservation policies. However, species traits and limited research efforts might decrease the accuracy of conservation assessments. *Scleria sumatrensis* has been considered a regionally extinct sedge plants in Taiwan, and the only, previous record of this species was from a collection provided by Urbain Faurie in 1914. More than one hundred years after Faurie's record, we confirm the current occurrence of *S. sumatrensis* in Taiwan via morphological and molecular authentications in the present study. The morphological details of *S. sumatrensis* are provided based on the descriptions and illustrations. The conservation status for this species is reassigned to the Critically Endangered (CR) category. Further conservation efforts and research on *S. sumatrensis* are also discussed.

KEY WORDS: Conservation status, Cyperaceae, red list, rediscovery, *Scleria sumatrensis,* species authentication.

INTRODUCTION

Accurately assessing the conservation status of a species is critical for the further species conservation efforts (Pimm *et al*., 2014; Humphreys *et al*., 2019; Wagner, 2020). An increasing number of species that were once classified as extinct have been rediscovered, thus demonstrating the difficulty in correctly assigning a conservation status to a species (Fisher and Blomberg, 2011; Lee *et al*., 2017; Humphreys *et al*., 2019). The species characteristics and search efforts affect the detectability and conservation status of a threatened species (Lee *et al*., 2017; Schmidt Silveira *et al*., 2019). With more search efforts and morphological investigations, 'extinct' species have been rediscovered (ex. Sheue *et al*., 2005; Wong *et al*., 2018; Borah *et al*., 2020). The DNA barcoding technique is a useful molecular tool for species authentication, and an increasing number of studies have applied this tool to authenticate the rediscovered species (Lee and Mohamed, 2016; Chang *et al*., 2017; Edwards *et al*., 2018; Hirano *et al*., 2018; Wahlsteen *et al*., 2020).

Scleria P. J. Bergius is a tropical to warm-temperate genus with approximately 200 species in the family Cyperaceae, and it is characterized by its unisexual flowers, paniculate or spike-like inflorescences, and achenes borne on a disc-like or cup-like hypogynium (Bergius, 1765; Koyama, 1978; Koyama *et al*., 2000; Zhang *et al*., 2010). Nine *Scleria* species have been reported in Taiwan (Koyama, 1978; Koyama *et al*., 2000; Leong and Kuoh, 2000; Editorial Committee of the Red List of Taiwan Plants, 2017). A recent assessment

showed that in Taiwan seven out of the nine *Scleria* species, i.e., *S. biflora* Roxb., *S. levis* Retz., *S. lithosperma* (L.) Sw., *S. radula* Hance, *S. rugosa* R. Br., *S. scrobiculata* Nees & Mey. ex Nees, and *S. terrestris* (L.) Fassett, were classified as the Least Concern (LC) species, *S. novae-hollandiae* Boeckeler was classified as the Endangered (EN), and *S. sumatrensis* was classified as the Regionally Extinct (RE) in Taiwan (Editorial Committee of the Red List of Taiwan Plants, 2017).

This presumed extinct species was first collected in Taiwan by Père Urbain Jean Faurie in 1914 from Bankinsing (Wanjin Villages, Wanluan Township, Pingtung County, Taiwan at present) and was described as a new species *S. fauriei* Ohwi (Ohwi, 1932). Koyama (1978) revised *Scleria* and identified Faurie's collections as *S. sumatrensis*. Except for Faurie's collection in 1914, no additional collections or observations have been reported in Taiwan until our rediscovery in 2020.

In this study, we aimed to authenticate *S. sumatrensis* in Taiwan using both morphological and molecular methodologies, provide an identification key to all Taiwanese *Scleria* species, and reassess the conservation status for *S. sumatrensis* in Taiwan.

MATERIALS AND METHODS

Sampling

To authenticate the regionally extinct species *S. sumatrensis* Retz., plant vouchers and leaf materials were collected during field surveys in Pingtung County, Taiwan. Three individuals were also planted in the research greenhouse at the Endemic Species Research

Table 1. PCR primers and their references. Optimal PCR conditions for the studied regions.

Primers		Primer Sequences (5' -->3')	References	PCR thermal cycling conditions					No. of cycles
				Initial denaturation	Denaturation	Annealing temperature	Elongation temperature	Final extension	
Nuclear region									
ITS	ITS_*Scleria*	ATGCTTAAACTCAGCGGGTA	This study	95°C, 5 min	95°C, 30 sec	58°C, 30 sec	72°C, 1 min	72°C, 5 min	30
	ITS_L	TCGTAACAAGGTTTCCGTAGGTG	Hsiao *et al.*, 1994						
Chloroplast regions									
rps1	rpsF	GTGGTAGAAAGCAACGTGCGACTT	Oxelman *et al.*, 1997	95°C, 5 min	95°C, 30 sec	64°C, 30 sec	72°C, 40 sec	72°C, 5 min	30
	rpsR2	TCGGGATCGAACATCAATTGCAAC							
ndhF	ndhF-A	TATGGTTACCTGATGCCATGGA	Hinchliff *et al.*, 2010	95°C, 5 min	95°C, 30 sec	58°C, 30 sec	72°C, 1 min	72°C, 5 min	30
	ndhF-D1	CTATRTAACCRCGATTATATGACCAA							

Institute (ESRI), Nantou, Taiwan, for the following morphological investigation. Leaf materials were dried in silica gel for molecular authentication. Five randomly selected individuals were included in the molecular analyses to identify the genetic diversity within the rediscovered population. Vouchers were deposited at Herbarium of National Taiwan Univesity (TAI), Taiwan Forestry Research Institute (TAIF) and Endemic Species Research Institute, Taiwan (TAIE) (Thiers, 2016) for further studies.

Morphological investigation

Morphological and phenological data of *S. sumatrensis* were collected both in the ESRI research greenhouse and in the field. Type specimens and additional herbarium specimens of S. *sumatrensis* and allied Taiwanese *Scleria* species were examined by visiting the Herbarium of the Biodiversity Research Center, Academia Sinica in Taiwan (HAST), Herbarium of Taiwan Forestry Research Institute in Taiwan (TAIF), and Herbarium of Endemic Species Research Institute in Taiwan (TAIE) (Thiers, 2016). On line information on the *Scleria* type specimens in the Kyoto University Herbarium in Japan (KYO), Lund University Herbarium in Sweden (LD), and Missouri Botanical Garden Herbarium in the USA (MO) was also searched (Thiers, 2016). In addition, the regional floras and relevant taxonomic literature on Taiwanese *Scleria* were reviewed (Bergius, 1765; Ohwi, 1932; Koyama, 1978; Koyama *et al.*, 2000; Leong and Kuoh, 2000; Zhang *et al.*, 2010).

