

Polystichum cavernicola, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae) from a karst cave in Guizhou, China and its phylogenetic affinities

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(Received January 25, 2010; Accepted August 10, 2010)

ABSTRACT. *Polystichum cavernicola* L. B. Zhang & H. He, a new pteridophyte species is described and illustrated from a karst cave in southern Guizhou, China. It is a member of *Polystichum* sect. *Haplopolystichum* (Dryopteridaceae). A phylogenetic analysis based on the chloroplast *trnL-F* sequences shows that the new species is most closely related with *P. speluncicola*, a species also described from a karst cave in southern Guizhou. Morphologically, *P. cavernicola* is most similar to *P. speluncicola*. The important morphological differences between *P. cavernicola* and *P. speluncicola* include that *P. cavernicola* has narrow-type microscales on the abaxial laminar surface, its pinnae are chartaceous and have auriculate acroscopic bases, and its lamina is broadest near the midpoint, whereas *P. speluncicola* has broad-type microscales on the abaxial laminar surface, its pinnae are subcoriaceous and have rounded, non-auriculate, acroscopic bases, and its lamina is broadest above the middle. The spores of *P. cavernicola* have verrucate sculpturing on the perispore, whereas those of *P. speluncicola* are cristate with numerous spinules on its perispore. *Polystichum cavernicola* is endemic to a single karst cave in southern Guizhou and is considered to be Critically Endangered (CR) based on IUCN Red List criteria.

Keywords: Cave flora; Fern; Dryopteridaceae; Guizhou; Phylogeny; *Polystichum cavernicola*; sect. *Haplopolystichum*; Spore morphology; *TrnL-F* sequence.

INTRODUCTION

During field work in 2008 we collected a few specimens and DNA samples of an undertermined species of *Polystichum* Roth (Dryopteridaceae) sect. *Haplopolystichum* Tagawa in a karst cave in Libo County, southern Guizhou, China. Like many of the species in sect. *Haplopolystichum*, it has a limited number of morphological characters available to infer its taxonomic identity and phylogenetic affinities. We therefore supplemented our macromorphological and palynological studies with a molecular analysis based on DNA sequences of the chloroplast *trnL-F* intergenic spacer region. We conclude that our collections represent an undescribed species, which we describe here.

MATERIAL AND METHODS

Materials examined. The morphological, palynological, and molecular data were based on the voucher specimens:

CHINA. Guizhou: Libo County, Wong'ang Town, Jilong Village, 4 Nov 2008, L. B. Zhang, H. He & C. B. Jiang 911 (CDBI, CTC, MO, Herb. Pei-Shan Wang).

Morphological study. The measurement of roots, petioles, rachises, scales, and indusia was conducted with a micrometer under a dissecting 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 Invitrogen™ (Shanghai, China).

Based on previous phylogenetic analyses (Driscoll and Barrington, 2007; Lu et al., 2007; Li et al., 2008; Zhang and He, 2010; Zhang et al., 2010), we included in the in-group 27 species of *Polystichum* sect. *Haplopolystichum* Tagawa sensu lato (s.l.; Zhang and He, 2009a) including sects. *Crucifilix* Tagawa, *Haplopolystichum*, and *Sphaenopolystichum* Ching ex W. M. Chu & Z. R. He, and genera *Cyrtogonellum* Ching and *Cyrtomidictyum* Ching,

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and *Cyrtomium* Presl subser. *Balansana* Ching & Shing (Zhang and He, 2009b), as well as 7 representatives of the monophyletic *Polystichum* s.s. (sensu Little and Barrington, 2003), were included as ingroup. Seven species of *Cyrtomium* s.s. sensu Lu et al. (2007) and two species of the neotropical *Phanerophlebia* C. Presl following Driscoll and Barrington (2007) were used as outgroups. A few species were represented by more than one accession. In total 51 accessions were included in the analysis. All sequences used in this study together with their GenBank accession numbers and voucher information or source publications are listed in Appendix 1.

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

Phylogenetic analysis followed the procedure in Zhang and Simmons (2006), Zhang and He (2010), and Zhang et al. (2010). Equally weighted parsimony tree searches were conducted using 1,000 tree-bisection-reconnection (TBR) searches in PAUP* 4.0b10 (Swofford, 2001) with a initial "Maxtrees" set to 1,000 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 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). Descriptive terminology of the spores follows Punt et al. (2007).

