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Two new tetraploid quillwort species, *Isoetes longpingii* and *I. xiangfei* from China (Isoëtaceae)

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Abstract: Two new tetraploid quillwort species, *Isoetes longpingii* and *I. xiangfei*, from China are described and illustrated. *I. longpingii* is a fully submerged plant found in a small pond of Hunan Province. It is morphologically similar to *I. sinensis*, but differs in its small and tuberculate-cristate megaspores and soft slender leaves that grow up to 60 cm. It is also similar to hexaploid *I. orientalis*, but differs in its 44 chromosomes and tuberculate-cristate megaspores. *I. xiangfei* is most similar to diploid *I. yunguiensis* in megaspore ornamentation, but differs in its microspore ornamentation, oblong sporangium, and 44 number of chromosomes. There are a few individuals of *I. longpingii* found in Ningxiang City of Hunan Province, and *I. xiangfei* is distributed in the wetlands of Tongdao and Huitong counties of Hunan Province. Because of their limited geographic ranges, small populations, fewer individuals and disturbed habitats, *I. longpingii* and *I. xiangfei* are evaluated as critically endangered (CR) and vulnerable (VU) category, respectively, according to the IUCN Red List criteria. A key to all the current known Chinese quillworts is also provided for further taxonomic identification and conservation of these rare and endangered plants in China.

Key words: new taxon, *Isoetes*, Lycopods, polyploid, heterosporous, aquatic plants

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中国水韭属两个四倍体新种

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摘要: 水韭属 (*Isoetes*) 是起源最为古老的水生维管植物, 全属物种均被列为国家一级重点保护植物。通过对全国水韭属植物的调查和研究发现, 不同产地的四倍体植株在形态上存在显著差异。基于形态学、孢粉学和细胞学证据, 将分布于中国湖南省长沙地区和怀化地区的四倍体居群分别命名为隆平水韭 (*Isoetes longpingüi*) 和湘妃水韭 (*I. xiangfei*), 并详细描述了其形态特征。隆平水韭形态上与中华水韭 (*I. sinensis*) 相似, 不同之处在于其大孢子具小的瘤状或冠状纹饰, 叶细长而柔弱, 长达 60 cm; 该种也与六倍体东方水韭 (*I. orientalis*) 相似, 不同之处在于其染色体 44 条, 大孢子具瘤状或冠状纹饰。湘妃水韭的大孢子纹饰虽与二倍体云贵水韭 (*I. yunguiensis*) 相似, 但在小孢子纹饰、孢子囊形状和染色体数目方面却不同。隆平水韭仅少数植株生长于湖南省宁乡市一处池塘, 完全沉水生长, 而湘妃水韭则分布于怀化市通道县和会同县的湿地。由于这两个新种的分布区狭窄, 野生居群数量和个体数较少, 栖息地环境受到人为干扰, 因此根据 IUCN 红色名录评估标准, 将隆平水韭评为极危 (CR) 等级, 湘妃水韭评为易危 (VU) 等级。所编制的中国已知水韭属物种的分种检索表, 为本属物种的鉴定和保护工作提供了重要参考。

关键词: 新分类群, 水韭属, 石松类植物, 多倍体, 异型孢子, 水生植物

Isoetes L. (1753: 1100) is the only extant genus of the family Isoëtaceae, with an extensive fossil record dating from the Devonian Period (Pigg, 2001; Wang et al., 2019) to rapid diversification in the Cenozoic Era (Pereira et al., 2017b, 2021; Wood et al., 2020). The genus contains about 250 species, widely distributed from tropical to subarctic regions, and grows in various habitats in most parts of the world (PPGI, 2016; Troia et al., 2016). However, many *Isoetes* species have restricted habitats and small populations, putting them at risk of extinction (Kang et al., 2005; Liu X et al., 2005; Gentili et al., 2010; Troia et al., 2016; Brunton & Troia, 2018; Singh et al., 2021). Interestingly, hybridization and polyploidization frequently occur in *Isoetes*, and more than half of the known species are polyploid ranging from tetraploid to dodecaploid (Hickey, 1984; Taylor & Hickey, 1992; Liu et al., 2004; Kim et al., 2010; Pereira, 2015; Troia et al., 2016; Grigoryan et al., 2020). Although this ancient

genus is widely distributed in various habitats and has a complex evolutionary history, its simple morphology — a tuft of linear sporophylls on the lobed rootstock — are remarkably similar among species, making interspecific identification difficult (Taylor & Hickey, 1992; Troia et al., 2016). *Isoetes* species are mostly distributed in America, southern Europe, Africa and Australia, with relatively few species in Asia (Troia et al., 2016; Pereira et al., 2017b, 2021; Brunton & Troia, 2018; Choi et al., 2018; Singh et al., 2021). The *Isoetes* species diversity in Asia is probably underestimated, and many cryptic species may not be identified (Schafran, 2019).