Genomic DNA extraction, amplification, and sequencing

Genomic DNA of putative *S. sumatrensis* leaves was extracted using the Plant Genomic DNA Extraction Miniprep System (Viogene, Taipei, Taiwan) and then stored at -20 °C before use.

To amplify the nuclear ribosomal internal transcribed spacer regions (ITS; ITS1 + 5.8S + ITS2) and two chloroplast regions (*ndhF* and *rps16*) successfully applied in an earlier *Scleria* study (Bauters *et al.*, 2016), we tested the PCR primers mentioned in the work of Bauters *et al.* (2016). We also designed more

economically efficient primers for the *Scleria* ITS region. The sequences of the PCR primers used in this study are listed in Table 1. A mixture of 1 μL genomic DNA, 1 μL 10 μM forward primer, 1 μL 10 μM reverse primer, 12.5 μL 2x Taq PCR Master Mix (Tiangen Biotech, Peijing, China), and 9.5 μL nuclease-free water was utilized for PCR amplification. The optimal PCR amplification conditions are provided in Table 1. For the ITS region, none of the polymorphic sites were detected in the direct sequencing of our *S. sumatrensis* samples. Hence, no cloning was conducted for the ITS region.

The PCR products were then purified and commercially sequenced in an ABI 3730xl DNA Analyzer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, U.S.) at the Genomics BioSci & Tech. Co., Ltd., New Taipei, Taiwan. GenBank accession numbers of the newly generated sequences are shown in Table 2.

In addition, the published DNA sequences of *S. sumatrensis* and other *Scleria* taxa in section *Elatae* C.B. Clarke were downloaded from GenBank. Sequences of additional *Scleria* taxa from 14 other sections analyzed in Bauters *et al.*'s (2016) work were selected for our molecular identification analyses. Two taxa (*Bisboeckelera microcephala* (Boeckeler) T. Koyama and *Calyptrocarya glomerulata* (Brongn.) Urb.) from a closely related tribe, Bisboeckelereae, were also applied to represent the outgroups in our analyses. All studied taxa and their GenBank accession numbers are available in Table 2.

Sequence alignment and phylogenetic analysis

The newly generated DNA reads were de novo assembled with Geneious Prime v.2020.2.4 (https://www.geneious.com). The contigs were then aligned in MAFFT v.7.453 (Katoh, 2002; Katoh and Standley, 2013) with DNA sequences from other *Scleria* taxa and outgroups (Table 2). The ITS, *ndhF*, and *rps16* alignments were manually adjusted in Mesquite v.3.61 (Maddison and Maddison, 2016) if needed.

Earlier studies have suggested that the plastome structures in angiosperms are highly conserved (ex. Burke *et al.*, 2016; Liu *et al.*, 2018; Jin *et al.*, 2020). If no

Table 2. List of taxa and DNA sequences with their GenBank accession numbers applied in this study. The newly generated sequences are indicated with asterisks, and missing data are shown with "--". *Scleria* sections were assigned by following the work of Bauters *et al.* (2016).

Taxa	*Scleria* Sections	ITS	*ndhF*	*rps16*
Outgroups				
Bisboeckelera microcephala (Boeckeler) T. Koyama		LN886916	LN887034	LN887145
Calyptrocarya glomerulata (Brongn.) Urb.		LN886843	GU075474	LN887070
***Scleria* species**				
Scleria baronii C.B. Clarke ex Cherm.	*Abortivae* Cherm. ex Bauters	LN886884	LN887000	--
Scleria catophylla C.B. Clarke	*Hypoporum* (Nees) Endl.	MG708555	LN886930	LN887048
Scleria ciliaris Nees	*Elatae* C.B. Clarke	LN886824	LN886935	LN887053
Scleria corymbosa Roxb.	*Margaleia* Raf.	LN886836	LN886947	LN887064
Scleria foliosa Hochst. ex A. Rich.	*Foveolidia* Raf.	LN886855	LN886967	LN887082
Scleria goossensii De Wild.	*Acriulus* (Ridl.) R.W. Haines & Lye	LN886857	LN886969	LN887084
Scleria kerrii Turrill	*Elatae* C.B. Clarke	LN886840	LN886951	LN887067
Scleria lacustris C. Wright	*Margaleia* Raf.	LN886806	LN886917	LN887035
Scleria latifolia Sw.	*Schizolepis* (Schrad. ex Nees) C.B. Clarke	LN886861	LN886973	LN887088
Scleria melanomphala Kunth	*Melanomphalae* Bauters	LN886848	LN886959	LN887075
Scleria microcarpa Nees ex Kunth	*Ophryoscleria* (Nees) C.B. Clarke	LN886844	LN886941	LN887058
Scleria naumanniana Boeckeler	*Naumannianae* Bauters	LN886866	LN886978	LN887093
Scleria oblata S.T. Blake	*Elatae* C.B. Clarke	LN886834	LN886945	LN887062
Scleria oligantha Michx.	*Trachylomia* (Nees) Bauters	LN886893	LN887010	LN887122
Scleria polycarpa Boeckeler	*Elatae* C.B. Clarke	--	LN886963	--
Scleria purpurascens Steud.	*Elatae* C.B. Clarke	LN886835	LN886946	LN887063
Scleria scabra Willd.	*Hymenolytrum* (Schrad. ex Nees) Core	LN886831	LN886942	LN887059
Scleria scrobiculata Nees & Meyen	*Elatae* C.B. Clarke	LN886839	LN886950	LN887066
Scleria sphacelata F. Muell.	*Browniae* C.B. Clarke	LN886911; LN886903	LN887029	LN887132
Scleria splitgerberiana Henrard ex Uittien	*Scleria* P.J. Bergius	LN886869	LN886981	LN887096
Scleria sumatrensis Retz. 1 #	*Elatae* C.B. Clarke	MW269765*	MW281549*	MW281554*
Scleria sumatrensis Retz. 2 #	*Elatae* C.B. Clarke	MW269766*	MW281550*	MW281555*
Scleria sumatrensis Retz. 3 #	*Elatae* C.B. Clarke	MW269767*	MW281551*	MW281556*
Scleria sumatrensis Retz. 4 #	*Elatae* C.B. Clarke	MW269768*	MW281552*	MW281557*
Scleria sumatrensis Retz. 5 #	*Elatae* C.B. Clarke	MW269769*	MW281553*	MW281558*
Scleria sumatrensis Retz.	*Elatae* C.B. Clarke	LN886837	LN886948	LN887065
Scleria terrestris (L.) Fassett	*Elatae* C.B. Clarke	LN886822	LN886933	LN887051

Samples were collected by Chau-Ching Huang (collection numbers: 201-1~ 201-5) and deposited at the TAI, TAIE and TAIF herbaria.

