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TONG L, SHEN JH, ZHAO X, et al., 2024. *Isoëtes changxingensis* (Isoëtaceae), a new tetraploid quillwort species from China [J]. Guihaia, 44(4): 611-618.

Isoëtes changxingensis (Isoëtaceae), a new tetraploid quillwort species from China

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Abstract: *Isoëtes changxingensis*, a new species from Zhejiang Province, China, is illustrated and described here. The new species morphologically resembles *I. baodongii*, but it differs from the latter by 44 chromosomes, megaspore 317 - 411 μm (mean = 360 μm) [vs. 22, 390 - 510 μm (mean = 450 μm)]. It is also similar to *I. longpingii* in terms of megaspore size, with the difference that *I. changxingensis* rhizome corms 3-lobed, leaves 2 - 3 mm wide at middle, megaspore echinate-cristate (vs. 2-lobed, 1 mm, tuberculate-cristate). This species is distinguished from *I. sinensis* by its smaller megaspore, different surface ornamentation, and leaf blade 20 - 60 cm [vs. 340 - 450 μm (mean = 409 μm), cristate, 15 - 30 cm]. According to Guidlines for Using the IUCN Red List Criteria, the conservation status of *I. changxingensis* should be regarded as endangered (EN).

Key words: new taxon, *Isoëtes*, lycophytes, endangered, spore ornamentation, key to *Isoëtes*

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长兴水韭,中国水韭属一个四倍体新种

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摘 要：水韭属是一类具有异形孢子的古老石松类植物，全属物种均被列入国家重点保护野生植物。该文报道了在浙江省长兴县新发现的一个四倍体水韭属居群。基于形态学、孢粉学和细胞学证据，将该物种命名为长兴水韭（*Isoëtes changxingensis*），并详细描述了其形态特征。长兴水韭与保东水韭（*I. baodongii*）在植株形态及孢子纹饰方面都较为相似，但不同之处在于其染色体为 44 条，大孢子极面直径为 317～411 μm（平均 360 μm）（vs. 染色体 22 条，大孢子极面直径 390～510 μm，平均 450 μm）。与同为 4 倍体的隆平水韭（*I. longpingii*）在孢子大小方面极为接近，不同之处在于其根状茎 3 裂，叶片中间宽 2～3 mm，大孢子表面具棘刺-脊条状纹饰（vs. 根状茎 2 裂，叶片中间宽度 1 mm，大孢子表面具瘤状-脊条状纹饰）。该种与中华水韭（*I. sinensis*）的区别在于其大孢子较小，表面纹饰不同，叶片长 20～60 cm（vs. 大孢子极面直径 340～450 μm，平均 409 μm，具脊条状突起纹饰，叶片长 15～30 cm）。长兴水韭目前仅分布于其模式产地的一处沟渠，由于其分布区狭窄，野生居群数量较少，栖息地环境受到人为干扰，因此根据 IUCN 红色名录评估标准，可将长兴水韭暂定为濒危（EN）等级。该种面临生境破坏和野外人为刈割的风险，因此很有必要开展迁地保护及野外回归等相关工作。该文编制了中国已报道的水韭属物种的分种检索表，为该属物种的鉴定和保护工作提供了参考。

关键词：新分类群，水韭属，石松类植物，濒危，孢子纹饰，水韭属检索表

Isoëtes L. (Isoëtaceae, Lycopodiopsida) is an ancient heterosporous genus of lycophytes. It originated between the Late Devonian and Early Carboniferous (Yang et al., 2022), occupying a key position in the evolution of terrestrial plants (Cui et al., 2022). The genus contains about 250 species which are widely distributed from the tropics to the sub-arctic and South America is considered a center of both morphological and taxonomical diversity with more than 60 cataloged species (Pereira et al., 2016; Troia et al., 2016). They thrive in diverse aquatic or wet soils habitats, including oligotrophic lakes, higher-altitude wetlands, seasonal pools, and intermittent streams (Pereira et al., 2016, 2017). The uncertainty in the assessment of species diversity of *Isoëtes* mainly comes from the difficulty in classification, which is related to a series of habitat adaptation, simple and conservative morphology, convergence and reticular evolution (Taylor & Hickey, 1992; Choi et al., 2008; Bagella et al., 2011; Freund et al., 2018; Gu et al., 2023a). The identification characteristics of *Isoëtes* mainly include habitat, leaf vein, ligule, megaspore size and texture, chromosome count and DNA sequence (Kott & Britton, 1983; Hickey, 1986, Taylor et al., 1993; Troia et al., 2016; Bolin et al., 2017; Brunton & Troia, 2018). The most reliable feature is megaspore morphology, but this is often difficult to achieve under field conditions (Hickey, 1986; Holmes et al., 2005; Brunton & Troia, 2018).