TAXONOMIC TREATMENT AND RESULTS

Polystichum cavernicola L. B. Zhang & H. He, sp. nov.—TYPE: CHINA. Guizhou Province: Libo County, Wong'ang Town, Jilong Village, Dongchang, Feihudong (Cave of the Flying Tiger), 25°12.51' N, 107°57.22' E, alt. 780 m, 4 Nov 2008, L. B. Zhang, H. He & C. B. Jiang 911 (Holotype: CDBI; Isotypes: CTC, HAST, MO, VT, Herb. Pei-Shan Wang). Figures 1, 2

Species affinis *P. speluncicola* L. B. Zhang et H. He, sed *microsquamis* *augustis* *base non-dilatatis*, *pinnis chartaceis* *base acroscopice auriculatis*, *parte latissime laminae circum medium locata*, *sculpturis perisporarum imbricatis differt*.

Plants perennial, evergreen, (4-)6-10 cm tall. *Rhizome* 0.4-0.8 cm long, ascending, densely covered with scales;

scales linear or subulate, brown, 1.0-1.3 mm long; roots brown when dry, up to 9 cm long, 0.2-0.5 mm in diam. *Leaves* cespitose, 3-7 per rhizome; petiole 1.0-2.5(-4.5) cm long, 0.2-0.9 mm in diam. at midpoint, canaliculate adaxially, green; basal petiole scales lanceolate, 3.0-4.5 × 0.4-0.9 mm, chartaceous, brown, margins with very few cilia, apex acuminate or caudate, matte; distal petiole scales similar but narrower, differing in size, lanceolate with dilated base, chartaceous, brown, margins regularly short-ciliate, apex caudate, matte. *Lamina* oblanceolate, contracted toward base, 1-pinnate, 4.2-9.4 cm long, 1.1-1.4 cm wide at midpoint, 1.2-1.7 cm wide at widest, apex acute; rachis 0.3-1.0 mm in diam. at midpoint, without proliferous buds, adaxially sulcate; scales of rachis subulate with dilated base, 1.6-3.6 mm long, base 0.3-0.6 mm wide, differing in size, chartaceous, brown, margins regularly short-ciliate, apex caudate, matte. *Pinnae* 10-20 pairs, not imbricate, angled acroscopically, basal two pairs 5.0-8.0 mm apart, basal pinnae deltate-ovate, median pairs 3.7-6.2 × 2.5-5.0 mm, largest pairs 4.0-7.0 × 2.6-5.3 mm and located slightly above middle of lamina, alternate, oblong, short-petioliolated with petiolules 0.2-0.5 mm long, chartaceous, acroscopic base slightly auriculate, basisopic base truncate and often forming a 75-120-degree angle with rachis, apex obtuse, acroscopic margin repand, abaxially scaly, adaxially lustrous and glabrous; microscales

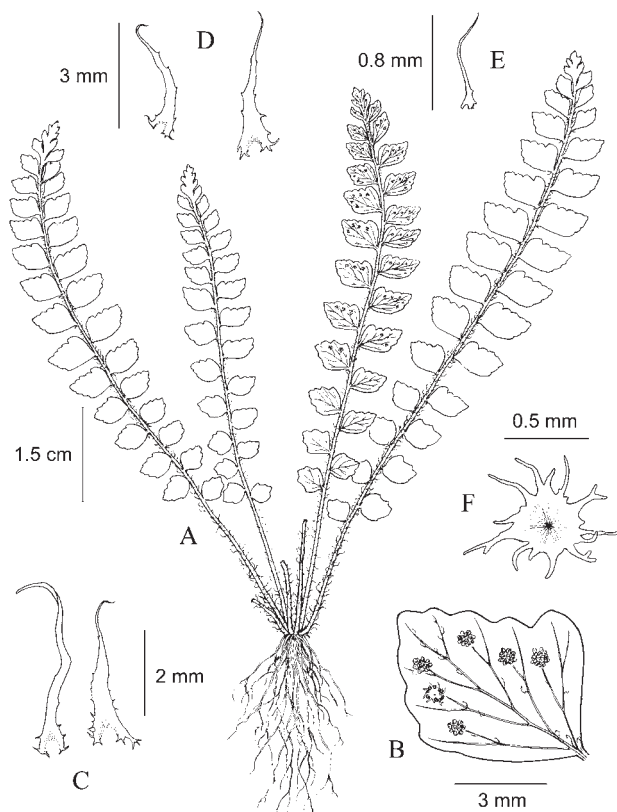


Figure 1. *Polystichum cavernicola* L. B. Zhang & H. He. A, Habit; B, Pinna; C, Scales from base of petiole; D, Rachis scales; E, Microscale; F, Indusium (based on the holotype, L. B. Zhang, H. He & C. B. Jiang 911, CDBI).

