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Two New Species of *Isoetes* (Isoetaceae) from Jeju Island, South Korea

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We name and describe two new species, *Isoetes jejuensis* and *I. hallasanensis*, from Jeju Island, South Korea. These taxa are compared with related species in East Asia, including *I. coreana* and *I. sinensis*. The rugulate megaspore ornamentation of *I. jejuensis* differs from those other *Isoetes* species. Furthermore, *I. hallasanensis* varies from *I. asiatica* in its microspore ornamentation and chromosome number, although both species have an echinate megaspore ornamentation. These new species are tetraploid (2n = 44), whereas *I. coreana* is hexaploid (2n = 66).

Keywords: Isoetes, Isoetes hallasanensis, Isoetes jejuensis, Jeju Island, new species

The number of named species of *Isoetes* throughout the world has increased due to more intensive searches for plants in the field and because of the re-evaluation of taxa using modern diagnostic techniques (Taylor and Hickey, 1992). Therefore, many taxa have recently been described, including those in East Asia (Wang et al., 2002; Romero et al., 2004; H. Liu et al., 2005). In Korea, only three species were previously recorded: *I. japonica* A. Braun (Park, 1942), *I. coreana* Chung & Choi (Chung and Choi, 1986; Jung, 2001), and *I. sinensis* (Palmer) var. *sinensis* (Takamiya, 2001). However, Korean *Isoetes* species are near extinction and are listed as endangered species due to habitat loss, an increase in agricultural land use, and an invasion of exotic species, phenomena also witnessed in other countries (X. Liu et al., 2005; Kim et al., 2008). During our systematic and conservational studies of the genus *Isoetes* in South Korea, we found two unknown species that are morphologically similar to *I. coreana* and *I. sinensis*, but differ in their megaspore ornamentations or chromosome numbers.

Although the worldwide monograph of genus *Isoetes* was done by Pfeiffer (1922), this taxonomic treatment is outdated because of recently described taxa. Therefore, we compared our two new species with regional species from China, Taiwan, and Japan (Table 1). Ten species are distributed in that area, but the new ones do not coincide with the previously reported taxa because they have rugulate megaspores (*I. jejuensis*) or echinate micro- and megaspores (*I. hallasanensis*).

Morphology. As diagnostic characters, we continue to use the number of corm lobes and shape of ligules, presence or absence of velum, and presence or absence of peripheral fiber strands in the sporophyll to distinguish among *Isoetes* species (Takamiya et al., 1997). Most of those from East Asia, including the two new species *I. jejuensis* and *I. hallasanensis*, typically have three-lobed corms whereas *I. asiatica* has a two-lobed corm (Table 1). Furthermore, the deltoid shape of the ligule and the presence of velum are unique in *I. asiatica* (Takamiya et al., 1997). The peripheral fiber strand is a mechanical tissue situated under the epidermis in the sporophyll. *Isoetes jejuensis* and *I. hallasanensis* have them at four sites (Figs. 1B and 2B) whereas no such strand is found in *I. taiwanensis*, *I. asiatica*, or *I. yunguiensis* (DeVol, 1972a, b; Takamiya et al., 1997; Wang et al., 2002).

The morphology of *Isoetes* species from East Asia is highly conserved, compounded by homoplasy and allopolyploid speciation (Takamiya et al., 1997; Taylor et al., 2004). Therefore, it is difficult to distinguish species by many morphological characters. This could be why we did not determine the key characters for delimitation of *I. jejuensis* or *I. hallasanensis* based on their external appearance.

Spore ornamentation. Spores of *I. jejuensis* and *I. hallasanensis* were obtained from specimens collected at the Dongbakdongsan, Bukjeju-gun and at 1100 Highland Wetland, Seoguipo city, Jeju Island, respectively. Observations, measurements, and photomicrographs were made with a Jeol JSM-6380 scanning electron microscope and Olympus BX40 microscopes.

Four types of megaspore ornamentation have been recorded for the *Isoetes* species of East Asia (Table 1): tuberculate, reticulate, echinate, and cristate (terminology for spore ornamentation follows that of Hickey, 1986). Although the phylogenetic significance of spore ornamentation in those species is controversial (Hickey, 1986; Rydin and Wikstrom, 2002), it was a useful diagnostic character when we identified and compared the two new species with other taxa of *Isoetes*.

The rugulate megaspores of *I. jejuensis* obviously differ from other East Asian species (Fig. 1G-I and Table 1). Megaspore ornamentation of *I. hallasanensis* is echinate-tuberculate in the proximal view and echinate in the distal view (Fig. 2G-I). Although it is similar to *I. asiatica*, a diploid species, it differs because of its microspore ornamentation, with *I. hallasanensis* being echinate (Fig. 2J) and *I. asiatica* levigate (Takamiya et al., 1997; Table 1).

