Ann. Bot. Fennici 49: 67–74 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 26 April 2012

Polystichum perpusillum (sect. *Haplopolystichum*, Dryopteridaceae), a new fern species from Guizhou, China

Li-Bing Zhang[1] & Hai He[2,*]

[1)] *Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu, Sichuan 610041, China; and Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, USA*
[2)] *College of Life Sciences, Chongqing Normal University, Shapingba, Chongqing 400047, China (*corresponding author's e-mail: hehaicq@gmail.com)*

Received 20 Dec. 2010, final version received 23 Mar. 2011, accepted 24 Mar. 2011

Zhang, L. B. & He, H. 2012: *Polystichum perpusillum* (sect. *Haplopolystichum*, Dryopteridaceae), a new fern species from Guizhou, China. — *Ann. Bot. Fennici* 49: 67–74.

Polystichum perpusillum L.B. Zhang & H. He, a new fern species of *Polystichum* sect. *Haplopolystichum* (Dryopteridaceae), is described and illustrated from the entrance to a karst cave in southern Guizhou, China. A phylogenetic analysis based on the chloroplast *trnL-F* sequences shows that it is phylogenetically isolated in the section with no close relatives. Morphologically, it is similar to *P. minutissimum*, but *P. perpusillum* has an acute lamina apex, up to 12 pairs of pinnae per lamina, and deltoid-ovate or ovate-lanceolate rachis scales, while *P. minutissimum* has a round lamina apex, 5–8 pairs of pinnae per lamina, and subulate or linear rachis scales. *Polystichum perpusillum* has a granulate sculpture with verrucae on its perispore, a sculpture rare in the genus. The species is considered to be critically endangered.

Introduction

Maolan National Nature Reserve (25°09´20´´–25°20´50´´N, 107°52´10´´–108°45´40´´E) in the south of Libo County, southern part of Guizhou Province, is dominated by a karst landscape. Unlike traditional karst topography, characterized by stone desertification, Maolan National Nature Reserve has a forest coverage of 91.59% (Zhou 1987). Its well preserved subtropical forest on a karst topography is unique in China, and globally a rarity.

During a field investigation in this area in 2008, we collected a few specimens of a species of the genus *Polystichum* growing on a limestone cliff at the entrance of a karst cave deep in the forest. Our molecular and morphological work shows that it is an undescribed species that is phylogenetically isolated.

Material and methods

The morphological, palynological, and molecular data are based on a single collection assignable to this species, cited below. We measured the roots, petiole, rachis, scales and indusia with a micrometer under microscope.

Molecular methods

Total genomic DNA was isolated from silica-dried leaves using Plant Genomic DNA Kits (TIANGEN BioTech., Beijing, China). The plastid *trnL-F* intergenic spacer was amplified using the universal primers *e* and *f* of Taberlet *et al*. (1991). The PCR protocols followed Zhang *et al*. (2001). Amplified fragments were purified with TIANquick Mini Purification Kits (TIANGEN). Purified PCR products were sequenced by InvitrogenTM (Shanghai, China).

Following previous phylogenetic analyses (Driscoll & Barrington 2007, Lu *et al*. 2007, Li *et al*. 2008, Zhang & He 2010, Zhang *et al*. 2010), 30 species of *Polystichum* sect. *Haplopolystichum s. lato* (Zhang & He 2009a) including sects. *Crucifilix*, *Haplopolystichum*, and *Sphaenopolystichum*, plus the genera *Cyrtogonellum* and *Cyrtomidictyum*, and *Cyrtomium* subser. *Balansana* (Zhang & He 2009a), as well as seven representatives of the monophyletic *Polystichum s. stricto* (*sensu* Little & Barrington 2003), were included as the ingroup. Seven species of *Cyrtomium s. stricto sensu* Lu *et al*. (2007) and two species of *Phanerophlebia*, following Driscoll and Barrington (2007), were used as outgroups. In total, we included 54 accessions in the analysis. All sequences used in this study together with their GenBank accession numbers and voucher information or source publications are listed in the Appendix.

The alignment of nucleotides was manually obtained using the alignment of the TreeBase (www.treebase.org) accession number M4534 (Zhang & He 2010) as a backbone. Gap characters were scored using modified complex indel coding (Simmons & Ochoterena 2000, Müller 2006).

Phylogenetic analysis followed the procedure in Zhang and He (2010) and Zhang *et al*. (2010). Equally weighted parsimony tree searches were conducted using 1000 tree-bisection-reconnection (TBR) searches in PAUP*4.0b10 (Swofford 2001) with an initial "Maxtrees" set to 1000 and auto-increased with 100. Parsimony jackknife analyses (Farris *et al*. 1996) were conducted using PAUP* with the removal probability set to approximately e-1 (37.073%), and "jac" resampling emulated. One thousand replicates were performed with ten TBR searches per replicate and a maximum of 100 trees held per TBR search.