Table 3. Summary characteristics and maximum parsimony statistics for the three studied alignments.

Alignment	No. of outgroups/ *Scleria* accessions	Length (bp)	Best-fit nucleotide substitution model (Akaike information criterion)	No. of parsimony-informative sites (%)	CI/RI/RC/HI [#]
Nuclear region (ITS)	2/26	683	GTR+G	201 bp (29.43 %)	0.67/0.66/0.44/0.33
Combined chloroplast regions (cp; *ndhF* + *rps16*)	2/27	2,234	GTR+I+G	386 bp (17.28 %)	0.74/0.78/0.58/0.26
cp + ITS	2/27	2,917	GTR+I+G	587 bp (20.12 %)	0.72/ 0.74/0.53/0.28

recombination occurred in the *Scleria* plastomes, we concatenated the two chloroplast alignments (*ndhF* and *rps16*) into one (cp alignment; Table 3) for the following analyses. Moreover, combining multiple DNA alignments to provide higher tree perception and resolution has been successfully applied in many earlier studies focusing on various plant groups (ex. Bauters *et al.*, 2016; Liu *et al.*, 2017; Phillips and Bytebier, 2020). We then concatenated the ITS and cp alignments into one alignment (cp + ITS alignment; Table 3) for our phylogenetic reconstructions. The alignment characteristics are given in Table 3.

The maximum likelihood (ML) and Bayesian inference (BI) algorithms were conducted to reconstruct the phylogenetic trees using RAxML 8.2.12 (Stamatakis, 2014) and MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist *et al.*, 2012), respectively, on the CIPRES Science Gateway (Miller *et al.*, 2010) based on the combined alignment (cp + ITS alignment; Table 3). The best-fit nucleotide substitution models were estimated using jModeltest 2.1.8 (Darriba *et al.*, 2012). A ML tree was generated for each alignment in the RAxML v.8.2.12 (Stamatakis, 2014) on the CIPRES Science Gateway (Miller *et al.*, 2010) with 1,000 rapid bootstrap procedures (bs) and the alignment's best-fit nucleotide substitution model. The consistency index (CI) and retention index (RI)

Table 4. Morphological comparison between ***Scleria scrobiculata*** and ***S. sumatrensis***.

Taxa	*Scleria scrobiculata*	*Scleria sumatrensis*
Leaves-arrangement	pseudo-whorls	pseudo-whorls
Contraligule	membranous at the margin, ciliate at the base	margin ciliate
Inflorescence	paniculate	paniculate
Spikelet	unisexual	unisexual
Hypogynium	disk-like	cup-like
Achene	white to grayish	dark reddish brown

were then estimated by applying Mesquite v.3.61 (Maddison and Maddison, 2016) to understand the degree of homoplasy of the phylogenies. The rescaled consistency (RC) and homoplasy index (HI) were also calculated (Table 3). Moreover, a 50% consensus BI tree was reconstructed based on the cp + ITS alignment (Table 3) in MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist *et al*., 2012) on the CIPRES Science Gateway (Miller *et al*., 2010). Two independent Markov chain Monte Carlo (MCMC) runs with 5,000,000 searches in each run were performed with the best-fit nucleotide substitution model, and the posterior probability (pp) at each branch was then computed by sampling every 1,000[th] search with 25% burn-in. Finally, both the ML and BI trees were imagined in FigTree 1.4.4 (Rambaut, 2014).

Conservation assessment

The conservation status of *S. sumatrensis* was assessed by following the IUCN v.3.1 and v.4.0, the Red List of Vascular Plants of Taiwan 2017, and the IUCN Standards and Petitions Subcommittee 2019 (IUCN, 2012a,b; Editorial Committee of the Red List of Taiwan Plants, 2017; IUCN Standards and Petitions Subcommittee, 2019). Criteria B was applied, and both the extent of occurrence (EOO) and the area of occupancy (AOO) of the species were estimated.

RESULTS AND DISCUSSION

Authentications for *S. sumatrensis* and comparison among Taiwanese *Scleria*

The morphological data of the studied plants were obtained from both the ESRI research greenhouse and the field. To identify the studied plant, regional floras and related studies on *Scleria* (Bergius, 1765; Koyama, 1978; Koyama *et al*., 2000; Leong and Kuoh, 2000; Zhang *et al*., 2010) were reviewed. Our morphological data strongly suggested that the studied plant is *S. sumatrensis*, which is characterized by its pseudo-whorl leaves, cup-likehypogynium, and dark reddish-brown achene. The specimens of the studied plant were also compared with the type specimens of *S. sumatrensis* (in the HAST, KYO, and LD herbaria) and the allied Taiwanese *Scleria* species *S. scrobiculata* Nees & Mey. ex Nees (in the MO herbarium). Both *S. sumatrensis* and *S. scrobiculata* have pseudo-whorls leaves while other Taiwanese *Scleria*

species have alternate leaves (Koyama, 1978; Koyama *et al*., 2000; Leong and Kuoh, 2000). Additional herbarium specimens of these two species collected in Taiwan and nearby countries were also examined by visiting the HAST, TAIF and TAIE herbaria. The examined specimens are listed at the end of the TAXONOMIC TREATMENT. Details of the morphological and phenological information of *S. sumatrensis* are provided in the TAXONOMIC TREATMENT and Figures 1 & 2.

Moreover, our DNA alignments indicate that the five individuals of the putative *S. sumatrensis* have completely the same sequences in the ITS and *ndhF* regions and have only six variant characters in the *rps16* region (see the alignment in Appendix 1). Each individual has its own haplotype in *rps16* region. The highly similar sequences among individuals suggest very low genetic diversity within the rediscovered population. The ML and BI trees based on the cp + ITS alignment show exactly the same topology. The tree files are available in Appendix 1. Hence, only the ML tree is shown here (Figure 3). Our trees suggest that the putative plants are grouped with *S. sumatrensis* with strong support (bs = 100; pp = 1.00; Figure 3). Therefore, the putative plants are very likely *S. sumatrensis*.