Chromosome Number. Plant materials for chromosome counts were collected from Dongbakdongsan, Bukjeju-gun and 1100 Highland Wetland, Seoguipo-city from Jeju

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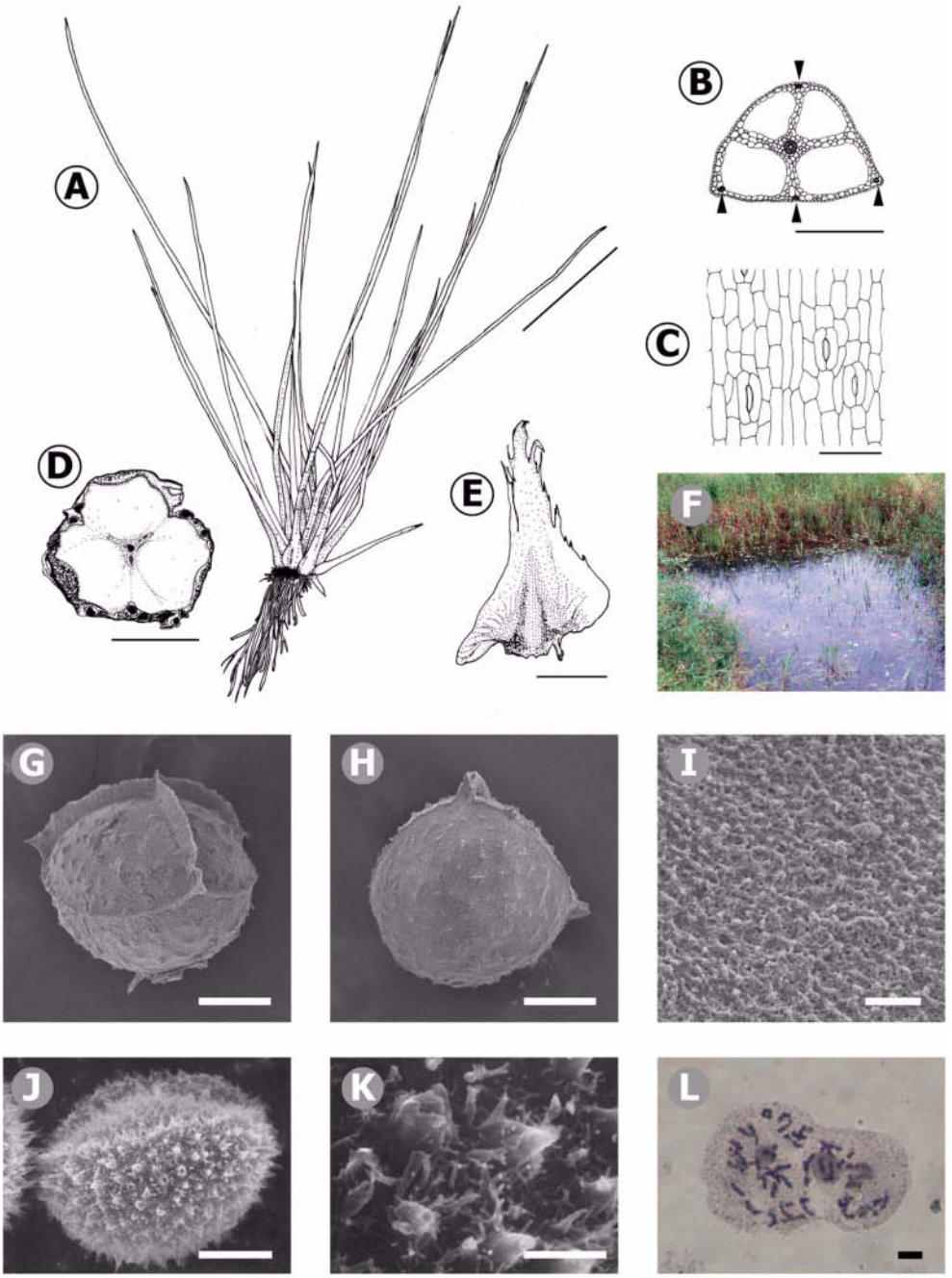

Figure 1. *Isoetes jejuensis* H.-K. Choi, C. Kim & J. Jung. **A**, Habit of plant. **B**, Cross section of sporophyll at middle length; arrowheads indicate peripheral fiber strands. **C**, Stomata. **D,** Cross section of corm. **E,** Ligule. **F,** Habitat(Namjeju-gun, Jeju Island, South Korea). **G-I,** Megaspore. **G,** Lateral view. **H,** Distal view. **I,** Ultrastructure of surface. **J,** Lateral view of microspore. **K,** Ultrastructure of microspore surface. **L,** Somatic chromosomes in mitotic root tip. Scale bars: A = 5 cm; B, E = 1 mm; C, G, H = 100 μm; D = 1 cm; I, L = 5 μm; J = 10 μm; K = 2 μm.