Spore morphology

The spore samples were attached onto a specimen stub with double-sided tape, and sputter-coated with gold-palladium. Observations were conducted using a JSE-5900LV Scanning Electron Microscope (SEM) (Electron Co., Tokyo, Japan) at 20 kV at the Sichuan University, Chengdu, China. Measurements were carried out using digital images of five spores with the measure tool in Adobe Photoshop (ver. 7.0.1; Adobe Systems Inc., San Jose, California). The descriptive terminology follows Punt *et al*. (2007).

Results and taxonomic treatment

Polystichum perpusillum L.B. Zhang & H. He, *sp. nova* (Fig. 1 and 2).

(*P.* sect. *Haplopolystichum*)

Type: China. Guizhou Province, Libo County, Lihua Town, Gaowang Village, Loudou Forest, 29°19´10.2´´N, 108°4´14.844´´E, 560 m, on limestone cliff at the entrance of a karst cave, 27 Oct. 2008 *L. B. Zhang, H. He & C. B. Jiang 801* (holotype CDBI; isotypes CTC, MO, VT).

Etymology: The epithet is from the Latin prefix *per-*, very, and the Latin *pusillum*, small, referring to the very small stature of the species.

Plants perennial, evergreen, 3.3–9.5 cm tall. Rhizome 0.3–0.5 cm long, ascending, densely covered with scales; scales deltoid-ovate, brown, 0.9–1.4 mm long; roots brown when dry, up to 4 cm long, ca. 0.3 mm in diam. Leaves caespitose, 4–7 per rhizome; petiole 1.2–4.0 cm long, 0.4–0.9 mm in diam. at middle, canaliculate adaxially, green; basal petiole scales deltoid-ovate, 2.0–2.5 × 0.5–1.2 mm, chartaceous, brown, matte, margin slightly long-ciliate, apex acuminate or caudate; distal petiole scales similar but narrower and shorter, differing in size, ovate-lanceolate, chartaceous, brown, matte, margin regularly short-ciliate, apex caudate. Lamina lanceolate, slightly contracted toward

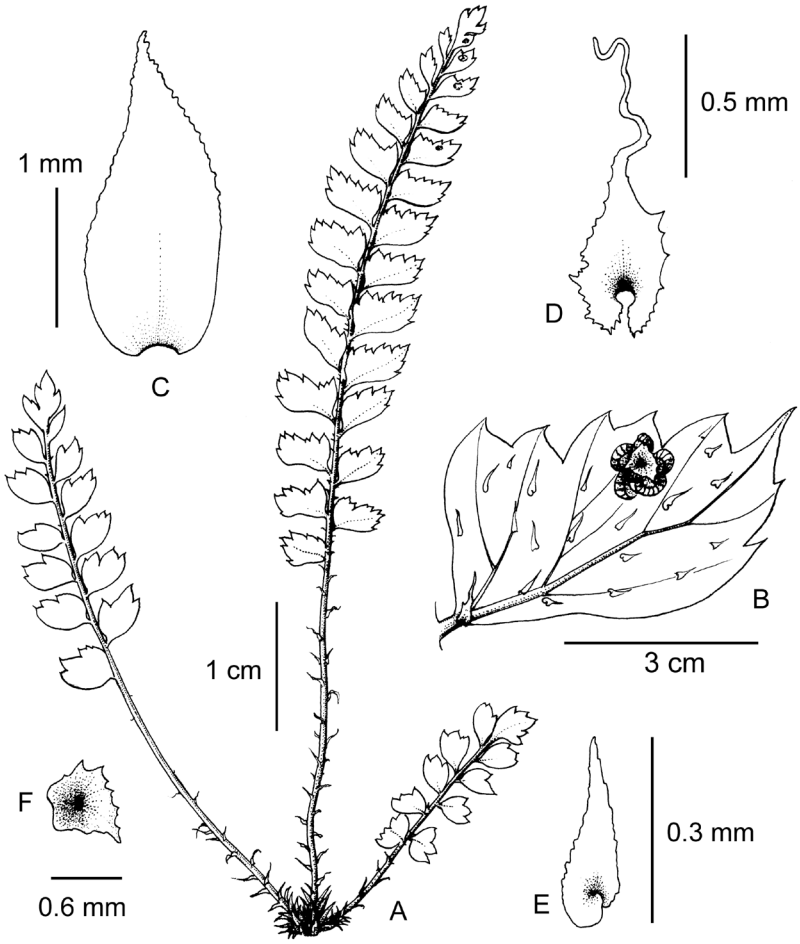

Fig. 1. *Polystichum perpusillum* (from an isotype in CDBI). — **A**: Frond. — **B**: Pinna from the middle of lamina. — **C**: Scale from base of petiole. — **D**: Rachis scale. — **E**: Microscales. — **F**: Indusium.