Characteristics such as habitat, velum, ligule, megaspore size and texture, chromosome count, and DNA sequences are generally employed to distinguish species of *Isoetes* (Hickey, 1986; Pereira, 2015; Troia et al., 2016); however, the characteristics of leaf morphology and anatomy are influenced by local

environmental conditions (Liu et al., 2006; Cavalheiro-Filho et al., 2021; Singh et al., 2021). Several species have recently been described and illustrated around the world (Mora-Olivo et al., 2016; Schafran et al., 2016; Pereira et al., 2016, 2017a, 2019; Li et al., 2019; Lu et al., 2021). In China, seven species — *I. yunguiensis* Q. F. Wang & W. C. Taylor, *I. hypsophila* Hand. - Mazz., *I. taiwanensis* De Vol, *I. shangrilaensis* X. Li & Y. Q. Huang, *I. baodongii* Y. F. Gu, Y. H. Yan & Yi J. Lu, *I. sinensis* T. C. Palmer and *I. orientalis* H. Liu & Q. F. Wang — have been reported (Handel-Mazzetti, 1923; Palmer, 1927; De Vol, 1972; Wang et al., 2002; Liu H et al., 2005; Zhang & Taylor, 2013; Li et al., 2019; Lu et al., 2021).

We conducted several field surveys of the genus in China and collected some tetraploid samples ($2n=4x=44$) previously considered as *I. sinensis* ($2n=4x=44$) with morphological and anatomical variation (Liu et al., 2004; Liu et al., 2006; Dai et al., 2020, 2021). By comparing morphological features and spore textures, these samples from Ningxiang City and Tongdao County in Hunan Province were found to be different from *I. sinensis*. In addition, our plastid phylogenomic analysis indicated that these samples formed monophyletic clades, which did not cluster together with *I. sinensis*. The sample from Ningxiang City, here named *I. longpingii*, is similar to *I. orientalis* ($2n=6x=66$) on megaspore ornamentation and that from Tongdao County, here named *I. xiangfei*, is similar to *I. yunguiensis* ($2n=2x=22$) on megaspore ornamentation. These two new species are described and illustrated in the present study.

1 Materials and Methods

Isoëtes species possess prominent spore morphological characteristics, and the spore ornamentation terminology established by Hickey (1986) was used in the present study. Megaspores and microspores of samples were examined under a scanning electron microscope. Spores were mounted on a double-sided adhesive tape attached to metal stubs, sputter-coated with platinum, and observed under a field emission scanning electron microscope (Quanta 250; FEI,

Hillsboro, Oregon, U. S. A.) at 30 kV. Megaspore and microspore sizes from 3 – 5 individuals were measured using Photoshop CS5 (Adobe Systems Inc., USA).

To determine the chromosome number, young root tips of the sporophytes were pretreated in a saturated aqueous solution of p-dichlorobenzene for 3–5 h and subsequently fixed in Carnoy's solution (1 : 3, glacial acetic acid : 95% ethanol) for 1 h at 4 °C. Subsequently, the samples were hydrolyzed with a mixture of 3% cellulase and 2.5% pectinase for 10 min at room temperature. They were then stained with carbol fuchsin. The chromosomes of the samples were counted and photographed using a Carl Zeiss Axio Scope A1 photomicroscope (Jena, Germany).

2 Taxonomic Treatment

2.1 *Isoëtes longpingii* Y.H. Yan, Y.F. Gu & J.P. Shu (Fig. 1, Fig. 3: A–D)

Diagnosis: — *Isoëtes longpingii* is similar to *I. sinensis*, but differs in its small megaspores (310–410 μm , mean = 350 μm , $n=20$), sparsely tuberculate-cristate [vs. larger (mean 410 μm), densely tuberculate-cristate] and bilobed rhizome corms (vs. trilobed in *I. sinensis*). It is also similar to hexaploid *I. orientalis*, but differs in its megaspores tuberculate-cristate, and 44 chromosomes (vs. 66 in *I. orientalis*) (Fig. 3, Fig. 4, Table 1).