Island, and cultivated in an artificial pond at Ajou University. Mitotic chromosome numbers were determined as described by Kott and Britton (1980). Both new species are tetraploid, with chromosome numbers of 2n = 44 (Figs. 1L and 2L). Of the 10 species of *Isoetes* earlier reported from East Asia, 4 are diploids (2n = 22: *I. yunguiensis*, *I. hypsophila*, *I. taiwanensis*, and *I. asiatica*), 1 is tetraploid (2n = 44: *I. sinensis*), 3 are hexaploid (2n = 66: *I. coreana*, *I. orientalis*, and *I. japonica*), 1 is heptaploid (2n = 77: *I. ×michinokuana*), and 1 is octoploid (2n = 88: *I. pseudojaponica*) (Table 1). Molecular data are now being used to investigate the close relationships among basic diploid and polyploid *Isoetes* species from East Asia (Taylor et al., 2004). Thus, our new tetraploid species may provide keys to clarifying this speciation process and the dispersal mechanism among *Isoetes* species of East Asia.

Key to the Taxa of *Isoetes* in East Asia

1a. Corms 2-lobed; sporangium covered by velum ····· ····· *I. asiatica*

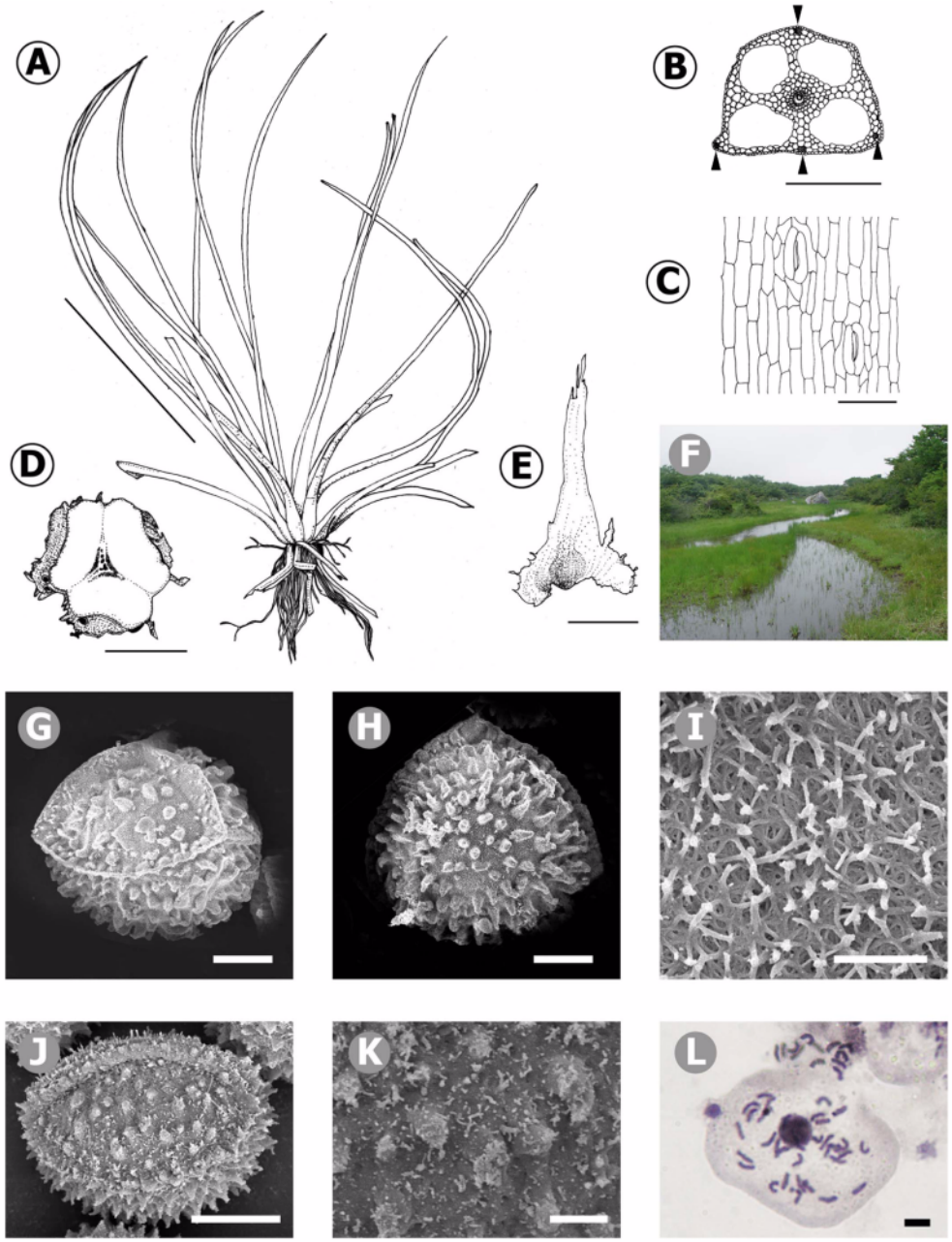

Figure 2. *Isoetes hallasanensis* H.-K. Choi, C. Kim & J. Jung. **A,** Habit of plant. **B,** Cross section of sporophyll at middle length; arrowheads indicate peripheral fiber strands. **C,** Stomata. **D,** Cross section of corm. **E,** Ligule. **F,** Habitat. **G-I,** Megaspore. **G,** Lateral view. **H,** Distal view. **I,** Ultrastructure of surface. **J,** Lateral view of microspore. **K,** Ultrastructure of microspore surface. **L,** Somatic chromosomes in mitotic root tip. Scale bars: A = 5 cm; B, E = 1 mm; C, G, H = 100 μm; D = 1 cm; I, L = 5 μm; J = 10 μm; K = 2 μm.

1b. Corms 3-lobed; sporangium not covered by velum
2a. Microspore ornamentation levigate ····· *I. japonica*
2b. Microspore ornamentation echinate
3a. Leaves without peripheral fiber strands; megaspore ornamentation tuberculate ····· *I. taiwanensis*
3b. Leaves with peripheral fiber strands; megaspore ornamentation cristate, rugulate, or echinate
4a. Megaspore ornamentation cristate
5a. Microspore length ≤ 20 μm ····· *I. sinensis*