base, 1-pinnate, 2.1–6.1 cm long, 0.85–1.25 cm wide at middle, apex acute; rachis 0.4–0.7 mm in diam. at middle, without proliferous buds, adaxially sulcate; rachis scales deltoid-ovate or ovate-lanceolate, 0.5–1.3 mm long, base 0.25–0.31 mm wide, differing in size, chartaceous, brown, margin regularly ciliate, apex caudate apically. Pinnae 5–12 pairs, not overlapping, ascendant except lowest pairs, basal two pairs 2.5–6.0 mm apart, alternate, oblong, middle (largest) pinnae 3.2–7.3 × 2.0–3.5 mm, short-petiolulate (petiolules 0.2–0.4 mm long), chartaceous, acroscopic base auriculate, basiscopic base cuneate and often forming an 80°–120° angle with rachis, apex acute, acroscopic margin with 1–4 teeth, abaxially scaly, adaxially lustrous and glabrous; microscales on abaxial surface subulate or linear (narrow-type microscales), 0.13–0.54 mm long, base 0.05–0.07 mm wide; venation pinnate; midrib abaxially slightly raised, adaxially flat; lateral veins free, 2–3 pairs from midrib per pinna, alternate, each lateral vein again dichotomous or not, abaxially slightly raised and distinct, adaxially indistinct. Sori terminal on veins of upper pinnae, 1–3 per fertile pinna, close to pinna margin (center of sorus 0.4–0.6 mm distant from pinna margin); indusia peltate, ca. 0.6 mm in diam., membranaceous, brown, erose on margin.

MORPHOLOGICAL DISTINCTIVENESS: In comparison with the morphologically most similar species *P. minutissimum*, *P. perpusillum* has a lamina with an acute apex and up to 12 pairs of pinnae per lamina and its rachis scales are deltoid-ovate or ovate-lanceolate, while *P. minutissimum* has a lamina with a round apex and 5–8 pairs of pinnae per lamina and its rachis scales are subulate or linear.