Type: — CHINA. Hunan Province, Changsha City, Ningxiang City, Yujia'ao Village, 28°14'27.91" N, 112°17'44.86" E, alt. 131 m, June 15, 2019. Zhiguo Ou YYH15160 (**holotype**, PE! barcode 02347221; **isotype**: NOCC!, IBSC!).

Description: — Plants aquatic. Rhizome corms: 2-lobed. Sporophylls: widely spreading, 40–60 in a tuft, white at base, green above, spirally arranged, 20–60 cm long, ca. 1 mm wide, flattened on the adaxial side, rounded on the abaxial side, base flat and alate, peripheral fibrous bundles present, central intrastelar canal 4. Sporangia: basal, obovate, 3.5–4.5×2.5–3.0 mm. Ligule: cuniform, 2.3–2.6×1.1–1.3 mm. Megaspores: white when dry, earthy yellow when wet, 310–410 μm (mean=350 μm , $n=20$) in diameter, proximal

Table 1 Spore features of *Isoëtes* from China

Species	Number of chromosomes	Megaspore		Microspore	
		Ornamentation	Size	Ornamentation	Size
<i>I. hypsophila</i> *	2n = 2x = 22	Levigate	290–400 μm (mean = 358 μm)	Echinate	19–25 μm (mean = 22 μm)
<i>I. shangrilaensis</i>	2n = 2x = 22	Tuberculate-rugulate	207.25–273.09 μm (mean = 244.71 μm)	Echinate-cristate	11.21–23.66 μm (mean = 19.41 μm)
<i>I. yunguiensis</i> *	2n = 2x = 22	Cristate-reticulate	340–430 μm (mean = 390 μm)	Levigate-granulate	20–25 μm (mean = 22 μm)
<i>I. taiwanensis</i> *	2n = 2x = 22	Tuberculate-cristate	280–340 μm (mean = 312 μm)	Echinate	20–28 μm (mean = 24 μm)
<i>I. sinensis</i> *	2n = 4x = 44	Cristate	340–450 μm (mean = 409 μm)	Echinate	23–32 μm (mean = 28 μm)
<i>I. orientalis</i> *	2n = 6x = 66	Cristate-reticulate	350–460 μm (mean = 420 μm)	Echinate-tuberculate	20–38 μm (mean = 34 μm)
<i>I. baodongii</i>	2n = 2x = 22	Echinate-cristate	390–510 μm (mean = 450 μm)	Echinate	22–27 μm (mean = 25 μm)
<i>I. longpingüi</i>	2n = 4x = 44	Tuberculate-cristate	310–410 μm (mean = 350 μm)	Echinate	27–30 μm (mean = 29 μm)
<i>I. xiangfei</i>	2n = 4x = 44	Cristate-reticulate	390–450 μm (mean = 430 μm)	Tuberculate	26–28 μm (mean = 27 μm)

Note: * Cited from Liu et al.(2008). Datas of *I. shangrilaensis* and *I. baodongii* are cited from Li et al.(2019) and Lu et al.(2021), respectively.

hemisphere tuberculate, distal hemisphere echinate-cristate. Microspores: gray *en masse*, elliptic, vertical axis length 27–30 μm (mean = 29 μm , $n = 20$), surface echinate. Chromosome numbers $2n = 4x = 44$.

Distribution: — Ningxiang City, Changsha City, Hunan Province.

Ecology: — Submerged in a small pond; alt. 131 m.

Etymology: — The specific epithet “*longpingüi*” is derived from the name of the late Prof. YUAN Longping, who devoted his entire life to hybrid rice research in Changsha City (where the species was discovered) and greatly contributed to global food security and poverty alleviation (Wang, 2021).

IUCN Red List category: — A single population of about 10 individuals is found in only one location in Yujia’ao Village, Ningxiang City, Hunan Province. *Isoëtes longpingüi* is critically endangered (CR) [A1a; B2ab(iv)c; C2a(ii); D(2)] according to the Red List Categories and Criteria (IUCN, 2022).