At present, ten species of *Isoëtes* have been described from China, including *I. hypsophila* Hand.-Mazz. (Handel-Mazzetti, 1923), *I. sinensis* T. C. Palmer (Palmer, 1927), *I. taiwanensis* De Vol (De Vol, 1972), *I. yunguiensis* Q. F. Wang & W. C. Taylor (Wang et al., 2002), *I. orientalis* H. Liu & Q. F. Wang (Liu et al., 2005), *I. shangrilaensis* X. Li, Y. Huang, X. Dai, & X. Liu (Li et al., 2019), *I. baodongii* Y. F. Gu, Y. H. Yan, & Yi J. Lu (Lu et al., 2021), *I. longpingii* Y. H. Yan, Y. F. Gu, & J. P. Shu, and *I. xiangfei* Y. H. Yan, Y. F. Gu, & J. P. Shu (Shu et al., 2022), and newly reported species *I. fengii* Y. F. Gu & Y. H. Yan (Gu et al., 2023b). According to the List of National Key Protected Wild Plants released in September 2021, all species of *Isoëtes* are list as first-level protection (http://www.forestry.gov.cn/main/5461/20210908/162515850572900.html). The distribution scopes of *Isoëtes* species are wide and the altitude spans are very large. *Isoëtes hypsophila is* located in the Qinghai-Tibet Plateau (QTP) while *I. sinensis* grows in the middle and low reaches of the Yangtze River (Li et al., 2019). In contrast to the United States, which is geographically at about the same latitude as China and has similar climatic conditions, there are more than 50 *Isoëtes* species in the United States, while only ten accepted *Isoëtes* species in China (Troia et al.,

2016). The relatively sparse number of *Isoëtes* species reported from China may be due to increased water pollution and urbanization, similar appearance between species, less taxonomic research and insufficient field exploration (Troia et al., 2016; Lu et al., 2021).

During field investigation in Changxing County, Huzhou City, Zhejiang Province, China, we discovered one population of *Isoëtes* similar to some populations found in eastern China, such as *I. baodongii* and *I. sinensis*. However, determination of different morphological traits, cytology and spore ornamentation provided convincing evidence that the Changxing population constituted a new species, as proposed below.

1 Materials and Methods

The megaspores and microspores of the samples were observed via scanning electron microscopy. Spores were mounted on doublesided adhesive tape attached to metal stubs, sputter-coated with platinum, and viewed under a scanning electron microscope FlexSEM 1000 Ⅱ (Hitachi, Japan) at 5 – 7 kV. In our study, a total of 20 megaspores and 30 microspores from three to five individuals were measured using ImageJ2 (National Institutes of Health, USA). Terms for describing the ornamentation of megaspores and microspores were taken from Hickey (1986).

Root tips of 1 cm in length were taken from the sporophytes and pretreated in a mixture of 0.1% colchicine and 0.002 mol · L^{-1} 8-hydroxyquinoline (1∶1) for 3.5 h at room temperature, rinsed with water for three times, and then fixed in Carnot's fixative of ethanol and glacial acetic acid (3∶1) for 6 h at 4 ℃. The samples were rinsed with water for three times, then dissociated with a mixture of 1 mol · L^{-1} HCl and 45% glacial acetic acid (1∶1) for 45 min in a thermostatic bath at 37 ℃. Finally, the samples were rinsed with water for three times, and then stained with modified carbonic acid magenta for 4 h, and then pressed into slices by the conventional tablet pressing method. The chromosomes of the samples were counted by Nikon ECLIPSE Ci-S Biomicroscope (Nikon, Japan) and photographed using a Digital Sight Ds-Fi2 Camera System (Nikon, Japan).