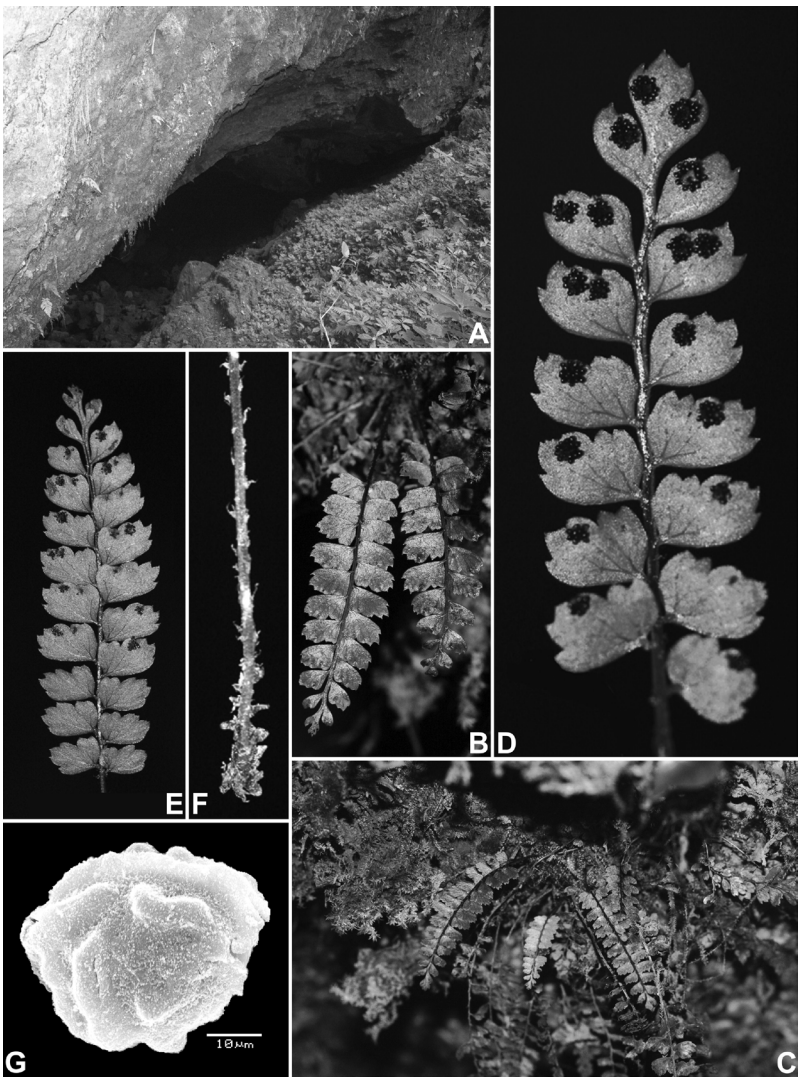

Fig. 2. *Polystichum perpusillum*. — **A**: Portion of a karst cave where the new species is discovered. — **B** and **C**: Two individuals in field. — **D** and **E**: Laminae. — **F**: Petiole. — **G**: Equatorial view of spore under SEM. (**D**–**G** from an isotype in CDBI).

MOLECULAR ANALYSIS: The *trnL-F* region of *P. perpusillum* sequenced was 341 bp in length (including a few basepairs of the *trnL* gene at the beginning). GC content was 34.6%. The length of the *trnL-F* intergenic spacer of *P. perpusillum* was 29 bp shorter than those of other *Polystichum* species available in GenBank submitted in our previous studies (Zhang & He 2010, Zhang *et al*. 2010) since the last pairs were not successfully sequenced. The aligned sequences were 398 basepairs long. A total of 12 informative indels were coded in the analysis, bringing the total number of characters included in the analysis to 410.

The maximum parsimony analysis yielded 13 361 most parsimonious trees with tree length = 276, consistency index = 0.7609, and retention index = 0.9207. Figure 3 shows one of the 13 361 most parsimonious trees as a phylogram with jackknife values (> 50%) below or next to branches. Species with doubtful identity, whose *trnL-F* sequence were downloaded from GenBank (mainly submitted by Li *et al*. 2004, 2007, 2008), are indicated with quotation marks in Fig. 3.

SPORE MORPHOLOGY: The spores are round in polar view and elliptic in equatorial view. The spore size is 38.1 × 45.2 μm (polar axis × equa-

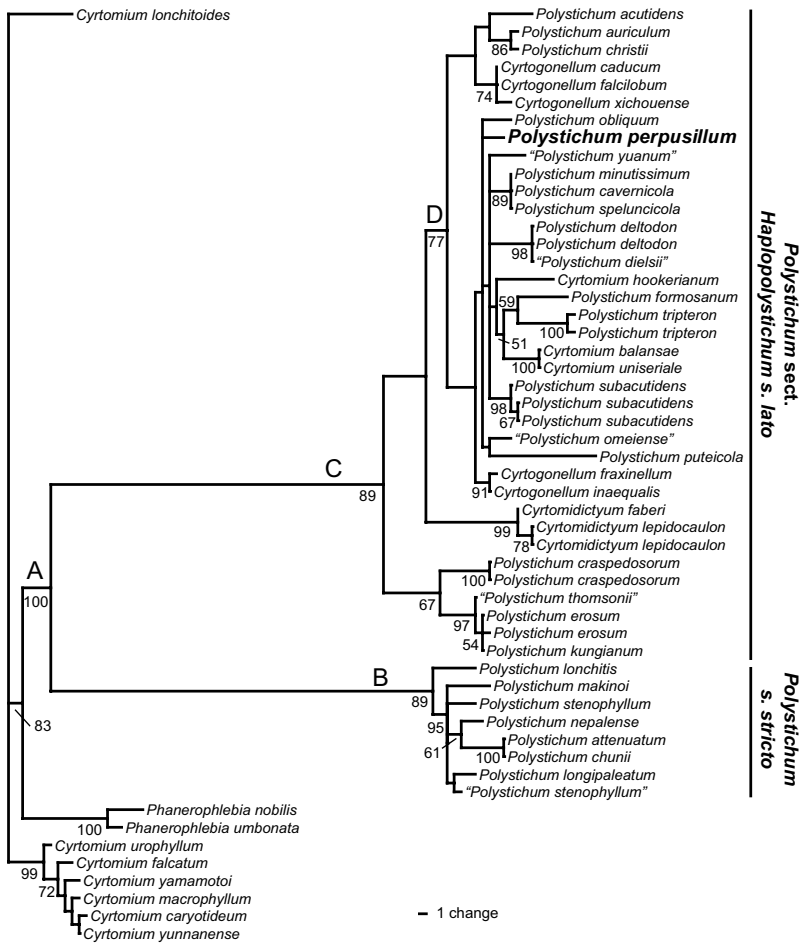

Fig. 3. One of the 13 361 most parsimonious trees based on DNA sequences of chloroplast *trnL-F* intergenic spacer. Tree length = 276, consistency index = 0.7609, and retention index = 0.9207. The numbers below or next to the branches are jackknife values. Species with doubtful identity are indicated with quotation marks. The species in boldface is the new one described in this study.