In summary, both our molecular and morphological data strongly support that the studied plant is *S. sumatrensis*. Specimens and relevant literature of all Taiwanese *Scleria* species examined are listed immediately after the TAXONOMIC TREATMENT. *Scleria sumatrensis* is closely related to *S. scrobiculata*. A morphological comparison between *S. sumatrensis* and *S. scrobiculata* is available in Table 4. A key to the nine *Scleria* species in Taiwan is also provided.

Conservation status of *S. sumatrensis*

The distribution of *S. sumatrensis* is very restricted in Taiwan. Currently, only one population has been reported (this study). The AOO of *S. sumatrensis* is less than 10 km^2, and its grassland habitat shows extremely fluctuations and is threatened because of human activities. Based on the criteria B2(c) i+ii, we reassigned the conservation status of *S. sumatrensis* to the Critically Endangered (CR) category (IUCN, 2012a; b; Editorial Committee of the Red List of Taiwan Plants, 2017; IUCN Standards and Petitions Subcommittee, 2019).
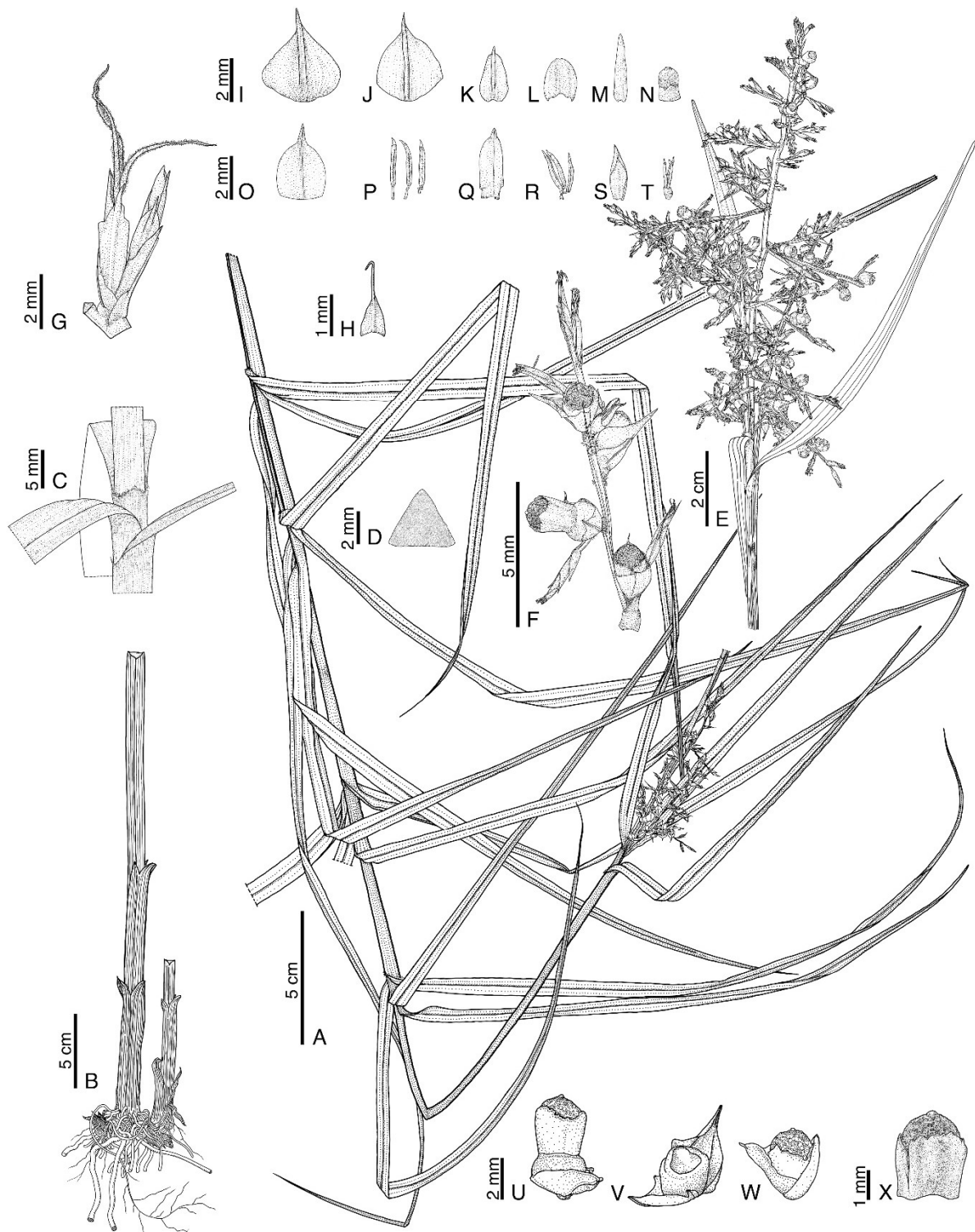
Fig. 1. *Scleria sumatrensis* Retz. **A**, habit; **B**, base of the plant; **C**, middle part of the culm, showing pseudo-whorled leaves and ciliate contraligule; **D**. cross-section of the culm; **E**, inflorescence; **F**, fruiting shoot; **G**, androgynous spikelet, including one pistillate spikelet and one staminate spikelet; **H**, bractlet of an androgynous spikelet; **I–M**, scales of pistillate spikelet (left to right: outside to inside); **N**, pistil with hypogynium; **O–T**, staminate scales and stamens (left to right: outside to inside); **U**, spikelet with fruit; **V**, disc with scales; **W**, early fruiting spikelet, with two outside scales removed; and **X**, achene with hypogynium. Illustration by C.-K. Liao.

Fig. 2. *Scleria sumatrensis* Retz. **A**, habit; **B**, part of the culm, showing the pseudo-whorled leaves and ciliate contraligule (arrowed); **C**, part of the inflorescence, showing pistillate and staminate spikelets; and **D**, achenes supported on hypogyniums. Photos taken by C.-K. Liao.

