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Asplenium delinghaense, a new species from western part of Qilian Mountains in Qinghai-Xizang Plateau

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Keywords: alpine plants, Aspleniaceae, China, East Asia, polyploidy.

Summary. A new species of the genus *Asplenium* L. from northern Qinghai, *A. delinghaense* S. Q. Liang et X. C. Zhang, is described and illustrated here. This new species resembles *A. iskardense* Viane et Reichst., *A. daghestanicum* Christ, and *A. neovarians* Ching but differs in frond and perispore morphology. Molecular phylogenetic evidence supported the close relationship of *A. delinghaense* with members of the *A. varians* complex and the *A. pekinense* complex and indicated an allotetraploid origin of it.

Asplenium delinghaense – новый вид из западной части хребта Циляньшань (Цинхай-Тибетское плато)

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Ключевые слова: высокогорные растения, высокогорья, Восточная Азия, Китай, полиплоидия, Aspleniaceae.

Аннотация. Описан и проиллюстрирован новый вид рода *Asplenium* L. из северного Цинхая – *A. delinghaense* S. Q. Liang et X. C. Zhang. Новый вид напоминает *A. iskardense* Viane et Reichstein, *A. daghestanicum* Christ и *A. neovarians* Ching, но отличается формой вай и морфологией перисория. Молекулярно-филогенетические данные подтверждают тесную связь *A. delinghaense* с видами комплексов *A. varians* и *A. pekinense* и указывают на его аллотетраплоидное происхождение.

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Introduction

Asplenium L. is one of the richest cosmopolitan genera among extant ferns, represented by a total of ca. 700 species (Kramer, Viane, 1990; Schneider et al., 2004; Rothfels et al., 2012; Lin, Viane, 2013). The Qinghai-Xizang Plateau is a diversification center for high altitudes species of *Asplenium*, especially the Himalayas which surround the western and southern edges of it (Kramer, Viane, 1990; Wu, 1999). In China, the field investigation of the Qinghai-Xizang Plateau has been mainly focused on Xizang and Sichuan, whereas Qinghai, a vast area showing a rugged landscape in the northeast of the Qinghai-Xizang Plateau, still lacks a more fully exploration. According to Flora Qinghaiica (Mei, 1997), only three *Asplenium* species were recorded in this province, which can hardly match the observed morphological diversity and variance. During a comprehensive field exploration throughout Qinghai organized recently by Mr. Sheng-Bang Zhang, a small *Asplenium* plant growing in dolomite-marble crevices was collected from the Cypress Mountain in Delingha. Cypress Mountain is located in the western section of Qilian Mountain and adjacent to Qaidam Basin in the south. Qilian Mountain lies on the northeastern edge of the Qinghai-Xizang Plateau, and abundant glaciers make it an important "solid water reservoir" nourishing the vegetation in surrounding regions. The climate in the Qaidam Basin is quite xeric and windy, therefore, ferns hardly survive there, except for very few drought-resisting species such as *Equisetum avense*. The present *Asplenium* specimen from Delingha resembles species of ser. *Variantia* Ching et S. H. Wu, a group including a complex assemblage of small ferns mainly distributed in the high-altitude area of the Qinghai-Xizang Plateau and adjacent temperate regions (Ching and Wu, 1985; Wu, 1999); it was different from known species by morphology, cytology, and molecular phylogeny. Therefore, we describe it as a new species named *Asplenium delinghaense* S. Q. Liang et X. C. Zhang and present the result here.

Material and methods

Morphological study

Specimens for morphological studies were obtained from our collections and herbarium PE. High resolution scanned images from websites of herbaria BR, K, and P were also used in morphological comparison. Morphological characteristics of frond were observed and photographed with a Leica S9D stereo microscope. Spore size and surface ornamentation were observed by light microscopy (LM) and scanning electron microscopy (SEM) using a Leica DM4000 microscope and a Hitachi S-4800 field emission SEM, respectively. For LM, untreated spores were collected and embedded in neutral balsam. We randomly selected more than 50 spores to measure the length of the exospore under LM; measurements are given in the following format: (minimum) mean minus standard deviation (*s*)–mean plus *s* (maximum). For SEM, unopened mature sporangia were broken on a specimen stub to release spores, then coated with platinum.

