Two Tetraploid New Species of Isoetes from China: Postprint

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Abstract

Isoëtes is the most anciently originated genus of aquatic vascular plants, with all species listed as National First-Class Protected Plants. Through nationwide investigation and research on Isoëtes plants, significant morphological differences were found among tetraploid individuals from different localities. Based on morphological, palynological, and cytological evidence, the tetraploid populations distributed in Changsha and Huaihua regions of Hunan Province, China, are designated as Isoëtes longpingii and I. xiangfei, respectively, with their morphological characteristics described in detail. Isoëtes longpingii is morphologically similar to I. sinensis, but differs in having megaspores with small tuberculate or cristate ornamentation, and slender, delicate leaves up to 60 cm in length; this species is also similar to the hexaploid I. orientalis, differing in having 44 chromosomes and megaspores with tuberculate or cristate ornamentation. The megaspore ornamentation of I. xiangfei is similar to that of the diploid I. yunguiensis, but differs in microspore ornamentation, sporangium shape, and chromosome number. Isoëtes longpingii consists of only a few individuals growing in a pond in Ningxiang City, Hunan Province, completely submerged, whereas I. xiangfei is distributed in wetlands of Tongdao and Huitong Counties in Huaihua City. Due to the narrow distribution range of these two new species, small number of wild populations and individuals, and habitat disturbance by human activities, I. longpingii is assessed as Critically Endangered (CR) and I. xiangfei as Vulnerable (VU) according to IUCN Red List criteria. The compiled dichotomous key for known Isoëtes species in China provides an important reference for the identification and conservation of species within this genus.

Full Text

Two New Tetraploid Quillwort Species, *Isoëtes longpingii* and *I. xiangfei* from China (Isoëtaceae)

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Abstract

Two new tetraploid quillwort species, *Isoëtes longpingii* and *I. xiangfei*, from China are described and illustrated. *I. longpingii* is a fully submerged plant found in a small pond in Hunan Province. It is morphologically similar to *I. sinensis* but differs in its small, tuberculate-cristate megaspores and soft, slender leaves that grow up to 60 cm. It is also similar to the hexaploid *I. orientalis* but differs in having 44 chromosomes and tuberculate-cristate megaspores. *I. xiangfei* is most similar to the diploid *I. yunguiensis* in megaspore ornamentation but differs in its microspore ornamentation, oblong sporangium, and 44 chromosomes. Only a few individuals of *I. longpingii* occur in Ningxiang County, Hunan Province, while *I. xiangfei* is distributed in the wetlands of Tongdao and Huitong counties in Hunan Province. Due to their limited geographic ranges, small populations, low individual numbers, and disturbed habitats, *I. longpingii* and *I. xiangfei* are evaluated as Critically Endangered (CR) and Vulnerable (VU), respectively, according to IUCN Red List criteria. A key to all currently known Chinese quillworts is also provided to facilitate further taxonomic identification and conservation of these rare and endangered plants in China.

Keywords: new taxon, *Isoëtes*, lycophytes, polyploid, heterosporous, aquatic

plants

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Isoëtes 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) through rapid diversification in the Cenozoic Era (Pereira et al., 2017b, 2021; Wood et al., 2020). The genus contains approximately 250 species widely distributed from tropical to subarctic regions, inhabiting various habitats across most parts of the world (PPGI, 2016; Troia et al., 2016). However, most *Isoëtes* species have restricted habitats and small populations, placing 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 occur frequently in *Isoëtes*, with more than half of known species being polyploids 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 across various habitats and has a complex evolutionary history, its simple morphology—a tuft of linear sporophylls on a lobed rootstock—is remarkably similar among species, making interspecific identification difficult (Taylor & Hickey, 1992; Troia et al., 2016). *Isoëtes* species are predominantly distributed in the Americas, 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 diversity of *Isoëtes* species in Asia is probably underestimated, and many cryptic species may remain unidentified (Schafran, 2019).