2 Taxonomic Treatment

Isoëtes changxingensis Y. F. Gu & J. H. Shen, sp. nov. (长兴水韭, 新拟, Fig. 1–Fig. 3)

Type: CHINA, Zhejiang Province, Changxing (长兴) County, Xiaopu (小浦) Town, in an artificial ditch, 31.014349° N, 119.779106° E, alt. 50 m, 27 August 2023. *Jiahao Shen & Ling Tong* 2515 (holotype: NAS!; isotype: NOCC!).

Diagnosis: *Isoëtes changxingensis* is a tetraploid that morphologically resembles *I. baodongii*, but differs in its 44 chromosomes [vs. 22 in *I. baodongii*]. It is also similar to *I. longpingii* in megaspore size, but differs in its trilobed rhizome corms, megaspore echinate-cristate [vs. bilobed, tuberculate-cristate in *I. longpingii*]. This species is distinguished from *I. sinensis* by its smaller megaspore (317 – 411 μm, mean =360 μm), different surface ornamentation, and leaf blade 20–60 cm [vs. megaspore 340 –450 μm, mean= 409 μm, cristate, 15–30 cm in *I. sinensis*]. (Fig. 1–Fig. 3, Table 1)

Description: Plants aquatic. Rhizome corms 3-lobed. Sporophylls white basally, green above, spirally arranged, widely spreading, 20–60 cm long, 2–3 mm wide at mid-length, in tufts of 50 to 90, flattened on the adaxial side, rounded on the abaxial side, base flat and alate, peripheral fibrous bundles present, central intrastelar canal 4. Ligule ovate-subtriangular, (3.0 – 3.5) mm × (2.0 – 2.5) mm. Sporangia basal, oblong, triangular ovate, (7.5 – 10.5) mm × (3.5 – 4.5) mm, sporangium wall clear. Megaspores rugulate, gray when wet, white when dry, ca. 317 – 411 μm (mean = 360 μm), proximal hemisphere echinate-cristate, distal hemisphere cristate. Microspores gray in mass, elliptic, vertical axis length 24 – 31 μm (mean = 27 μm), echinate. Megaspores and microspores in different sporangia.

Distribution: *Isoëtes changxingensis* is known only from Changxing County, Huzhou City of Zhejiang Province, China.

Table 1 Critical character differences between *Isoëtes changxingensis* and the other ten reported *Isoëtes* species in China

Character		*I. changxingensis*	*I. sinensis* *	*I. longpingii* *	*I. xiangfei* *	*I. orientalis* *	*I. baodongii*	*I. fengii*	*I. yunguiensis* *	*I. taiwanensis* *	*I. hypsophila* *	*I. shangrilaensis*
Number of chromosomes		2n = 4x = 44	2n = 4x = 44	2n = 4x = 44	2n = 4x = 44	2n = 6x = 66	2n = 2x = 22	2n = 6x = 66	2n = 2x = 22	2n = 2x = 22	2n = 2x = 22	Unknown
Rhizome corms		3	3	2	3	3	3	3	3	2–4	2–3	3
Sporophyll	Length (cm)	20–60	15–30	20–60	15–35	10–20	15–45	15–30	10–60	7–25	3–4.5	2.8–17
	Width at middle (mm)	2–3	1–2	1	2–3	2	3	2–3	5–10	1–2	1	1
Megaspore	Size (μm)	317–411	340–450	310–410	390–450	350–450	390–510	470–500	360–450	280–340	290–400	207–273
	Mean (μm)	360	409	350	330	420	450	480	390	312	358	245
	Ornamentation	Echinate-cristate	Cristate	Tuberculate-cristate	Cristate-reticulate	Cristate-reticulate	Echinate-cristate	Reticulate	Cristate-reticulate	Tuberculate-cristate	Levigate	Tuberculate-rugulate
Microspore	Size (μm)	24–31	23–32	27–30	26–28	19–29	22–27	25–35	20–25	20–28	19–25	11–24
	Mean (μm)	27	28	29	27	22	25	30	22	24	22	19
	Ornamentation	Echinate	Echinate	Echinate	Tuberculate	Echinate-tuberculate	Echinate	Echinate	Levigate-granulate	Echinate	Rugulate	Echinate-cristate