Suggestions for further conservation efforts and future studies

Following the rediscovery of *S. sumatrensis*, its conservation status reassignment, and the almost identical sequences among the sampled individuals, we here highlight the urgent needs for both in-situ and ex-situ conservation for this species. Mature seeds of *S. sumatrensis* collected in 2020 have been preserved in the seed bank of the Endemic Species Research Institute (ESRI), Nantou, Taiwan, for ex-situ conservation, and a few living individuals are maintained in the research greenhouse at ESRI. Seed bank storage and living material maintaince for *S. sumatrensis* in additional research institutions should be performed. Unfortunately, the rediscovered population of *S. sumatrensis* is located in an area with frequent human activities, which hinders ex situ conservation. Currently, specific laws have not been enacted to protect the threatened species in the Red List in Taiwan nowadays. The only laws to limit human activities and land uses in this area are the Catchment Area Regulation (https://www.wrasb.gov.tw/en/KeyOperations/AreaRegul ation.aspx) and Soil and Water Conservation Law (https://law.moj.gov.tw/eng/LawClass/LawAll.aspx?pcod

e=M0110001) in Taiwan. To reduce the possible artificial disturbances stirring up by this rediscovery, we here reveal no locality details for the rediscovered population.

Incorporating our data with limited information from earlier studies on *S. sumatrensis* (Ohwi, 1932; Koyama, 1978; Koyama *et al*., 2000; Zhang *et al*., 2010), we are still not able to tell whether the rediscovered population is the same population Faurie collected from in 1914, which represents an undiscovered population that has been persisted for a long time, or a population newly recolonized after the species went extinct in Taiwan (Carlquist, 1967; Blake *et al*., 2012; Larridon *et al*., 2021). The Faurie's plant hunting itinerary rearranged based on his collections does not show collection locations further south beyond Bankinsing (Wanjin Villages, Wanluan Township, Pingtung County, Taiwan at present) and Raisha (Laiyi Villages, Laiyi Township, Pingtung County, Taiwan at present) (Kakuta, 1992). It is likely that Faurie assigned all his collections from the southern part of Taiwan to these two localities. Therefore, although the rediscovered *S. sumatrensis* population is approximately 50-km linear distance away from Bankinsing, which is where the locality Faurie's *S.*
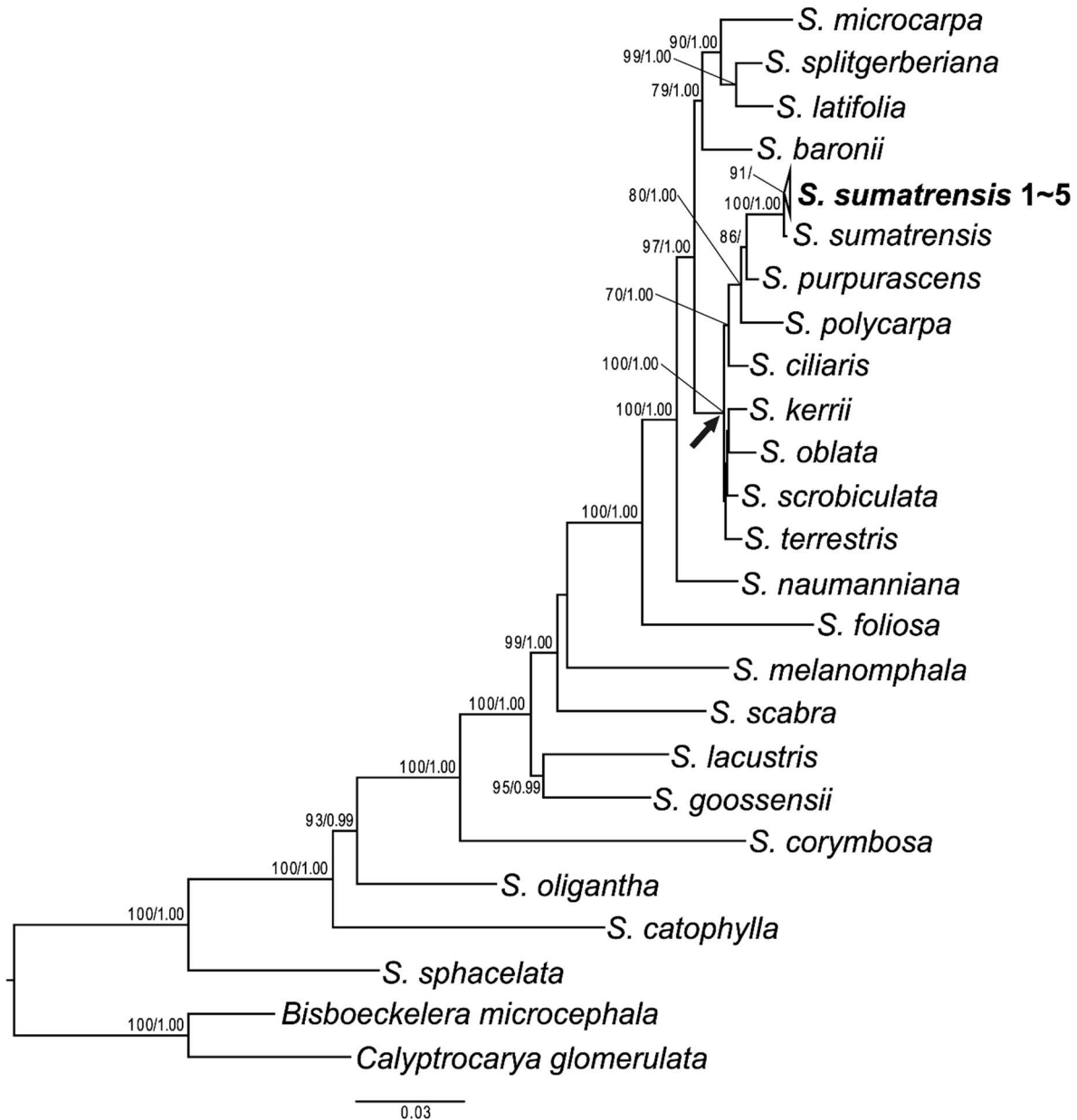
403

Fig. 3. Molecular identification of ***Scleria sumatrensis*** Retz. based on the phylogenetic analyses applying the combined nuclear and chloroplast regions (ITS + *ndhF* + *rps16*). Numbers at nodes indicate the branch supports when the bootstrap percentages are greater than 70% and Bayesian posterior probabilities are greater than 0.95. The arrow denotes the crown node of sect. *Elatae*. Numbers after *S. sumatrensis* refer to the voucher information shown in Table 2.

sumatrensis collection was assigned, the possibility that the rediscovered population is the one Faurie or his helpers visited in 1914 cannot be eliminated. Moreover, due to the lack of earlier comprehensive vegetationinvestigations in the surrounding area of the rediscovered population, how long this population has been extant is difficult to determine. Further seed bank analyses based on soil samples might fill this gap (e.g. Jacquemyn *et al*., 2011; Stroh *et al*., 2012; Saatkamp *et al*., 2018). Additional DNA samples of *S. sumatrensis* from Faurie's collections from Taiwan, other earlier

herbarium collections, and current populations from other countries might be also helpful to determine whether or not the rediscovered population has recolonized (e.g. Ge *et al*., 2012; Stewart *et al*., 2017; Bieker and Martin, 2018; Sugita *et al*., 2020).