torial axis). The ratio of length of the polar axis to that of the equatorial axis is 0.84. The perispore sculpture is granulate with a few verrucae (Fig. 2G).

Distribution and habitat: *Polystichum perpusillum* is known only from the type locality in the Maolan Karst Nature Reserve, Libo County, southern Guizhou, China. Previous studies (e.g. Wang & Wang 1994, 1997, 2001) and our own observations with cave ferns (Zhang & He 2009b, 2010, He & Zhang 2010, Zhang *et al*. 2010) suggest that *P. perpusillum* is most probably endemic to that single cave. It grows on a wet limestone cliff at the cave entrance. The cliff is about 30 × 8 m in size. The cool and humid air from the cave must influence the temperature and moisture of the habitat. The well protected forest in the nature reserve provides shade to the habitat necessary for the survival of the species. *Polystichum perpusillum* was found 2–3 m above the ground in low-light conditions. The associated plants include the ferns *Adiantum malesianum*, *Pteris deltodon*, and *Tectaria coadunata*, the lycophyte species *Selaginella* sp., the seed plants *Begonia* sp., *Ficus* sp., *Elatostema* sp., and a few bryophytes such as *Reboulia* sp.

Conservation assessments: Only one population with ca. 30 individuals was found. The status of the new species clearly should be CR — Critically Endangered, following the IUCN (The International Union for Conservation of Nature and Natural Resources) guidelines (IUCN 2008). The cave is located deep in the Loudou Forest in Maolan National Nature Reserve, and thus is less susceptible to human disturbance. Although a hiking trail runs nearby, it is not heavily used. The protected vegetation is essential for maintaining the habitat.

Discussion

Our phylogenetic analysis based on *trnL-F* sequences resolved the genus *Phanerophlebia* as sister to *Polystichum s. lato* (clade A in Fig. 3) with relatively high jackknife support (83%). Such a resolution has never been previously recovered using DNA sequences. This support, however, is possibly superficial, resulting from the small sampling of *Polystichum s. stricto* (*sensu* Little & Barrington 2003, clade B in our Fig. 3) and outgroups.

Our analysis showed that the new species described here, *P. perpusillum*, is isolated on the phylogenetic tree on a relatively long branch (Fig. 3) judging from the fact that there is no difference in the *trnL-F* sequences between the morphologically highly divergent species pair in *P.* sect. *Haplopolystichum s. lato* (Zhang and He 2009), *P. cavernicola* and *P. speluncicola* (Zhang & He 2010), and among the morphologically distinctive *P. capillipes*, *P. erosum*, *P. kungianum*, and *P. tiankengicola* (Q. Luo & L. B. Zhang unpubl. data). This is suggestive that *P. perpusillum* is not closely related to any species in the genus sampled. The relationships of *P. perpusillum* with the other 22 species of sect. *Haplopolystichum s. lato* (Zhang & He 2009a, clade C in our Fig. 3) sampled are not well resolved although this 23-species clade (clade D in Fig. 3) is relatively strongly supported as a monophyletic group with a jackknife value of 77%, which is consistent with our previous findings (Zhang & He 2010, Zhang *et al*. 2010). Some of the morphologically divergent species of *Polystichum* with identical *trnL-F* sequences can be allopolyploids which share maternal origin since the cpDNA is maternally inherited.

The small stature of *P. perpusillum* is reminiscent of that of *P. minutissimum*, the smallest fern in the genus known so far (Zhang & He 2009b). Both are shorter than 10 cm and their pinnae are smaller than 3.2–7.3 × 2.0–3.5 mm. However, *P. perpusillum* has a lamina with an acute apex and not contracted towards the base, its pinnae can be up to 12 pairs per lamina, its pinna margins have acute teeth, and its rachis scales are deltoid-ovate or ovate-lanceolate and with a base 0.25–0.31 mm wide, while *P. minutissimum* has a lamina with a round apex and contracted towards lamina base, its pinnae can be 4–6(–8) pairs per lamina, its pinna margins have obtuse teeth, and its scales on rachis are subulate or linear and with a base 0.07–0.14 mm wide (Zhang & He 2009b).