2.2 *Isoëtes xiangfei* Y.H. Yan, Y.F. Gu & J.P. Shu (Fig. 2, Fig. 4: A–D)

Diagnosis: — *Isoëtes xiangfei* is a tetraploid, morphologically similar to the diploid *I. yunguiensis* and the tetraploid *I. sinensis* in megaspore ornamentation, but differs in its less reticulate, lower megaspore

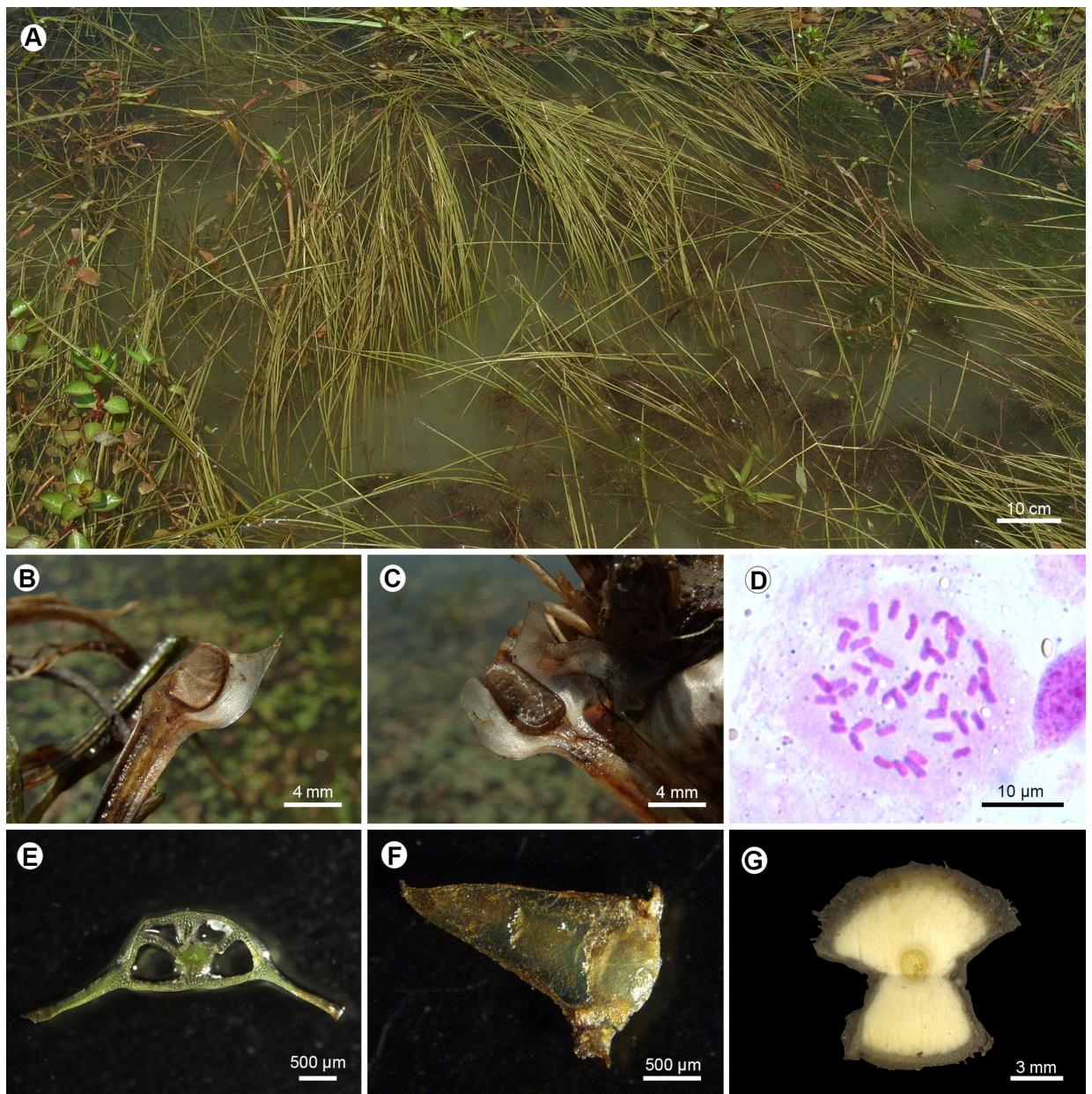
ornamentation, and densely low-tuberculate (vs. sparsely tuberculate-echinate) microspores (Fig. 3, Fig. 4, Table 1).