CONCLUSIONS

In conclusion, with both of molecular and morphological authentications, we affirm the rediscovery of *S. sumatrensis* in Taiwan. Description, photos, and

illustrations of *S. sumatrensis* are provided. A morphological comparison among all Taiwanese *Scleria* is also given to facilitate further relevant studies on this genus. The conservation status of *S. sumatrensis* is reassigned to the Critically Endangered (CR) category. Additionally, here, we call attention to the need for both in-situ and ex-situ conservation efforts for *S. sumatrensis* in Taiwan.

TAXONOMIC TREATMENT

Key to *Scleria* of Taiwan

1a. Inflorescence loosely narrow panicles; hypogynium reduced .... *S. lithosperma*
1b. Inflorescence paniculate; hypogynium well developed 2
2a. Leaves crowded in pseudo-whorls of 3–5 in the middle part of the culms ....….......................... 3
3a. Contraligules with ciliate margin; hypogynium cup-like; achenes dark reddish-brown .... *S. sumatrensis*
3b. Contraligules with membranous margins; hypogynium 3-lobed disk-like; achenes white to slightly grayish .... *S. scrobiculata*
2b. Leaves distinctly alternate 4
4a. Slender annual, rhizomes inconspicuous; culms 10–70 cm tall 5
5a. Hypogynium deeply 3-lobed, lobes lanceolate, acute at apex ....…... *S. biflora*
5b. Hypogynium shallowly 3-lobed, lobes orbicular, obtuse at apex ... 6
6a. Culms 10-35 cm tall, achenes globose, 1.25–1.75 mm long, without darker brown marking .... *S. rugosa*
6b. Culms 25-45 cm tall, achenes ellipsoid to oblong-ellipsoid, 2.25–2.75 mm long, with darker brown marking ...... *S. novae-hollandiae*
4b. Perennial, rhizomes woody; culms 30–200 cm tall 7
7a. Hypogynium deeply 3-lobed, lobes ovate to lanceolate, acute at apex; culms 30-90 cm tall ....…..…............ *S. levis*
7b. Hypogynium shallowly 3-lobed, lobes round to ovate, obtuse at apex; culms 60-200 cm tall 8
8a. Leaves pale green, pubescent, apex acute, not elongated; spikelets greenish to olivaceous brown .... *S. laeviformis*
8b. Leaves dark green, glabrous, apex acuminate to attenuate, elongated; spikelets purplish to dark brown .... *S. terrestris*

Scleria sumatrensis Retz., Oberv. Bot. 5:19, pl. 2. 1789. T. Koyama, Fl. Taiwan 5: 195. 1978. T. Koyama, C. S. Kuoh & W. C. Leong, Fl. Taiwan 2ed 5: 313. 2000.

印尼珍珠茅 **Figs. 1 & 2**

Scleria fauriei Ohwi, Acta Phytotax. Geobot. 1(1): 78–79. 1932.

Perennial. Rhizomes woody, short creeping, oblique, stout. Culms erect and robust, loosely caespitose, 1–4 m tall, 6-8 mm thick, trigonous, glabrous or scabrous on the ridges, leafy. Leaves cauline, crowded in pseudo-whorls of 3–5 in the middle parts of the culms, linear, 30–100 cm long, 7–15 mm wide, herbaceous to coriaceous, glabrous, margins scabrous, apex acuminate to apiculate ; sheaths trigonous, scabrous on ridges, 10–16 cm long, brown toward base, the middle sheaths winged occasionally, the lower sheaths fuscous or purple-brown, slightly disintegrating into reticulate fibers; contraligules ciliate margin, ca. 0.5 mm. Inflorescences consisting of 2–4 compound panicles, elliptical to oblong, 10–35 cm long, 3–10 cm wide, branched; lateral branches single, elliptic to oblong, 3–6 × 2–4 cm; peduncles of inflorescence branches rigid, triquetrous; bractlets glumelike, ovate, awned, only basal one with short sheath, others sheathless.

Pistillate and staminate spikelets solitary, brown to reddish brown; pistillate spikelets ovoid, ca. 4 mm long, sessile; staminate spikelets lanceolate, ca. 4 mm long, sessile or short pedunclled; stamens 3, filaments short, basifixed, ca. 0.5 mm long, anthers short linear, ca. 2 mm long; pistil globose, ca. 1 mm in diameters, hypogynium cup-like, irregular crenate in the upper edges, stigmas 3. Achenes depressed globose, 2–2.5 mm in diameters, dark reddish brown, shining, sparsely puberulent; hypogynium cup-like, crenate in upper edges, dark reddish brown. Flowering and fruiting for the whole year. Wet forest margins and marshes at low altitudes in Taiwan.

Geographical distribution: Japan (Ryukyu), Taiwan, southern China, southeastern Asia, Australia, and western Carolines.

Note: *Scleria sumatrensis* is morphologically similar to *S. scrobiculata* in their arrangement and shape of leaves, height of culm, and composition of inflorescence. However, the former can be distinguished from the latter by its contraligules ciliated (*vs.* contraligules with membranous margin and ciliate at the base), cup-like hypogynium (*vs.* 3-lobed disk-like hypogynium), and dark reddish brown achenes (*vs.* white to slightly grayish achenes).

Specimens examined:

Scleria sumatrensis Retz. **INDONESIA**: E Sumatra, *Wennerberg s.n.* (Holotype: LD). **PHILIPPINES**: Negros Oriental, Sibulan, Lake Balinsasayao, *Hsu 6977* (TAIF). **TAIWAN**: Pintung County: Mudan, *Huang 201* (TAI, TAIE, TAIF).

Scleria fauriei Ohwi. **TAIWAN**: Pintung County: Bankinsing, *Faurie 5* (Holotype: KYO, Isotype: HAST).