5b. Microspore length ≥ 30 μm ····· *I. coreana*
4b. Megaspore ornamentation rugulate or echinate
6a. Megaspore ornamentation rugulate and ultrastructure dense ····· *I. jejuensis*
6b. Megaspore ornamentation echinate and ultrastructure fibrous ····· *I. hallasanensis*

The following is a distinct description of two previously unnamed species of *Isoetes*:

Isoetes jejuensis H.-K. Choi, C. Kim & J. Jung, sp. nov. TYPE: South Korea. Jeju Island: Namjeju-gun, 33° 23 N, 126° 48 E, ca. 4 km NE of Pyosunmyun, elev. 125 m, 5 Aug. 2000, *J. Jung 2000157* (holotype, AJOU; isotype, AJOU). Figure 1.

Table 1. Comparison of diagnostic morphological characters and chromosome numbers among *Isoetes* species from East Asia

Species	No. of corm lobes	Megaspore (μm) †	Microspore (μm) ‡	Chromosome number (2n)	Voucher locality	Literature Cited
I. jejuensis	3	rugulate (325-425)	echinate (26-32)	44	South Korea	In this study
I. hallasanensis	3	echinate (356-464)	echinate (26-31)	44	South Korea	In this study
I. coreana	3	cristate (355-484)	echinate (31-38)	66*	South Korea	Chung and Choi, 1986
I. yunguiensis	3	cristate-reticulate (340-430)	levigate (20-25)	22	China	Wang et al., 2002
I. orientalis	3	reticulate (350-450)	tuberculate-echinate (19-29)	66	China	H. Liu et al. 2005
I. hypsophila	3	reticulate (ca. 320)	perforate (15-18)	22	China	Palmer, 1927
I. sinensis	3	cristate (330-462)	echinate (19-20)	44	China	Palmer, 1927
I. taiwanensis	3 (rarely, 4 or 5)	tuberculate (310-390)	echinate (ca. 25)	22	Taiwan	DeVol, 1972a
I. asiatica	2	echinate (413-563)	levigate (21-33)	22	Japan	Takamiya et al., 1997
I. japonica	3 (rarely, 2)	reticulate (300-563)	levigate (25-38)	66	Japan	Takamiya et al., 1997
I. ×michinokuana	3	reticulate (338-539)	echinate (25-38)	77	Japan	Takamiya et al., 1997
I. pseudojaponica	3	reticulate (375-600)	echinate (26-38)	88	Japan	Takamiya et al., 1997

†Classification of megaspore ornamentation follows that of Hickey (1986).
‡Terminology for microspore ornamentation follows their original descriptions.
*The chromosome number for *I. coreana* was determined in this study as 2n=66 (Photo not shown)

Korean name: Je-ju-mul-bu-chu.