Polystichum perpusillum and *P. minutissimum* grow in different habitats. *Polystichum minutissimum* grows on dripping limestone walls and stalactites inside a cave in twilight conditions, whereas *P. perpusillum* occurs on a drier limestone cliff at the entrance of a cave in brighter conditions.

The spores of *P. perpusillum* have a granulate sculpture with a few verrucae on the perispore (Fig. 2: G), a sculpture rare among the species of *Polystichum* (Xiang 1992, Zhang & Kung 1994). The perispore sculpture of *P. minutissimum* is unknown.

Polystichum perpusillum also resembles *P. liui*, a species originally described from Nanchuan County, Chongqing, China (Ching & Liu 1983) and distributed in the Chingqing, Guizhou, Hunan, and Sichuan Provinces of China (Kung *et al*. 2001). *Polystichum perpusillum* can be easily distinguished from *P. liui* by having pinnae without spinulose margins and 1–2(–3) sori per fertile pinna. In contrast, *P. liui* has pinnae with distinctly spinulose margins and up to eight sori per fertile pinna.

Key to *Polystichum perpusillum* and its allied species

1. Pinnae with spinulose margins; fertile pinnae with up to 8 sori per pinna ..... *P. liui*
1. Pinnae without spinulose margins; fertile pinnae with 1–3(–4) sori per pinna 2
2. Lamina with acute apex and not contracted towards lamina base; pinnae up to 12 pairs; pinna margins with acute teeth; rachis scales deltoid-ovate or ovate-lanceolate and with a base 0.25–0.31 mm wide ..... *P. perpusillum*
2. Lamina with round apex and contracted towards lamina base; pinnae 4–6(–8) pairs; pinna margins with obtuse teeth; rachis scales subulate or linear and with a base 0.07–0.14 mm wide ..... *P. minutissimum*

Acknowledgements

This study was supported by the National Natural Science Foundation of China (proj. no. 31070187), the Chongqing Municipality Science and Technology commission (proj. no.

cstc2011jjA00006), the Open Laboratory of Ecological Restoration and Biodiversity Conservation of the Chengdu Institute of Biology, the Chinese Academy of Sciences, and the National Geographic Society, USA, grant to LBZ. Chunbao Jiang and Kai Huang helped with the field work. Yu Wang helped with DNA sequencing and Bo Xu with SEM work. Yingbo Sun prepared the line drawing. The Administration of Maolan National Nature Reserve granted the collection permission. Helpful comments were received from Peishan Wang and an anonumous reviewer. Special thanks go to Ning Wu and Xinfen Gao for their support. The curators of the herbaria CDBI, CTC, and MO are appreciated for giving access to the material in their care.

References

Ching, R. C. & Liu, Z. Y. 1983: [New ferns of Jinfoshan, Nanchuan, Sichuan]. — *Bulletin of Botanical Research* (Harbin) 3(4): 1–37. [In Chinese].

Driscoll, H. E. & Barrington, D. S. 2007: Origin of Hawaiian *Polystichum* (Dryopteridaceae) in the context of a world phylogeny. — *American Journal of Botany* 94: 1413–1424.

Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D. & Kluge, A. G. 1996: Parsimony jackknifing outperforms neighbor-joining. — *Cladistics* 12: 99–124.

He, H. & Zhang, L. B. 2010: *Polystichum kungianum*, sp. nov. (sect. *Mastigopteris*, Dryopteridaceae) from Chongqing, China. — *Botanical Studies* 51: 395–401.

He, H. & Zhang, L. B. 2011: *Polystichum cavernicola*, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae) from a karst cave in Guizhou, China and its phylogenetic affinities. — *Botanical Studies* 52: 121–127.

IUCN (International Union for Conservation of Nature and Natural Resources) 2008: *IUCN Red List Categories and Criteria*, ver. 7. — IUCN, Gland & Cambridge.

Kung, H. S., Chu, W. M., He, Z. R. & Zhang, L. B. 2001: *Polystichum*. — In: Wu, C. Y. (ed.), *Flora Reipublicae Popularis Sinicae*, vol. 5(2): 1–246. Science Press, Beijing. [In Chinese].

Li, C. X., Lu, S. G. & Barrington, D. S. 2008: Phylogeny of Chinese *Polystichum* (Dryopteridaceae) based on chloroplast DNA sequence data (*trnL-F* and *rps4–trnS*). — *Journal of Plant Research* 121: 19–26.