Scleria scrobiculata Nees & Mey. ex Nees. **JAPAN:** Okinawa: Iriomote Island, Shirahama, *Wang, Hsu, Moore, Liu 11807* (TAIE), Kuira-gawa river, *Weng 2327* (HAST). **PHILIPPINES:** Luzon: Cagayan, *Rubite 109* (HAST); Manilla: *Mertens s.n.* (Syntype: MO). **TAIWAN:** Kaohsiung County: Liouguei District: Hsinliao Village, *Hsu 953* (TAIF), Mt. Shibaluohan, *Yang 200* (TAIE); Pintung County: Manzhou Township, Nanjenshan Working Station, *Hsu 1747* (TAIF), Nanren Lake, *Yang 268* (TAIE), Mudan Township, Xuhai, *Yang 169* (TAIE); Taipei City: Hokuto, *Y. Simada 3271* (HAST); Taitung County: Changbin Township, Shuimuting, *Hsu 10506* (TAIF), Lanyu Township, *Chiou 11164* (TAIF), Hsiaotienchih, *Chiu & Chen 04110* (TAIF), Tienchi, *Leong 2265* (HAST), *Huang 2429* (HAST), Mt. Huoshao, *Chen 6319* (TAIE), Lyudao Township, *Leong 3296* (HAST), Haisenping *Peng 7584* (HAST), Kuoshan Ancient Trail, *Hsu & Su s.n.* (TAIF); Taoyuan County: Daman, *K. Takiya s.n.* (TAIF).

ACKNOWLEDGMENTS

Research Institute, Council of Agriculture, Executive Yuan, Taiwan (108B012-01) to CCH and CKL.

LITERATURE CITED

Bauters, K., P. Asselman, D.A. Simpson, A.M. Muasya, P. Goetghebeur and I. Larridon. 2016. Phylogenetics, ancestral state reconstruction, and a new infrageneric classification of *Scleria* (Cyperaceae) based on three DNA markers. Taxon **65(3):** 444–466.

Bergius, P.J. 1765. *Scleria*, Et nytt Orte-genus ifrán America, framlagt och befkrifvet. Kongl. Svenska Vetensk Acad. Handl. **26:** 142–148.

Bieker, V.C. and M.D. Martin. 2018. Implications and future prospects for evolutionary analyses of DNA in historical herbarium collections. Bot. Lett. **165(3-4):** 409–418.

Blake, S., M. Wikelski, F. Cabrera, A. Guezou, M. Silva, E. Sadeghayobi, C.B. Yackulic and P. Jaramillo. 2012. Seed dispersal by Galápagos tortoises. J. Biogeogr. **39(11):** 1961–1972.

Borah, D., N. Tanaka, L.V. Averyanov, M. Taram and D.K. Roy. 2020. Rediscovery of *Tupistra stoliczkana* (Asparagaceae) in northeastern India and the identity of *T. ashihoi*. Phytotaxa **443(2):** 207–210.

Burke, S.V., W.P. Wysocki, F.O. Zuloaga, J.M. Craine, J.C. Pires, P.P. Edger, D. Mayfield-Jones, L.G. Clark, S.A. Kelchner and M.R. Duvall. 2016. Evolutionary relationships in Panicoid grasses based on plastome phylogenomics (Panicoideae; Poaceae). BMC Plant Biol. **16(1):** 140.

Carlquist, S. 1967. The biota of long-distance dispersal. V. Plant dispersal to Pacific Islands. Bull. Torrey Bot. Club **94(3):** 129.

Chang, C.-H., K.-T. Shao, H.-Y. Lin, Y.-C. Chiu, M.-Y. Lee, S.-H. Liu and P.-L. Lin. 2017. DNA barcodes of the native ray-finned fishes in Taiwan. Mol. Ecol. Resour. **17(4):** 796–805.

Darriba, D., G.L. Taboada, R. Doallo and D. Posada. 2012. jModelTest 2: more models; new heuristics and parallel computing. Nat. Methods **9(8):** 772.

Editorial Committee of the Red List of Taiwan Plants. 2017. The Red List of Vascular Plants of Taiwan. Endemic Species Research Institute, Forestry Bureau, Council of Agriculture, Executive Yuan and Taiwan Society of Plant Systematics, Taiwan. pp.5–12, 73.

Edwards, C.E., B. Bassüner, C. Birkinshaw, C. Camara, A. Lehavana, P.P. Lowry, J.S. Miller, A. Wyatt and P.W. Jackson. 2018. A botanical mystery solved by phylogenetic analysis of botanical garden collections: the rediscovery of the presumed-extinct Dracaena umbraculifera. Oryx **52(3):** 427–436.

Fisher, D.O. and S.P. Blomberg. 2011. Correlates of rediscovery and the detectability of extinction in mammals. Proc. Roy. Soc. B-Biol. Sci. **278(1708):** 1090–1097.

Ge, X.-J., T.-W. Hsu, K.-H. Hung, C.-J. Lin, C.-C. Huang, C.-C. Huang, Y.-C. Chiang and T.-Y. Chiang. 2012. Inferring multiple refugia and phylogeographical patterns in *Pinus massoniana* based on nucleotide sequence variation and DNA fingerprinting. PLoS ONE **7(8):** e43717.

Hinchliff, C.E., A.E. Lliully A., T. Carey and E.H. Roalson. 2010. The origins of *Eleocharis* (Cyperaceae) and the status of Websteria, Egleria, and Chillania. Taxon **59(3):** 709–719.

Hirano, T., S. Wada, H. Mori, S. Uchida, T. Saito and S. Chiba. 2018. Genetic and morphometric rediscovery of an extinct land snail on oceanic islands. J. Mollus. Stud. **84(2):** 148–156.

Hsiao, C., N.J. Chatterton, K.H. Asay and K.B. Jensen. 1994. Phylogenetic relationships of 10 grass species: an assessment of phylogenetic utility of the internal transcribed spacer region in nuclear ribosomal DNA in monocots. Genome **37(1):** 112–120.

Huelsenbeck, J. P. and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics **17(8):** 754–755.

Humphreys, A.M., R. Govaerts, S.Z. Ficinski, E. Nic Lughadha and M.S. Vorontsova. 2019. Global dataset shows geography and life form predict modern plant extinction and rediscovery. Nat. Ecol. Evol. **3(7):** 1043–1047.

IUCN. 2012a. Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. IUCN, Gland, Switzerland and Cambridge, UK.

IUCN. 2012b. IUCN Red List Categories and Criteria: Version 3.1. 2nd. IUCN, Gland, Switzerland and Cambridge, UK.

IUCN Standards and Petitions Subcommittee. 2019. Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. (http://www.iucnredlist.org/documents/RedListGuidelines.pdf. accessed on 10.12.2020)

Jacquemyn, H., C. Van Mechelen, R. Brys and O. Honnay. 2011. Management effects on the vegetation and soil seed bank of calcareous grasslands: an 11-year experiment. Biol. Conserv. **144(1):** 416–422.