Cytological study

In *Asplenium*, monoploid nuclear DNA content (1Cx-value) is relatively stable among related species, therefore, we inferred the ploidy level of our samples through comparing the 2C-value estimated by flow cytometry (FCM) with the data of well-studied species. FCM investigations were performed with a BD LSRFortessa flow cytometer. We selected *Zea mays* ssp. *mays* "B73" (5.64 pg/2C, Díez et al., 2013) or *Capsicum annuum* var. *annuum* (6.76 pg/2C, Moscone et al., 2003) as the internal standard and propidium iodide as the nucleic acid dye. Suspension of cell nuclei was prepared by chopping tissues of silica gel-dried sample with fresh internal standard in a petri dish containing modified Galbraith's buffer (provided by the Plant Science Facility of IBCAS). For each sample, the measurement was repeated for three times. Mean and *s* were both calculated to represent the nuclear DNA content of the sample.

Phylogenetic analyses

We included a total of 16 individuals, representing 13 species of *Asplenium* ser. *Variantia* Ching et S. H. Wu from China and adjacent regions, including *A. tenuicaule*, *A. neovarians*, *A. varians*, *A. kukkonenii*, *A. altajense*, *A. kansuense*, *A. anogrammoides*, *A. pekinense*, *A. sarelii*, *A. fugax*, *A. capillipes*, *A. pulcherrimum*, and *A. tenuifolium*. *A. incisum* was chosen as an outgroup. Detailed voucher information and GenBank accession numbers were listed in Appendix.

Total genomic DNA was isolated from silica gel-dried material using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol. Two plastid DNA fragments (*rbcL* and *rpl32-trnP*) and one nuclear gene fragment (*pgiC*) were amplified by PCR and sequenced for phylogenetic analyses. Primers, experiment conditions and alignment processes

were all followed Liang et al. (2019). IQ-TREE v.1.6.8 (Nguyen et al., 2015) was used to reconstruct maximum likelihood (ML) phylogeny based on concatenated plastid DNA sequences and nuclear gene sequences, respectively.

Results and discussion

Based on gross morphology (Fig. 1), *Asplenium delinghaense* is very similar to *A. iskardense* Viane et Reichst. (holotype: K001092507, image online!)