Type: — CHINA. Hunan Province: Huaihua City, Tongdao County, 26°14'56.82" N, 109°50'0.33" E, alt. 298 m, December 28, 2019. Juan Yang Fern08928 (**holotype**: PE! barcode 02347220; **isotype**: NOCC!, IBSC!).

Description: — Plants aquatic. Rhizome corms 3-lobed. Sporophylls 20–60 in a tuft, widely spreading, white at the base, green above, spirally arranged, 15–35 cm long, 2–3 mm wide, flattened on the adaxial side, rounded on the abaxial side, base flat and alate, peripheral fibrous bundles present, central intrastelar canal 4. Sporangia: basal, oblong, 4.4–5.0 × 2.9–3.3 mm. Ligule: oval triangle, 2.5–2.7 × 1.4–1.6 mm. Megaspores: white when dry, earthy yellow when wet, 390–450 μm (mean = 430 μm , $n = 20$) in diameter; proximal hemisphere cristate, distal hemisphere reticulate. Microspores: gray *en masse*, elliptic, monolete, vertical axis 26–28 μm (mean = 27 μm , $n = 20$), surface echinate. Chromosome numbers $2n = 4x = 44$.

Distribution: — Tongdao and Huitong counties, Huaihua City, Hunan Province.

Ecology: — Wetlands and small gullies; alt. 298 m.



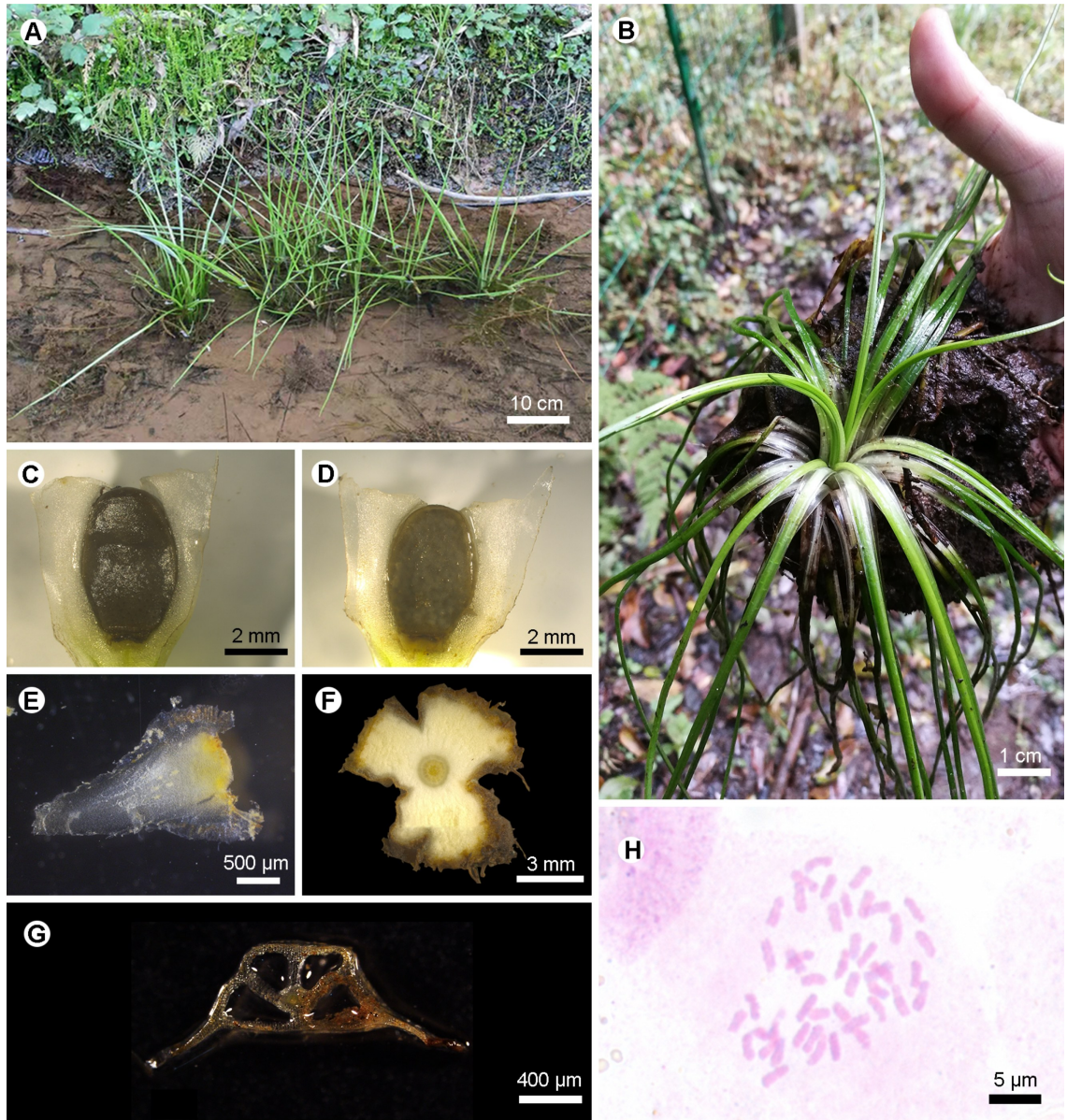
A. Habitat; B. Microsporangium; C. Megasporangium; D. Chromosomes ($2n=4x=44$); E. Transection of leaf base; F. Ligule; G. Transection of rhizome (2-lobed).