Note: * indicates cite from Liu et al. (2008) and Shu et al. (2022). Data of *I. shangrilaensis*, *I. baodongii* and *I. fengii* are cited from Li et al. (2019), Lu et al. (2021) and Gu et al. (2023a, b), respectively.

Etymology: The specific epithet is derived from its type locality, Changxing County.

Cytology: *Isoëtes changxingensis* is a tetraploid species with a chromosome number of 2n = 4x = 44. (Fig. 3)

Ecology: Ditches beside farmland at an altitude of 50 m, it has been submerged in the upstream ditch at a higher water level while standing up in the ditch at a lower water level. It often blends in with associated species such as *Pteris multifida* Poir., *Oenanthe javanica* (Blume) DC., *Pouzolzia zeylanica* (L.) Benn., *Commelina benghalensis* L. and *Gynostemma pentaphyllum* (Thunb.) Makino at the distribution site.

IUCN Red List category: *Isoëtes changxingensis* is only found in Changxing County, Huzhou City, Zhejiang Province. There are about 300 individuals, but the ditches in which they are distributed are close to villagers' farmlands, which poses a risk of pesticide contamination, vine shading, and farmers' cutting. Based on currently available information, we proposed that *I. changxingensis* should be considered as 'Endangered (EN)' [A2a; B2ab (iii); C2a (i)] according to the Red List Categories and Criteria (IUCN, 2022). This species is confronted with habitat destruction and wild human mowing, therefore, it is necessary to carry out the related conservation work such as *ex situ* conservation and field return, etc.

Additional specimen examined (Paratype): CHINA, Nanjing Botanical Garden Mem. Sun Yat-Sen, cultivated plant, collected from type locality, 12 January 2024, *Jiahao Shen* 2707 (NAS!).