Characteristics such as habitat, velum, ligule, megaspore size and texture, chromosome count, and DNA sequences are generally employed to distinguish *Isoëtes* species (Hickey, 1986; Pereira, 2015; Troia et al., 2016). However, 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 worldwide (Mora-Olivo et al., 2016; Pereira et al., 2016, 2017a, 2019; Schafran et al., 2016; Li et al., 2019; Lu et al., 2021). In China, seven species have been reported: *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 (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 extensive field surveys of the genus across China and collected tetraploid samples (2n=4x=44) previously identified as *I. sinensis* (2n=4x=44) that exhibited morphological and anatomical variation (Liu et al., 2004; Liu et al., 2006; Dai et al., 2020, 2021). By comparing morphological features and spore texture, samples from Ningxiang and Tongdao counties in Hunan Province were found to differ from *I. sinensis*. Additionally, our plastid phylogenomic analysis indicated that these samples formed monophyletic clades that did not cluster with *I. sinensis* (Gu et al., unpublished). The sample from Ningxiang County, here named *I. longpingii*, resembles *I. orientalis* (2n=6x=66) in megaspore ornamentation, while the sample from Tongdao County, here named *I. xiangfei*, resembles *I. yunguiensis* (2n=2x=22) in megaspore ornamentation. These two new species are described and illustrated in the present study.

Materials and Methods

Isoëtes species possess prominent spore morphological characteristics, and the spore ornamentation terminology established by Hickey (1986) was used in this study. Megaspores and microspores were examined under a scanning electron microscope. Spores were mounted on double-sided adhesive tape attached to metal stubs, sputter-coated with platinum, and observed under a field emission scanning electron microscope (Quanta250; 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 chromosome number, young root tips of 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. The samples were then hydrolyzed with a mixture of 3% cellulase and 2.5% pectinase for 10 min at room temperature and stained with carbol fuchsin. Chromosomes were counted and photographed using a Carl Zeiss Axio Scope A1 photomicroscope (Jena, Germany).

Taxonomic Treatment

***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 m, mean = 350 m, n = 20) that are sparsely tuberculate-cristate [vs. larger (mean 410 μm) and densely tuberculate-cristate] and bilobed rhizome corms (vs. trilobed in *I. sinensis*). It is also similar to the hexaploid *I.*

orientalis but differs in having tuberculate-cristate megaspores and 44 chromosomes (vs. 66 in *I. orientalis*) (Fig. 3, Fig. 4, Table 1).

Type: CHINA. Hunan Province, Changsha City, Ningxiang County, Yujia' ao Village, 28°14' 27.91'' N, 112°17' 44.86'' E, alt. 131 m, June 15, 2019. Zhiguo Ou YYH15160 (holotype, PE!; 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 cuneiform, 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 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 number 2n = 4x = 44.

Distribution: Ningxiang County, Changsha City, Hunan Province.

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

Etymology: The specific epithet "longpingii" honors the late Prof. Long-Ping Yuan, who devoted his 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 ten individuals is found at only one location in Yujia' ao Village, Ningxiang County, Hunan Province. *Isoëtes longpingii* is Critically Endangered (CR) [A1a; B2ab(iv)c; C2a(ii); D(2)] according to the Red List Categories and Criteria (Subcommittee, 2019).

Fig. 1 Morphological and anatomic characteristics of *Isoëtes longpingii* Y.H. Yan, Y.F. Gu & J.P. Shu. A. Habit; B. Microsporangia; C. Megasporangia; D. Chromosomes (2n = 4x = 44); E. Transection of leaf; F. Ligule; G. Transection of rhizome (2-lobed).

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 microspores (vs. sparsely tuberculate-echinate) (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!; 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-triangular, 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 number 2n = 4x = 44.