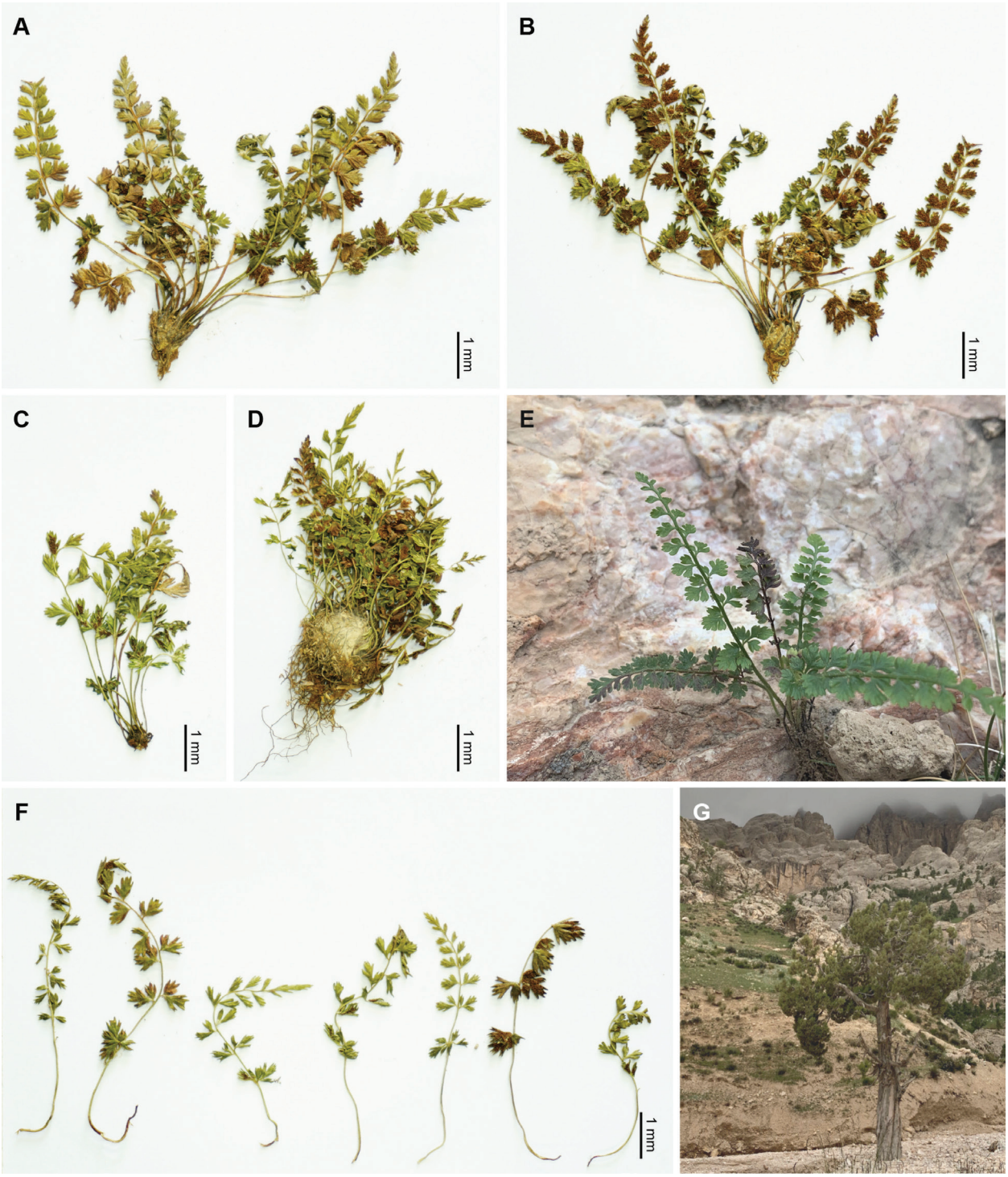

Fig. 1. *Asplenium delinghaense* S. Q. Liang et X. C. Zhang, sp. nov.: **A–B** – Adaxial and abaxial views of individual 1 of the holotype, Sheng-Bang Zhang et al. 7617 (PE); **C–D** – Individual 2 and 3 of the holotype; **E** – Habit of a living plant in the original habitat; **F** – Variation in frond morphology of the holotype; **G** – Habitat of the species in Delingha, Qinghai.

and *A. daghestanicum* Christ (holotype: P00622782, image online!; isotype: BR0000024934701, image online!), two species in need of further field investigation to clarify the distribution range. Up to now, *A. iskardense* is only known from the type locality in Pakistan (Viane, Reichstein, 2003); *A. daghestanicum* has been found in Daghestan and Chad (Viane, 1987), thus, it is expected to have a wider distribution. *A. delinghaense* can be distinguished from them by the stipe a little bit shorter than lamina, larger spore size and different perispore morphology (Fig. 2H). *A. delinghaense* also resembles *A. neovarians* Ching (holotype: PE01895940!), a species known only from few collections in NW China (Ching, Wu, 1985; Lin, Viane, 2013). However, our phylogenetic results indicated that they are diverged on genetic level (Figs. 3–4). In addition, they are also different in exospore size, perispore morphology and number of pinnae pairs.