3 Updated Key to *Isoëtes* Species in China

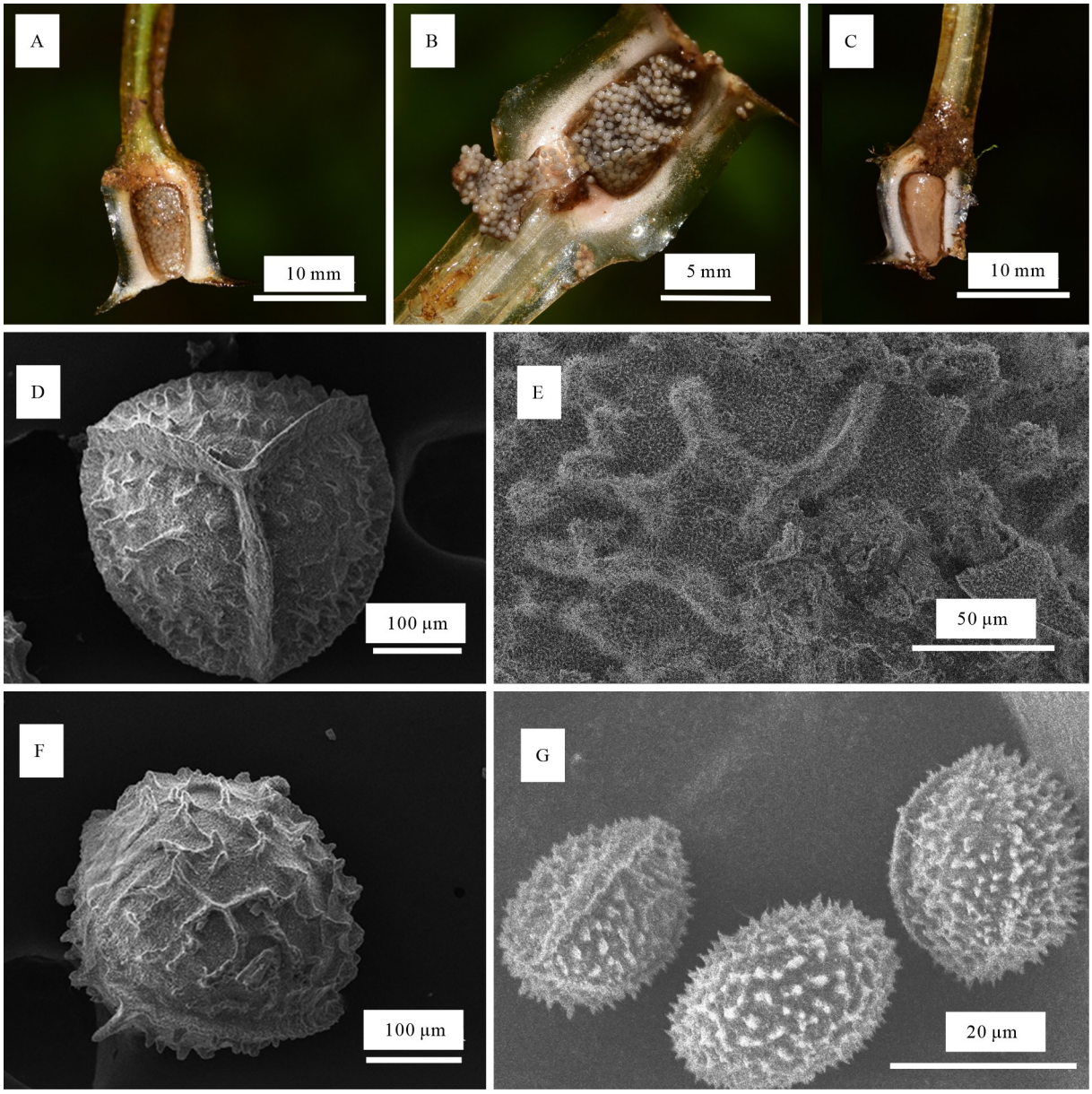
Key to *Isoëtes* species from China

1. Small ferns, mostly found at high altitudes (H > 2 000 m) ····· 2
1. Taller plants, mostly found at low to medium altitudes (H < 2 000 m) ····· 3
2. Megaspore laevigate, microspore rugulate ····· *I. hypsophila*
2. Megaspore tuberculate-rugulate, microspore echinate to cristate ····· *I. shangrilaensis*
3. Leaves 1 – 2 mm wide at middle ····· 4
3. Leaves 2 – 10 mm wide at middle ····· 7

A. Seedlings reproduced by spores; **B**. Plant *in situ*; **C**. Roots; **D**. Three-lobed rhizome corm.

Fig. 1　Morphological characteristics of *Isoëtes changxingensis* Y. F. Gu & J. H. Shen

4. Megaspore cristate-reticulate, microspore echinate-tuberculate …… *I. orientalis*
4. Megaspore cristate or tuberculate-cristate, microspore echinate …… 5
5. Megaspore cristate, 340 – 450 μm (mean = 409 μm) in diameter on the proximal face …… *I. sinensis*
5. Megaspore tuberculate-cristate, 280 – 410 μm in diameter on the proximal face …… 6
6. Individual diploid (2n = 22), just distributing in Taiwan …… *I. taiwanensis*
6. Individual tetraploid (2n = 44), just distributing in Hunan …… *I. longpingii*
7. Microspore tuberculate or levigate-granulate …… 8
7. Microspore echinate …… 9
8. Individual tetraploid (2n = 44), just distributing in Hunan …… *I. xiangfei*



A. Megasporangium; **B**. Megaspores in megasporangium; **C**. Microsporangium; **D**. Proximal view of megaspore; **E**. Detail surface ornamentation of megaspore; **F**. Distal view of megaspore; **G**. Proximal view and distal view of microspores.