Fig. 1 Morphological and anatomic characteristics of *Isoetes longpingii* Y.H. Yan, Y.F. Gu & J.P. Shu

Etymology: — The specific epithet “*xiangfei*” is derived from the name of Xiangfei, who are two respectable goddesses of the Xiangjiang River in Hunan Province, where the aquatic plant was discovered. “Xiang” is the name of the longest river in Hunan Province, and “fei” is the wife of the king in Chinese. Xiangfei, two sisters, married the legendary

King Shun at the same time.

IUCN Red List category: — *Isoetes xiangfei* is found in Tongdao and Huitong counties, Hunan Province. There are about 200 individuals, which are well conserved by the local forestry department. It is vulnerable (VU) [B2ab(iii); C2a(i)] according to the Red List Categories and Criteria (IUCN, 2022).

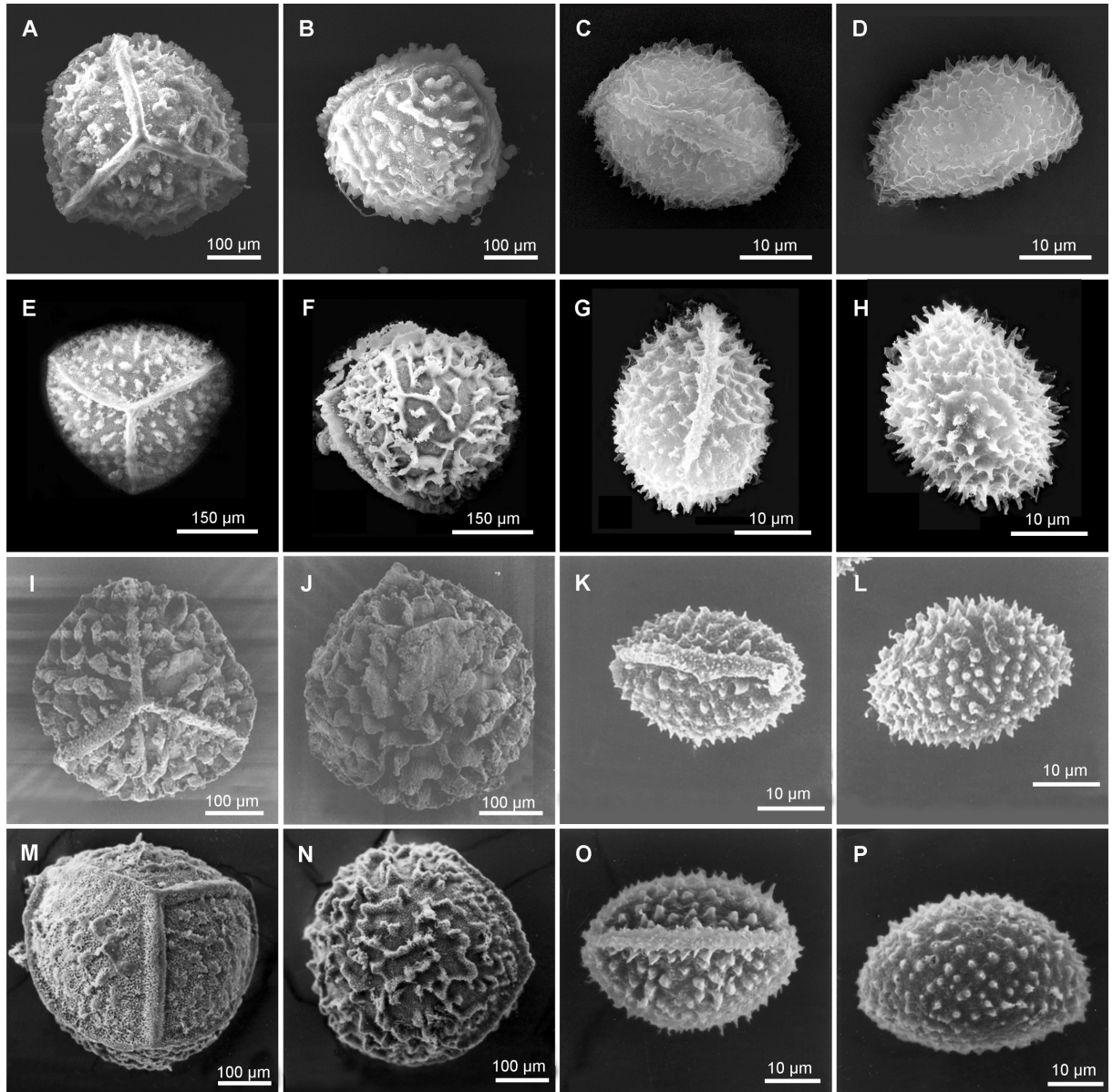


A. Habit; B. Plants with wide wing at the base of leaf; C. Microsporangium; D. Megasporangium; E. Ligule; F. Transection of rhizome (3-lobed); G. Transection of leaf base; H. Chromosomes.

Fig. 2 Morphological and anatomic characteristics of *Isoetes xiangfei* Y.H. Yan, Y.F. Gu & J.P. Shu

3 Key to *Isoetes* of China

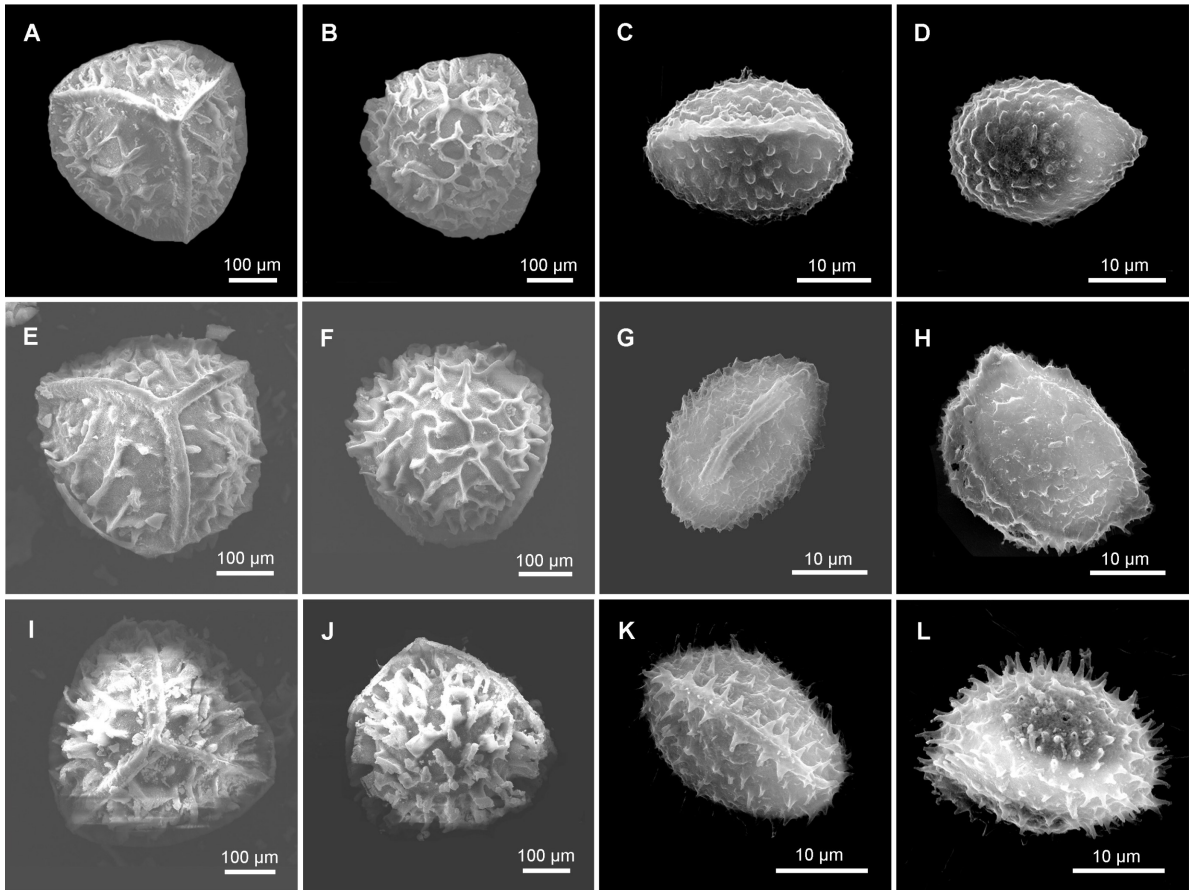
- (1) Individual diploid. (2)
 (1) Individual polyploid. (6)
 (2) Megaspore levigate or just tuberculate-regulate. (3)
 (2) Megaspore with various types of ornamentation. (4)
 (3) Leaves 2–5 cm long, microspores echinate. *I. hypsophila*
 (3) Leaves 3–18 cm long, microspores echinate to cristate. *I. shangrilaensis*
 (4) Megaspore tuberculate-cristate, just distributing in Taiwan. *I. taiwanensis*