Li, C. X., Lu, S. G. & Yang, Q. 2004: [Asian origin for *Polystichum* (Dryopteridaceae) based on *rbcL* sequences]. — *Chinese Science Bulletin* 49: 1146–1150. [In Chinese].

Li, C. X., Lu, S. G. & Yang, Q. 2007: Phylogeny and biogeography of Chinese and Australasian *Polystichum* ferns as inferred from chloroplast *trnL-F* and *rps4–trnS* sequence data. — *Palaeoworld* 16: 294–300.

Little, D. P. & Barrington, D. S. 2003: Major evolutionary events in the origin and diversification of the fern genus *Polystichum* (Dryopteridaceae). — *American Journal of Botany* 90: 508–514.

Liu, H. M., Zhang, X. C, Wang, W. & Zeng, H. 2010: Phylogeny and systematic position of two endemic fern genera *Cyrtomidictyum* and *Cyrtogonellum* (Dryopteridaceae) from East Asia: evidence from four chloroplast DNA markers. — *Organisms Diversity & Evolution* 10: 57–68.

Lu, J. M., Barrington, D. S. & Li, D. Z. 2007: Molecular phylogeny of the polystichoid ferns in Asia based on *rbcL* sequences. — *Systematic Botany* 32: 26–34.

Lu, J. M., Li, D. Z., Gao, L. M., Cheng, X. & Wu, D. 2005: Paraphyly of *Cyrtomium* (Dryopteridaceae): evidence from *rbcL* and *trnL-F* sequence data. — *Journal of Plant Research* 118: 129–135.

Müller, K. F. 2006: Incorporating information from length-mutational events into phylogenetic analysis. — *Molecular Phylogenetics and Evolution* 38: 667–676.

Punt, W., Hoen, P. P., Blackmore, S., Nilsson, S. & Thomas, A. L. 2007: Glossary of pollen and spore terminology. — *Review of Palaeobotany and Palynology* 143: 1–81.

Simmons, M. P. & Ochoterena, H. 2000: Gaps as characters in sequence-based phylogenetic analyses. — *Systematic Biology* 49: 369–381.

Swofford, D. L. 2001: *PAUP*: Phylogenetic analysis using parsimony (*and other methods)*. — Sinauer, Sunderland, Mass.

Taberlet, P., Gielly, L., Pautou G. & Bouvet, J. 1991: Universal primers for amplification of three non-coding regions of chloroplast DNA. — *Plant Molecular Biology* 17: 1105–1109.

Wang, P. S. & Wang, X. Y. 2001: [*Pteridophyte flora of Guizhou*]. — Guizhou Science & Technology Press, Guiyang. [In Chinese].

Wang, X. Y. & Wang, P. S. 1994: [Studies on pteridophytes in Guizhou (II)]. — *Acta Botanica Yunnanica* 12: 53–57. [In Chinese].

Wang, X. Y. & Wang, P. S. 1997: [New materials for *Polystichum* from Guizhou]. — *Acta Botanica Yunnanica* 19: 41–42. [In Chinese].

Xiang, L. L. 1992: Studies on the spore morphology of the genus *Polystichum* from Yunnan. — *Yushania* 9: 93–116.

Zhang, L. B. & He, H. 2009a: *Polystichum peishanii* (sect. *Haplopolystichum*, Dryopteridaceae): A new fern species from limestone area in Guizhou, China. — *Botanical Studies* 50: 101–106.

Zhang, L. B. & He, H. 2009b: *Polystichum minutissimum*, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae): The smallest *Polystichum* found in a karst cave in China. — *Botanical Studies* 50: 353–358.

Zhang, L. B. & He, H. 2010: *Polystichum speluncicola sp. nov*. (sect. *Haplopolystichum*, Dryopteridaceae) based on morphological, palynological, and molecular evidence with reference to the non-monophyly of *Cyrtogonellum*. — *Systematic Botany* 35: 13–19.

Zhang, L. B. & Kung, H. S. 1994: Studies on the spore morphology of Chinese sect. *Metapolystichum* (*Polystichum*, Dryopteridaceae). — *Acta Botanica Yunnanica* 16: 273–278.

Zhang, L. B., Comes, H. P. & Kadereit, J. W. 2001: Phylogeny and Quaternary history of the European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. — *American Journal of Botany* 88: 2331–2345.

Zhang, L. B., He, H. & Luo, Q. 2010: *Polystichum puteicola*, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae) from

a karst sink hole in Guizhou, China based on molecular, palynological, and morphological evidence. — *Botanical Studies* 51: 127–136.

Zhou, Z. X. 1987: *Scientific survey of the Maolan karst forest*. — The People's Publishing House of Guizhou, Guiyang.