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

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

Etymology: The specific epithet "xiangfei" derives from the name of the Xiangfei, two revered goddesses of the Xiangjiang River in Hunan Province where this aquatic plant was discovered. "Xiang"is the longest river in Hunan Province, and "fei" means wife in Chinese. According to legend, the Xiangfei sisters married King Shun simultaneously.

IUCN Red List category: *Isoëtes xiangfei* occurs in Tongdao and Huitong counties, Hunan Province, with approximately 200 individuals that are well conserved by the local forestry department. It is Vulnerable (VU) [B2ab(iii); C2a(i)] according to the Red List Categories and Criteria (Subcommittee, 2019).

Fig. 2 Morphological and anatomic characteristics of *Isoëtes xiangfei* Y.H. Yan, Y.F. Gu & J.P. Shu. A. Habit; B. Plants with wide wing at the base of leaf; C. Microsporangia; D. Megasporangia; E. Ligule; F. Transection of rhizome (3-lobed); G. Transection of leaf; H. Chromosomes.

Fig. 3 Palynological comparison between *Isoëtes longpingii* and *I. sinensis*. A–D. *Isoëtes 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. 4 Palynological comparison between *Isoëtes xiangfei*, *I. yunguiensis* and *I. orientalis*. A–D. *Isoëtes 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.

Table 1 Spore features of *Isoëtes* from China

Species	Chromosome number	Megaspore	Microspore
		Ornamentation	Size
I. hypsophila	2n = 2x = 22	Levigate	290–400 μm
I. shangri-laensis	2n = 2x = 22	Tuberculate-rugulate	207.25–273.09 μm (mean = 244.71 μm)
I. yunguiensis	2n = 2x = 22	Cristate-reticulate	340–430 μm (mean = 390 μm)
I. taiwanensis	2n = 2x = 22	Tuberculate-cristate	280–340 μm (mean = 312 μm)
I. sinensis	2n = 4x = 44	Cristate	340–450 μm (mean = 409 μm)
I. orientalis	2n = 6x = 66	Cristate-reticulate	350–460 μm (mean = 420 μm)
I. baodongii	2n = 2x = 22	Echinate-cristate	290–400 μm (mean = 358 μm)
I. longpingii	2n = 4x = 44	Tuberculate-cristate	310–410 μm (mean = 350 μm)
I. xiangfei	2n = 4x = 44	Cristate-reticulate	390–450 μm (mean = 430 μm)

Note: Data for *I. hypsophila*, *I. yunguiensis*, *I. taiwanensis*, *I. sinensis*, and *I. orientalis* were cited from Liu et al. (2008). Data for *I. shangrilaensis* and *I. baodongii* were cited from Li et al. (2019) and Lu et al. (2021), respectively.

Key to *Isoëtes* of China

1. Individual diploid → 2
2. Individual polyploid → 6
3. Megaspore levigate or just tuberculate-regulate → 3
4. Megaspore with various types of ornamentation → 4
5. Leaves 2–5 cm long, microspores echinate → *I. hypsophila*
6. Leaves 3–18 cm long, microspores echinate to cristate → *I. shangrilaensis*
7. Megaspore tuberculate-cristate, just distributing in Taiwan → *I. taiwanensis*
8. Megaspore echinate or cristate-reticulate → 5
9. Microspore echinate, distributing in Zhejiang → *I. baodongii*
10. Microspore levigate-granulate, distributing in Yunnan and Guizhou → *I. yunguiensis*
11. Leaves ca. 1 mm wide at mid-length → 7
12. Leaves 2–3 mm wide at mid-length → *I. xiangfei*
13. Megaspore ornamentation cristate-reticulate, chromosome number 66 → *I. orientalis*
14. Megaspore without reticulate ornamentation, chromosome number 44 → 8
15. Rhizome 2-lobed, megaspore 310–410 μm (mean = 350 μm) → *I. longpingii*
16. Rhizome 3-lobed, megaspore 340–450 μm (mean = 409 μm) → *I. sinensis*

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Note: Figure translations are in progress. See original paper for figures.