A–D. *Isoetes longpingii* (YYH15160. A. Proximal view of megaspore; B. Distal view of megaspore; C. Proximal view of microspore; D. Distal view of microspore). E–H. *I. sinensis* from China [cited from Liu et al. (2008)]. E. Proximal view of megaspore; F. Distal view of megaspore; G. Proximal view of microspore; H. Distal view of microspore]. I–L. *I. sinensis* from Japan [cited from Watanabe et al. (1996)]. I. Proximal view of megaspore; J. Distal view of megaspore; K. Proximal view of microspore; L. Distal view of microspore]. M–P. *I. sinensis* from Korea [cited from Takamiya (2001)]. M. Proximal view of megaspore; N. Distal view of megaspore; O. Proximal view of microspore; P. Distal view of microspore].

Fig. 3 Palynological comparison between *Isoetes longpingii* and *I. sinensis*

- (4) Megaspore echinate or cristate-reticulate. (5)
 (5) Microspore echinate, distributing in Zhejiang. *I. baodongii*
 (5) Microspore levigate-granulate, distributing in Yunnan and Guizhou. *I. yunguiensis*
 (6) Leaves ca. 1 mm wide at mid-length. (7)
 (6) Leaves 2–3 mm wide at mid-length. *I. xiangfei*
 (7) Megaspore ornamentation cristate-reticulate, chromosome numbers 66. *I. orientalis*



A–D. *Isoetes xiangfei* (Fern08928. **A.** Proximal view of megaspore; **B.** Distal view of megaspore; **C.** Proximal view of microspore; **D.** Distal view of microspore). **E–H.** *I. yunguiensis* (YYH15163. **E.** Proximal view of megaspore; **F.** Distal view of megaspore; **G.** Proximal view of microspore; **H.** Distal view of microspore). **I–L.** *I. orientalis* (Fern08748. **I.** Proximal view of megaspore; **J.** Distal view of megaspore; **K.** Proximal view of microspore; **L.** Distal view of microspore).

Fig. 4 Palynological comparison between *Isoetes xiangfei*, *I. yunguiensis* and *I. orientalis*

- (7) Megaspore without reticulate ornamentation, chromosome numbers 44. (8)
 (8) Rhizome 2-lobed, megaspore 310–410 μm (mean = 350 μm). *I. longpingii*
 (8) Rhizome 3-lobed, megaspore 340–450 μm (mean = 409 μm). *I. sinensis*

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