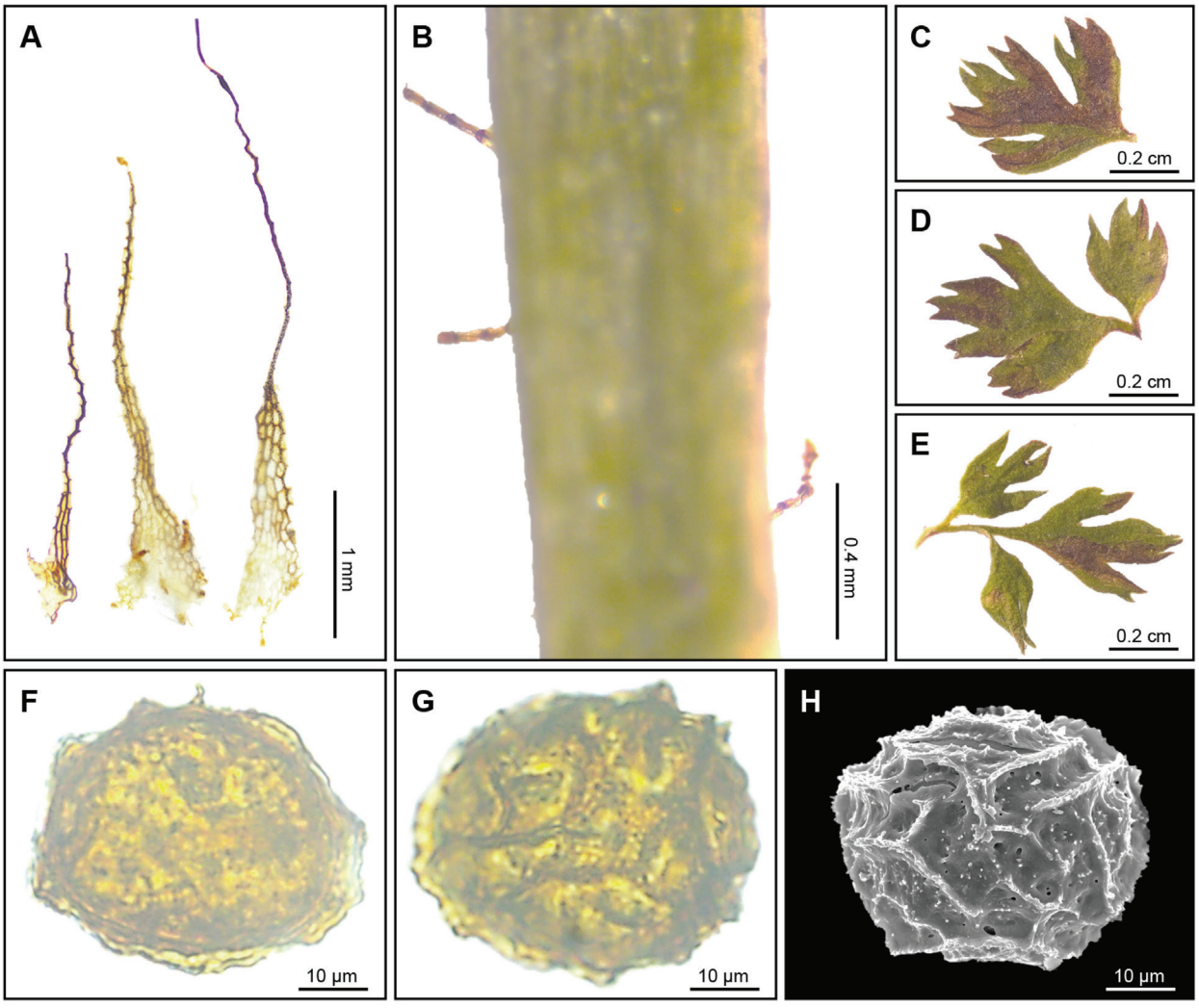

Fig. 2. Micromorphology of *Asplenium delinghaense* S. Q. Liang et X. C. Zhang, sp. nov. (Sheng-Bang Zhang et al. 7617 (PE), individual 1): **A** – Rhizome scales; **B** – Fibrillar scales on the lower stipe; **C–E** – Differences in the degree of division among the third, second and basal pinnae; F – Optical section of spore showing exospore contour and the thickness of perispore folds; **G–H** – LM and SEM photos of spore showing perispore morphology.