Megasporae rugatae, canae, 325-425 μm diam., hemisphaerium proximalis rugatum, hemisphaerium distalis rugatum, crista radialis undulata, crista aequatoria rudimentaria, ultrastructura densa. Microsporae echinatae, brunneae, 26-32 μm longae. Chromosomatum numerus 2n = 44.

Plant amphibious. Corm 3-lobed, 10-20 mm diameter. Sporophylls ascending or inclined, white basally, green above, half-terete, 9-26 cm long, 1.5-2.5 mm wide at mid-length, base alate. Stomata anomocytic, 58-88 μm long, 28-40 μm wide, with 5-7 peristomatic neighboring cells. Velum absent. Ligule hastiform or sub-triangular, 2-4 mm long, 2-2.5 mm wide. Sporangium wall clear. Megaspore rugulate, grey, 325-425 μm diameter, proximal and distal hemisphere rugulate, radial ridge undulate, equatorial ridge rudimentary, ultrastructure dense. Microspore echinate, brown, 26-32 μm long. Chromosomes: 2n = 44.

Distribution: This species occurs on clay soils in shallow ponds and swamps (elev. ca. 125 m; Fig. 1F) in the low land of Mt. Halla at Bukjeju-gun and Namjeju-gun of Jeju Island, South Korea.

Etymology: The epithet is derived from the name for Jeju Island, the province from which the new species was collected.

Paratype. South Korea. Jeju Island. Namjeju-gun, Namwon-eup, 5 Aug. 2000, *J. Jung 2000154, 2000155* (AJOU).

This species can be distinguished from other East Asian *Isoetes* species by its rugulate megaspore ornamentation.

Isoetes hallasanensis H.-K. Choi, C. Kim & J. Jung, sp. nov. TYPE: South Korea. Jeju Island: Seoguipo city, 33° 21 N, 126° 27 E, ca. 6.7 km W of top of Mt. Halla, elev. 1070 m, 13 Jul. 2006, *H.-K. Choi 20061380* (holotype, AJOU; isotype, AJOU). Figure 2.

Korean name: Hal-la-mul-bu-chu.

Megasporae echinata, canae, 356-464 μm diam., hemisp- haerium proximalis echinatum et tuberculatum, hemisphaerium distalis echinatum, crista radialis et crista aequatoria undulata, ultra-structura fibrosa. Microsporae echinatae, brunneae, 26-31 μm longae. Chromosomatum numerus 2n = 44.

Plant amphibious. Corm 3-lobed, 10-18 mm diameter. Sporophylls ascending, white basally, green above, half-terete, 5-19 cm long, 1.5-2.5 mm wide at mid-length, base alate. Stomata anomocytic, 44-77 μm long, 23-34 μm wide, 5-7 peristomatic neighboring cells. Velum absent. Ligule hastiform or sub-triangular, 3-4 mm long, 1.5-2 mm wide. Sporangium wall clear. Megaspore echinate, grey, 356-464 μm diameter, proximal hemisphere echinate and tuberculate, distal hemisphere echinate, radial- and equatorial ridge undulate, ultrastructure fibrous. Microspore echinate, brown, 26-31 μm long. Chromosomes: 2n = 44.

Distribution: This species grows on sandy-clay or clay soils of shallow streams in a high-mountain area (elev. ca. 1100 m; Fig. 2F) at Seoguipo city on Jeju Island, Korea.

Etymology: The epithet is derived from the name for Mt. Halla, on which the new species was found.

Paratype. South Korea. Jeju Island. Seoguipo city, 3 May 2007, *C. Kim 200784* (AJOU), 30 Aug. 2007, *C. Kim 200798* (AJOU).

This species differ from all other East Asian *Isoetes* except *I. asiatica* because of its echinate megaspores. However, it can be distinguished from *I. asiatica* by its echinate microspores and sporophyll with peripheral fiber strands.

Like all other *Isoetes* species in Korea, *I. jejuensis* and *I. hallasanensis* are extremely rare and highly endangered. Populations of *I. jejuensis* and *I. hallasanensis* have been found at only two and one site, respectively, where they comprise fewer than 20 individuals. These small and isolated communities might be the result of fragmentation and habitat destruction through human activities. Therefore, protection of the remaining plants is essential, and additional populations must be searched for in the future.

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