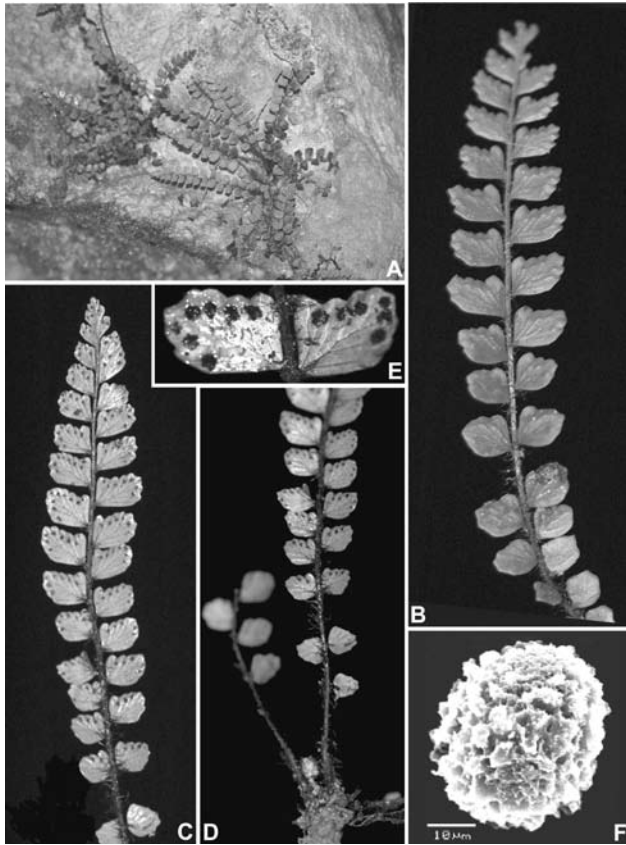


Figure 2. *Polystichum cavernicola* L. B. Zhang & H. He. A, Two individuals in the field; B, Adaxial view of lamina; C, Abaxial view of lamina; D, Lower portion of plant; E, Abaxial view of pair of pinnae showing sori; F, Equatorial view of spore under SEM.

on abaxial surface subulate without dilated base (narrow-type microscales), (0.3-)0.5-1.8 mm long, base ca. 0.1 mm wide, with a few tortuous cilia on margin of base; venation pinnate; midrib abaxially slightly raised, adaxially flat; lateral veins free, 4-5 pairs from midrib per pinna, nearly opposite, each lateral vein further dichotomous, abaxially slightly raised and distinct, adaxially indistinct. *Sori* terminal on veins of distal pinnae, (1-)4-8 per fertile pinna, close to pinna margin, center of sorus 1.0-1.6 mm from pinna margin; *indusia* peltate, ca. 0.9 mm in diam., membranaceous, fimbriate, brown (Figures 1, 2).

Molecular phylogenetics. The *trnL-F* intergenic spacer of *P. cavernicola* was 375 bp in length (including a few basepairs of *trnL* and *trnF* genes at the ends). The GC content was 37.1%. The length and GC content of the *trnL-F* intergenic spacer of *P. cavernicola* are comparable with those of other *Polystichum* species available in GenBank submitted in our previous studies (Zhang and He, 2010; Zhang et al., 2010). The aligned sequences were 398 base-pairs long and in total 11 informative indels were coded in the analysis.

The maximum parsimony analysis yielded 1,670 most parsimonious trees with tree length = 256, consistency index = 0.7791, and retention index = 0.9306. One of the

1,670 most parsimonious trees is shown in Figure 3. Species with doubtful identity, whose *trnL-F* sequences we downloaded from GenBank (mainly submitted by Li et al., 2004, 2007, 2008), are indicated with quotation marks in Figure 3.