FCM results showed that the nuclear DNA content of *A. delinghaense* is 19.4 ± 1.3 pg/2C, which is similar to the level of related tetraploid species in Liang et al. (2021). As cytotypes can often be distinguished by spore sizes (Sleep, Reichstein, 1984), the length of exospore, (29)35–41(47) µm, is also an indicator of high ploidy of this species comparing with related taxa (Lin, Viane, 2013). In the plastid DNA phylogeny (Fig. 3), *A. delinghaense* showed close maternal relationship with *A. fugax*, *A. capillipes*, and members of the *A. pekinense* complex (Lin, Viane, 2013; Liang et al., 2021). However, in the

nuclear DNA phylogeny, two different copies were detected. One of them showed a position similar to that of the plastid result, whereas the other clustered with *A. tenuicaule* var. *subvarians* and one of its putative tetraploid offspring, *A. kansuense* (Liang et al., 2021) (Fig. 4). According to the above evidence, we concluded that *A. delinghaense* is probably an allotetraploid that originated from hybridization of *A. tenuicaule* and an unknown taxon closely related to *A. fugax*, *A. capillipes*, or to members of the *A. pekinense* complex.

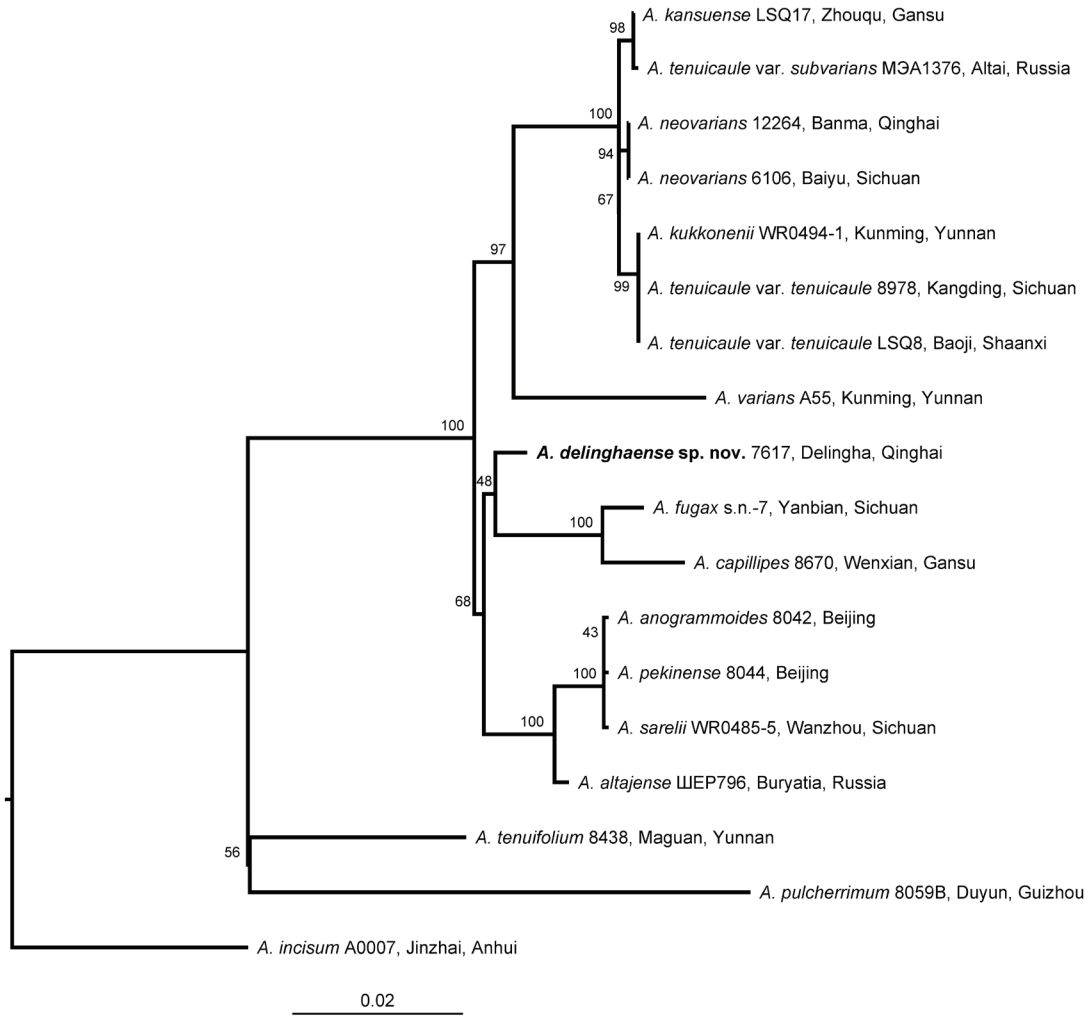

Fig. 3. Maximum likelihood (ML) phylogeny of plastid DNA dataset showing the position of *Asplenium delinghaense*. Bootstrap support values are shown above branches.

Taxonomic treatment

Asplenium delinghaense S. Q. Liang et X. C. Zhang, **sp. nov.** (Figs. 1–2).

Diagnosis. *Asplenium delinghaense* resembles *A. iskardense* and *A. daghestanicum* but can be distinguished by its lophate perispore with costate-cristate ridges (with narrow dentate crests and swollen fold base) and some perforations near the base. In comparison, ridges of perispore are costate and costate-cristate with narrower fold base in the latter two species. The ratio between the length of lamina and stipe is around 1.0 in *A. delinghaense*, whereas the ratio is ca. 0.5 and ca. 1.4 in *A. iskardense* and *A. daghestanicum*, respectively. *A. delinghaense* is also similar to *A. neovarians*, and their distribution overlapped in NW China. Comparing with *A. neovarians*, *A. delinghaense* has longer stipes, more pinnae pairs and perispore with narrower and crests broader folds. In addition, the spore size of *A. delinghaense* is the largest among all four species, though they were all estimated to be tetraploid.