Jin, D.-M., J.-J. Jin and T.-S. Yi. 2020. Plastome structural conservation and evolution in the Clusioid clade of Malpighiales. Sci. Rep. **10(1):** 9091.

Kakuta, M. 1992. Abbé Urbain Faurie and an itinerary calendar of his plant hunting. Acta Phytotaxon. Geobot. **43:** 59–74. [In Japanese]

Katoh, K. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. **30(14):** 3059–3066.

Katoh, K. and D.M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and isability. Mol. Biol. Evol. **30(4):** 772–780.

Koyama, T. 1978. *Scleria*. In: Lee, H.-L. *et al*. (eds.), Flora of Taiwan **5:** 195–196. Editorial Committee, Dept. Bot., NTU, Taipei, Taiwan.

Koyama, T., C.-S. Kuoh, and W.-C. Leong. 2000. *Scleria*. In: Huang, T.-C. *et al*. (eds.), Flora of Taiwan, 2nd ed. **5:** 309–313. Editorial Committee, Dept. Bot., NTU, Taipei, Taiwan.

Larridon, I., J. Galán Díaz, K. Bauters and M. Escudero. 2021. What drives diversification in a pantropical plant lineage with extraordinary capacity for long-distance dispersal and colonization? J. Biogeogr. **48(1):** 64–77.

Lee, S.Y. and R. Mohamed. 2016. Rediscovery of *Aquilaria rostrata* (Thymelaeaceae), a species thought to be extinct, and notes on *Aquilaria* conservation in Peninsular Malaysia. Blumea **61(1):** 13–19.

Lee, T.E., D.O. Fisher, S.P. Blomberg and B.A. Wintle. 2017. Extinct or still out there? Disentangling influences on extinction and rediscovery helps to clarify the fate of species on the edge. Global Change Biol. **23(2):** 621–634.

Leong, W.-C. and C.-S. Kuoh. 2000. Two new records of *Scleria* Bergius (Cyperaceae) in Taiwan. Taiwania **45(4):** 334–339.

Liu, S.-H., C.E. Edwards, P.C. Hoch, P.H. Raven and J.C. Barber. 2018. Genome skimming provides new insight into the relationships in *Ludwigia* section *Macrocarpon*, a polyploid complex. Am. J. Bot. **105(5):** 875–887.

Liu, S.-H., P.C. Hoch, M. Diazgranados, P.H. Raven and J.C. Barber. 2017. Multi-locus phylogeny of *Ludwigia* (Onagraceae): Insights on infrageneric relationships and the current classification of the genus. Taxon **66(5):** 1112–1127.

Maddison, W.P. and D.R. Maddison. 2016. Mesquite: a modular system for evolutionary analysis. Version 3.11.

Miller, M.A., W. Pfeiffer and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 1–8. Gateway Computing Environments Workshop (GCE), New Orleans, Louisiana, USA.

Ohwi, J. 1932. Symbolae ad Floram Asiae Orientalis IV. Acta Phytotax. Geobot. **1:** 66–87.

Oxelman, B., M. Liden and D. Berglund. 1997. Chloroplastrps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). Plant Syst. Evol. **206(1-4):** 393–410.

Phillips, D.P. and B. Bytebier. 2020. A morphometric and molecular phylogenetic analysis of the African orchid genus *Stenoglottis* (Orchidaceae: Orchidoideae). Bot. J. Linn. Soc. **193(3):** 340–362.

Pimm, S.L., C.N. Jenkins, R. Abell, T.M. Brooks, J.L. Gittleman, L.N. Joppa, P.H. Raven, C.M. Roberts and J.O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344(6187):** 1246752.

Rambaut, A. 2014. FigTree v1.4.2 http://tree.bio.ed.ac.uk/software/figtree/.

Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M.A. Suchard and J.P Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61(3):** 539–542.

Saatkamp, A., F. Henry and T. Dutoit. 2018. Vegetation and soil seed bank in a 23-year grazing exclusion chronosequence in a Mediterranean dry grassland. Plant Biosyst. **152(5):** 1020–1030.

Schmidt Silveira, F., A.A. Schneider and L.R. de Moura Baptista. 2019. The role of a local rediscovery in the evaluation of the conservation status of a plant species: Testing the hypothesis of the biodiversity knowledge gap. J. Nat. Conserv. **48:** 91–98.

Sheue, C. R., J. W. Yong and Y. P. Yang. 2005. The *Bruguiera* (Rhizophoraceae) species in the mangroves of Singapore, especially on the new record and the rediscovery. Taiwania **50(4):** 251–260.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics **30(9):** 1312–1313.

Stewart, F.E.C., J.P. Volpe, J.S. Taylor, J. Bowman, P.J. Thomas, M.J. Pybus and J.T. Fisher. 2017. Distinguishing reintroduction from recolonization with genetic testing. Biol. Conserv. **214:** 242–249.

Stroh, P.A., F.M. Hughes, T.H. Sparks and J.O. Mountford. 2012. The influence of time on the soil seed bank and vegetation across a landscape-scale wetland restoration project. Restor. Ecol. **20(1):** 103–112.

Sugita, N., A. Ebihara, T. Hosoya, U. Jinbo, S. Kaneko, T. Kurosawa, M. Nakae and T. Yukawa. 2020. Non-destructive DNA extraction from herbarium specimens: a method particularly suitable for plants with small and fragile leaves. J. Plant Res. **133(1):** 133–141.

Thiers, B. 2016. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/

Wagner, D. L. 2020. Insect declines in the anthropocene. Annu. Rev. Entomol. **65(1):** 457–480.

Wahlsteen, E., W. Zhou, Q. (Jenny) Xiang and K. Rushforth. 2020. Rediscovery of the lost little dogwood *Cornus wardiana* (Cornaceae) - Its phylogenetic and morphological distinction and implication in the origin of the Arctic-Sino-Himalayan disjunction. J. Syst. Evo. **59(2):** 405–416

Wong, K.J., P.K. Ng and M.S. Jeng. 2018. From an old eroded carapace: rediscovery of the majid crab *Leptomithrax sinensis* Rathbun, 1916 (Crustacea, Brachyura, Majidae) from Taiwan and Japan. Zool. Stud. **57:** 49.

Zhang, S., G.C. Tucker and D.A. Simpson. 2010. *Scleria*. In: Wu Z.-Y., P.H. Raven and D.Y. Hong (eds.) Flora of China. **vol. 23.** Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp. 260–268.

Supplementary materials are available from Journal Website.