Appendix. Voucher information, GenBank accession numbers, and source publications.

Cyrtogonellum caducum Ching, AY736350, Lu *et al*. (2005); *C. falcilobum* Ching *ex* Y. T. Hsieh, DQ202409, Li *et al*. (2008); *C. fraxinellum* (Christ) Ching, AY736349, Lu *et al*. (2005); *C. inaequalis* Ching, AY736351, Lu *et al*. (2005); *C. xichouense* S. K. Wu & Mitsuda, EU106595, Li *et al*. (2008); *Cyrtomidictyum faberi* (Bak.) Ching, EF540697, Liu *et al*. (2010); *C. lepidocaulon* (Hook.) Ching, EF177266, Driscoll and Barrington (2007), DQ150392, Li *et al*. (2007); *Cyrtomium balansae* (Christ) C. Chr., DQ202411, Li *et al*. (2008); *C. caryotideum* (Wall.) C. Presl, EF177267, Driscoll and Barrington (2007); *C. falcatum* (L. f.) C. Presl, EF177268, Driscoll and Barrington (2007); *C. hookerianum* (C. Presl) C. Chr., DQ202414, Li *et al*. (2008); *C. lonchitoides* H. Christ, AY736336, Lu *et al*. (2005); *C. macrophyllum* (Makino) Tagawa, EU106596, Li *et al*. (2008); *C. uniseriale* Ching, DQ202415, Li *et al*. (2008); *C. urophyllum* Ching, DQ202416, Li *et al*. (2008); *C. yamamotoi* Tagawa, DQ202417, Li *et al*. (2008); *C. yunnanense* Ching, DQ202418, Li *et al*. (2008); *Polystichum acutidens* Christ, DQ202419, Li *et al*. (2008); *P. attenuatum* Tagawa & Iwatsuki, DQ150396, Li *et al*. (2007); *P. auriculum* Ching, DQ150397, Li *et al*. (2007); *P. cavernicola* L. B. Zhang & H. He. China. Guizhou Province, Libo County, *L. B. Zhang, H. He, & C. B. Jiang 911* (CDBI, CTC, MO), JF713056, He & Zhang (2011); *P. christii* Ching, DQ150399, *Li et al*. (2007); *P. chunii* Ching, DQ202421, Li *et al*. (2008); *P. craspedosorum* (Maxim.) Diels, EF177288, Driscoll and Barrington (2007), DQ202422, Li *et al*. (2008); *P. deltodon* (Baker) Diels, EF177289, Driscoll and Barrington (2007), DQ202424, Li *et al*. (2008); "*P. dielsii* Christ", DQ150400, Li *et al*. (2007); *P. erosum* Ching & Shing, DQ150403, Li *et al*. (2007), DQ202425, Li *et al*. (2008); *P. formosanum* Rosenst., EF177307, Driscoll and Barrington (2007); *P. kungianum* H. He & L. B. Zhang. China. Chongqing Municipality, Wuxi County, *H. He & Y. Q. Yang 791* (CDBI, CTC, MO), GQ244336; *P. lonchitis* (L.) Roth, AY736354, Lu *et al*. (2005); *P. longipaleatum* Christ, AY736353, Lu *et al*. (2005); *P. makinoi* (Tagawa) Tagawa, DQ202431, Li *et al*. (2008); *P. nepalense* (Spreng.) C. Chr. China. Sichuan Province, Shimian County, *L. B. Zhang 4723* (CDBI), JF713057; *P. obliquum* (Don) Moore, EF177284, Driscoll and Barrington (2007); "*P. omeiense* C. Chr.", DQ202434, Li *et al*. (2008); *P. perpusillum* L. B. Zhang & H. He. China. Guizhou Province, Libo County, *L. B. Zhang, H. He & C. B. Jiang 801* (CDBI, CTC, MO), JF713058; *P. speluncicola* L. B. Zhang & H. He, GQ244334, Zhang and He (2010); "*P. stenophyllum* Christ", EF177296, Driscoll and Barrington (2007); *P. subacutidens* Ching *ex* L. L. Xiang, AY534749, Li *et al*. (2004), DQ514518, Lu *et al*. (2007), DQ150418, Li *et al*. (2007); "*P. thomsonii* (Hook. f.) Bedd.", EU106597, Li *et al*. (2008); *P. tripteron* (Kunze) C. Presl, EF177298, Driscoll and Barrington (2007). China. Chongqing Municipality, Nanchuan District, *L. Zhang* 200 (CDBI), JF713059; "*P. yuanum* Ching", DQ150421, Li *et al*. (2007).