Fig. 4. ML phylogeny of nuclear DNA dataset showing the position of two different copies of *Asplenium delinghaense*. Bootstrap support values are shown above branches.

Holotype: "China, Qinghai, Delingha, Baishushan (Cypress Mountain) 37°29′24″N, 97°22′19″E, alt. 3610 m. V 2021. Sheng-Bang Zhang et al. 7617" (PE!).

Description. Plants 5–8 cm tall. Rhizome erect, short, apex scaly; rhizome scales (1.5)2.1–4.0(4.4) × (0.3)0.4–0.6(0.7) mm wide at the base, brown, narrowly triangular to linear-subulate, base

cordate, margin nearly entire, apex acuminate, terminating in a long apical tail. Fronds firmly herbaceous, green, brown green when dry, more than ten usually, tufted; stipes 3–5 cm long, generally a little bit shorter than laminae, slender, adaxially sulcate, abaxially dark brown at the base, upward green, base with few narrowly triangular scales, toward the rachis covered with more sparsely fibrillar scales; rachis structure similar to that of distal part of stipe. Laminae 32–52 × 9–14 mm, narrowly triangular, base bipinnate and nearly symmetrical, becoming pinnate toward the apex, apex acute-acuminate with triangular apical segment ca. 6–10 × 2–5 mm; pinnae 6 to 8 pairs, opposite to subopposite, deltate-ovate to rhombic, shortly (up to 0.5 mm) stalked, basal pinnae pinnate, 0.8–1.1 cm remote from the next pair and usually slightly larger, the second, third, fourth, and fifth pinnae slightly smaller and pinnatisect to pinnatilobate; only basal pinnae with a pair of independent pinnules, the basal acroscopic pinnules largest, sessile or slightly stipitate, base broadly cuneate, apex serrate with long and sharp teeth, apex obtuse. Veins slightly raised adaxially, biforked or simple, not reaching margin. Sori 1–3 per segment, linear, 1–3 mm, submedial on veins, confluent at maturity but not covering the upper half of the segment; indusia white-gray, semi-elliptic, membranous, entire, opening toward costa or costule, persistent. Spores dark brown, perispore lophate with costate-cristate folds, average exospore length (29)35–41(47) μm.

Etymology. *Delinghaense* is derived from the type locality, Delingha, a transliteration of a Mongolian word means "golden world".