Fig. 2 Palynological characteristics of *Isoëtes changxingensis* Y. F. Gu & J. H. Shen

8. Individual diploid (2n=22), distributing in Yunnan and Guizhou ····· *I. yunguiensis*
9. Megaspore echinate-cristate ····· 10
9. Megaspore reticulate ····· *I. fengii*
10. Individual diploid (2n=22), megaspore 390 – 510 μm (mean=450 μm) in diameter on the proximal face ····· *I. baodongii*
10. Individual tetraploid (2n=44), megaspore 317–411 μm (mean=360 μm) in diameter on the proximal face ····· *I. changxingensis*

中国水韭属检索表

1. 植株较矮小，多分布于高海拔地区（H > 2 000 m） …… 2
1. 植株较高大，多分布于中低海拔地区（H < 2 000 m） …… 3
2. 大孢子表面光滑，小孢子表面具突起纹饰 …… 高寒水韭 *I. hypsophila*
2. 大孢子表面具瘤状–突起纹饰，小孢子表面具棘刺至脊条状突起纹饰 …… 香格里拉水韭 *I. shangrilaensis*
3. 叶片中部较窄，宽度 1~2 mm …… 4
3. 叶片中部较宽，宽度 2~10 mm …… 7
4. 大孢子表面具网络状纹饰；小孢子表面具棘刺状瘤状突起纹饰 …… 东方水韭 *I. orientalis*
4. 大孢子表面具脊条状或瘤状突起纹饰；小孢子表面具棘刺状突起纹饰 …… 5
5. 大孢子表面具脊条状突起纹饰，平均极面直径 409 μm …… 中华水韭 *I. sinensis*
5. 大孢子表面具瘤状–脊条状突起纹饰，平均极面直径小于 400 μm …… 6
6. 染色体数目为 22，分布于台湾 …… 台湾水韭 *I. taiwanensis*
6. 染色体数目为 44，分布于湖南 …… 隆平水韭 *I. longpingii*
7. 小孢子表面具瘤状突起纹饰或具疣状颗粒至近乎光滑 …… 8
7. 小孢子表面具棘刺状突起纹饰 …… 9
8. 染色体数目为 44，分布于湖南 …… 湘妃水韭 *I. xiangfei*
8. 染色体数目为 22，分布于云南和贵州 …… 云贵水韭 *I. yunguiensis*
9. 大孢子表面具棘刺–脊条状突起纹饰 …… 10
9. 大孢子表面具网络状纹饰 …… 青锋水韭 *I. fengii*
10. 染色体数目为 22，大孢子平均极面直径为 450 μm …… 保东水韭 *I. baodongii*
10. 染色体数目为 44，大孢子平均极面直径为 360 μm …… 长兴水韭 *I. changxingensis*

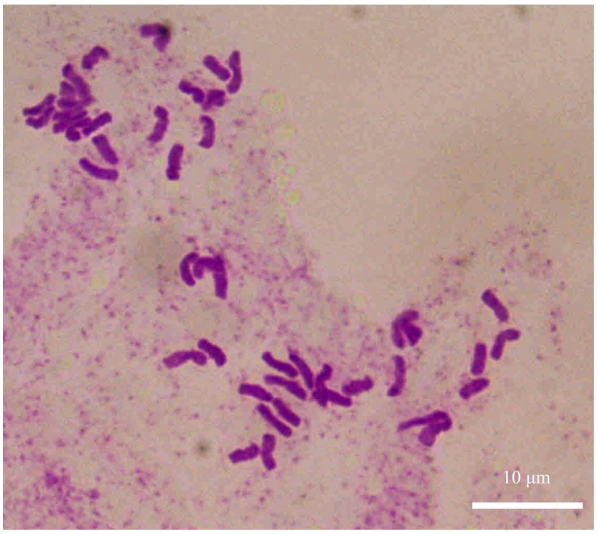


Fig. 3　Chromosomes of *Isoëtes changxingensis* Y. F. Gu & J. H. Shen（2n = 4x = 44）

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