Spore morphology. The spores are monolete, circular in polar view and elliptic in equatorial view, and dark brown in color when fresh. The spore size is ca. $39.1 \times 40.6 \mu\text{m}$ (polar axis \times equatorial axis). The ratio of length of the polar axis to that of the equatorial axis is ca. 0.96. The perispore sculpturing is verrucate (Figure 2F).

Geographical distribution. *Polystichum cavernicola* is known only from the type locality in the Maolan Karst Nature Reserve, Libo County, southern Guizhou, China (Figure 4). Previous studies involving ferns of caves and sinkholes (e.g. Wang and Wang, 1994, 1997) and our own experience with cave ferns (Zhang and He, 2009b, 2010; He and Zhang, 2010) suggest that *P. cavernicola* is highly likely endemic to that single cave.

Ecology. In Libo County, Guizhou, the new species occurs on the side of a slow-growing and large-sized stalagmite facing the mouth inside a karst cave. The stalagmite is ca. $15 \times 1.8 \text{ m}$. *Polystichum cavernicola* grows in the

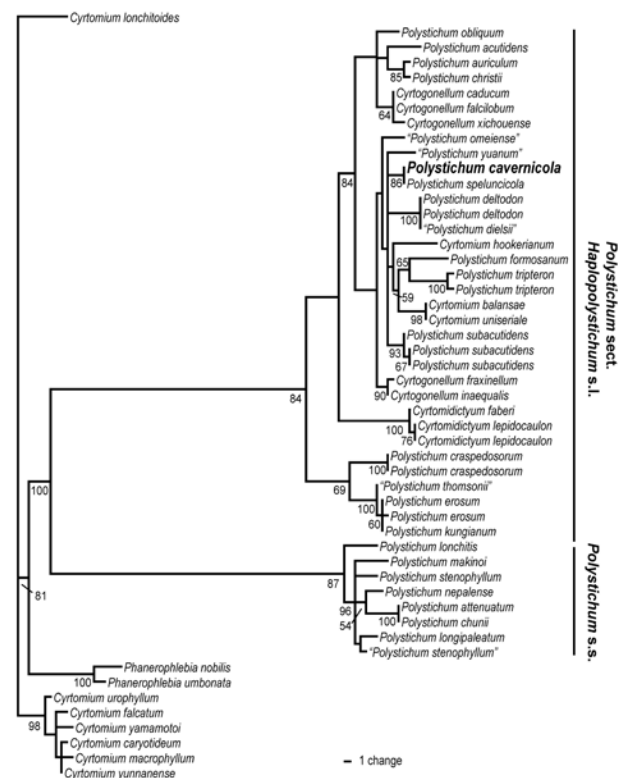


Figure 3. One of the 1,670 most parsimonious trees based on DNA sequences of chloroplast *trnL-F* intergenic spacer. Tree length = 256, consistency index = 0.7791, and retention index = 0.9306. The numbers below or next to the branches are jackknife values. Species with doubtful identity are indicated with quotation marks. The bar indicates one change. The species in bold face is the new one described in this study.

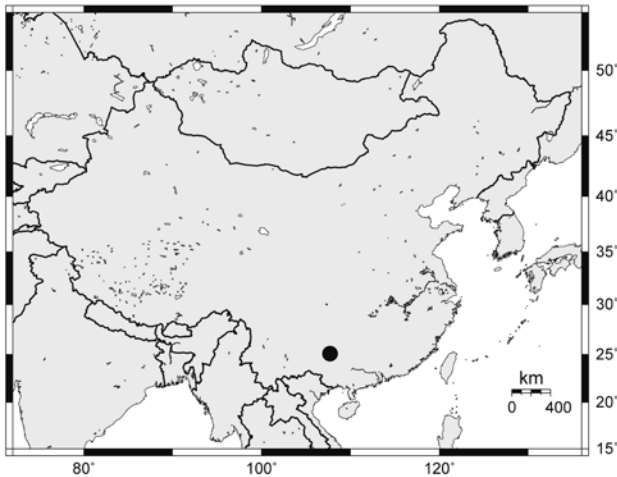


Figure 4. Geographical distribution of *Polystichum cavernicola* L. B. Zhang & H. He (solid circle) in southern Guizhou Province, China.

places 5-10 m from the cave mouth and 0.2-1.5 m above the cave ground, with twilight conditions, at alt. 780 m. The humidity of the cave entrance relies largely on the water dripping from the ceiling of the cave. The vegetation around and beyond the cave is essential for the survival of *P. cavernicola*.