Distribution and habitat. *Asplenium delinghaense* is known only from the Cypress Mountain (Baishushan) geopark north of Delingha, Qinghai, NW China, growing on steep dolomite-marble, at an elevation of 3610 m.

Conservation status. *Asplenium delinghaense* is known only from its type locality, Cypress Mountain (Baishushan) geopark north of Delingha, located at the northeastern edge of the Qaidam Basin, where is quite xeric. Exact population size of this new species is unknown, because it is adaptive to the barren rocky south face slope of the alpine karst mountain, which is massive and difficult for people to climb.

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Appendix

Specimen information and GenBank accession number. Asterisks (*) and en-dashes (–) indicate newly generated and missing sequences, respectively.

Taxon	Voucher	Country	*rbcL*	*rpl32-trnP*	*pgiC*
Asplenium altajense (Komar.) Grubov	A. I. Shmakov et al. IIIEP796 (PE)	Tunkinsky, Buryatia Republic, Russia	–	MK828788	MK828931, MK828932
Asplenium anogrammoides Christ	X.-C. Zhang 8042 (PE)	Beijing, China	–	MK828796	MK828859, MK828860
Asplenium capillipes Makino	X.-C. Zhang et S.-Q. Liang 8670 (PE)	Wenxian, Gansu, China	MN688475	MN688506	MN688448
Asplenium delinghaense S. Q. Liang et X. C. Zhang	S.-B. Zhang et al. 7617 (PE)	Delingha, Qinghai, China	OP795813*	OP795810*	OP795818*, OP795819*
Asplenium fugax Christ	X.-H. Jin s. n.-7 (PE)	Yanbian, Sichuan, China	MN688476	MN688507	MN688449
Asplenium incisum Thunb.	C.-F. Zhao A0007 (PE)	Jinzhai, Anhui, China	–	MK828792	MK828854
Asplenium kansuense Ching	X.-C. Zhang et S.-Q. Liang LSQ17 (PE)	Zhouqu, Gansu, China	–	MK828812	MK828878, MK828879
Asplenium kukkonenii Viane et Reichst.	R. Wei & Q.-P. Xiang WR0494-1 (PE)	Kunming, Yunnan, China	–	MK828829	MK828900, MK828901
Asplenium neovarians Ching	S.-B. Zhang et al. 12264 (PE)	Banma, Qinghai, China	OP795811*	OP795808*	OP795814*, OP795815*
Asplenium neovarians Ching	X.-C. Zhang 6106 (PE)	Baiyu, Sichuan, China	OP795812*	OP795809*	OP795816*, OP795817*
Asplenium pekinense Hance	X.-C. Zhang 8044 (PE)	Beijing, China	–	MK828798	MK828861, MK828862
Asplenium pulcherrimum (Baker) Ching ex Tardieu	Z.-Y. Guo 8059B (PE)	Duyun, Guizhou, China	MN688469	MN688498	MN688429, MN688430
Asplenium sarelii Hook.	R. Wei WR0485-5 (PE)	Wanzhou, Chongqing, China	–	MK828807	MK828871, MK828872

Appendix (continued)

Taxon	Voucher	Country	*rbcL*	*rpl32-trnP*	*pgiC*
Asplenium tenuicaule var. *subvarians* (Ching) Viane	A. I. Shmakov et al. MƏA1376 (PE)	Chemalsky, Altai Republic, Russia	–	MK828846	MK828925
Asplenium tenuicaule var. *tenuicaule* Hayata	X.-C. Zhang et al. 8978 (PE)	Kangding, Sichuan, China	–	MK828821	MK828893
Asplenium tenuicaule var. *tenuicaule* Hayata	S.-Q. Liang LSQ8 (PE)	Baoji, Shaanxi, China	–	MK828818	MK828889
Asplenium tenuifolium D. Don	X.-C. Zhang et al. 8438 (PE)	Maguan, Yunnan, China	MN688478	MK828791	MK828922
Asplenium varians Wall. ex Hook. et Grev.	C.-F. Zhao A55 (PE)	Kunming, Yunnan, China	–	MK828837	MK828909, MK828910