The associated plants include *Elatostema sublineare* W. T. Wang (Urticaceae), *Ctenitis membranifolia* Ching & C. H. Wang (Dryopteridaceae), *Pteris* sp. (Pteridaceae), and a few mosses.

Conservation assessments. Only one population with ca. 30 individuals was found. Assuming that other populations do not exist, this taxon should clearly be classified as CR - Critically Endangered category following the IUCN (The International Union for Conservation of Nature and Natural Resources) guidelines (IUCN, 2008).

The karst cave where the new species is found contains various beautiful karst stalactites and stalagmites and is well known locally, but it was not developed for tourism yet in 2008. Several western documentary film-makers have explored the cave in recent years. The day when the cave becomes touristic likely will be the day when *P. cavernicola* goes extinct. This raises serious conservation concerns.

Etymology. From the Latin *caverna*, cave, and the Latin suffix *-cola*, dweller, referring to the cave-dwelling habit of the species.

DISCUSSION

Our phylogenetic analysis based on *trnL-F* sequences showed that *P. cavernicola* formed a relatively strongly supported clade with *P. speluncicola* (86% jackknife support) (Figure 3). These two species do share similar stature, but they can be easily distinguished from each other. *Polystichum cavernicola* has narrow-type microscales on the abaxial laminar surface, its pinnae are chartaceous and

have round acroscopic bases, and the broadest part of the lamina is located medially, whereas *P. speluncicola* has broad-type microscales on the abaxial laminar surface, its pinnae are subcoriaceous and have auriculate acroscopic bases, and the broadest part of the lamina is located above the midpoint.

In addition, *Polystichum cavernicola* has oblong pinnae which are normally not overlapping, its rachis scales are 0.3-0.6 mm wide at their bases, and the basispic base of the pinnae and the rachis often form a 75-120-degree angle, whereas *P. speluncicola* has deltate-ovate pinnae which are proximate and often imbricate, its rachis scales are 0.4-1.0 mm wide at their bases, and the basispic base of the pinnae and the rachis often form a 20-60-degree angle.

Palynologically, *Polystichum cavernicola* has verrucate perispore sculpturing without any perforations (Figure 2F), whereas *P. speluncicola* has cristate sculpturing with numerous spinules (Zhang and He, 2010). So far, no other species in *Polystichum* for which perispore sculpturing has been documented (Xiang, 1992; Zhang and Kung, 1994) has sculpturing similar to that of *P. cavernicola*.

Interestingly, the two caves harboring the two cave species, *P. cavernicola* and *P. speluncicola*, are only separated by ca. 20 km (by air). If the two species are indeed sister to each other as shown in our phylogenetic analysis, their most recent common ancestor is likely to have lived in the neighborhood of the two caves.

Other morphologically similar species in China include *P. liui* Ching and *P. jinfoshanense* Ching & Z. Y. Liu, both described from Nanchuan County, Chongqing, China (Ching and Liu, 1983). *Polystichum liui* is distributed in Chongqing, Guizhou, Hunan, and Sichuan in China, whereas *P. jinfoshanense* occurs in Chongqing, Guizhou, Sichuan, and Yunnan (Kung et al., 2001). *Polystichum cavernicola* is distinct from these two species by having bluntly serrate acroscopic pinna margins, whereas both *P. liui* and *P. jinfoshanense* have sharply serrate acroscopic pinna margins (Kung et al., 2001; Zhang and He, 2010).

These four species discussed above can be distinguished from one another using the following key:

Key to *Polystichum cavernicola* and its allies

1. Acroscopic pinna margins sharply serrate.
 2. Pinnae thinly papery, not aristate on margins
.....*P. jinfoshanense*
 2. Pinnae nearly coriaceous, aristate on margins ...*P. liui*
1. Acroscopic pinna margins bluntly serrate.
 3. Broadest part of lamina well above the midpoint; rachis scales 0.4-1.0 mm wide at base; basispic base of pinnae and rachis often forming a 20-60-degree angle; pinnae subcoriaceous, deltate-ovate, proximate and often overlapping, acroscopic base round; microscales broad-type *P. speluncicola*
 3. Broadest part of lamina near the midpoint; rachis

scales 0.3-0.6 mm wide at base; basiscopic base of pinnae and rachis often forming a 75-120-degree angle; pinnae chartaceous, oblong, normally not overlapping; acroscopic base auriculate; microscales narrow-type *P. cavernicola*

Acknowledgements. This project was partly supported by funding from the Open Laboratory of Ecological Restoration and Biodiversity Conservation of Chengdu Institute of Biology, Chinese Academy of Sciences, a National Geographic Society, USA, grant to LBZ, and the National Natural Science Foundation of China (31070187) to HH. Peishan Wang, Chunbao Jiang, Kai Huang, and Liang Zhang helped with the field work. Yu Wang helped with DNA sequencing and Bo Xu with SEM work. Musen Guo prepared the line drawing. Hongmei Liu provided an unreleased sequence. The Administration of Maolan National Nature Reserve granted the collection permission. Helpful comments were received from Peishan Wang, George Yatskievych, and an anonymous reviewer. Special thanks go to Ning Wu and Xinfen Gao for their support. We thank the curators of the herbaria CDBI, CTC, and MO for providing access to the material in their care.

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Appendix 1. 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.) Presl, EF177267, Driscoll and Barrington (2007); *C. falcatum* (L. f.) Presl, EF177268, Driscoll and Barrington (2007); *C. hookerianum* (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); *Phanerophlebia nobilis* (Schlecht. & Cham.) Presl, EF177269, Driscoll and Barrington (2007); *P. umbonata* Underw., EF177270, Driscoll & Barrington (2007); *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. Guizhou: Libo, L. B. Zhang, H. He, & C. B. Jiang 911 (CDBI, CTC, MO); *P. christii* Ching, DQ150399, Li et al. (2007); *P. chunii* Ching, DQ202421, Li et al. (2008); *P. craspedosorum* (Maxim.) Diels, EF177288, Driscoll & Barrington (2007), DQ202422, Li et al. (2008); *P. deltodon* (Baker) Diels, EF177289, Driscoll & 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 & Barrington (2007); *P. kungianum* H. He & L. B. Zhang. Chongqing: Wuxi, 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. Sichuan: Shimian, L. B. Zhang 4723; *P. obliquum* (Don) Moore, EF177284, Driscoll & Barrington (2007); "*P. omeiense* C. Chr.", DQ202434, Li et al. (2008); *P. speluncicola* L. B. Zhang & H. He, GQ244334, Zhang & He (2010); "*P. stenophyllum* Christ", EF177296, Driscoll & Barrington (2007), DQ202439, Li et al. (2008); *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) Presl, EF177298, Driscoll & Barrington (2007). Chongqing: Nanchuan, L. Zhang 200; "*P. yuanum* Ching", DQ150421, Li et al. (2007).

中國貴州喀斯特岩洞耳蕨屬一新種—洞生耳蕨（半開羽耳蕨組，鱗毛蕨科）及其系統親緣

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本文描述了在中國貴州南部一喀斯特岩洞中發現的耳蕨屬半開羽耳蕨組 (*Polystichum* sect. *Haplopolystichum*) 一新種：洞生耳蕨 (*P. cavernicola*)，並提供線繪圖與照片以資辨識。基於 *trnL-F* 基因間區序列的系統發育分析表明，洞生耳蕨與另一髮現於岩洞中的岩穴耳蕨 (*P. speluncicola*) 親緣最近。從形態上看，洞生耳蕨與岩穴耳蕨也最接近，但洞生耳蕨羽片背面的小鱗片為窄型，羽片質地為草質，基部下側為有耳狀突起，葉片最寬處位於葉片中部附近，而岩穴耳蕨羽片背面的小鱗片為寬型，羽片質地為近革質，基部下側為圓形，葉片最寬處位於葉片中部以上。從孢子形態來看，洞生耳蕨的周壁紋飾為覆瓦狀，而岩穴耳蕨的周壁紋飾為冠狀。洞生耳蕨僅見於貴州南部一喀斯特岩洞，瀕臨絕滅。

關鍵詞： 洞穴植物；鱗毛蕨科；貴州；系統發育；洞生耳蕨；半開羽耳蕨組；孢子形態；*TrnL-F* 序列。

