Molecular phylogeny and the biogeographic origin of East Asian Isoëtes (Isoëtaceae)

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ABSTRACT: Isoëtes L. (Isoëtaceae) is a cosmopolitan genus of heterosporous lycopods containing ca. 200 species being found in lakes, streams, and wetlands of terrestrial habitats. Despite its ancient origin, worldwide distribution, and adaptation to diverse environment, species in Isoëtes show remarkable morphological simplicity and convergence. Allopolyploidy appears to be a significant speciation process in the genus. These characteristics have made it difficult to assess the phylogenetic relationships and biogeographic history of Isoëtes species. In recent years, these difficulties have somewhat been reduced by employing multiple molecular markers. Here, we reconstruct the phylogenetic relationships in East Asian Isoëtes species. We also provide their divergence time and biogeographic origin using a fossil calibrated chronogram. East Asian Isoëtes species are divided into two clades: I. asiatica and the remaining species. Isoëtes asiatica from Hokkaido forms a clade with northeastern Russian and western North American Isoëtes species. In clade I, western North America is the source area for the dispersal of Isoëtes to Hokkaido and northeastern Russia via the Bering land bridge during the late Miocene. The remaining Isoëtes species (I. sinensis, I. yunguiensis, I. hypsophila, I. orientalis, I. japonica, I. coreana, I. taiwanensis, I. jejuensis, I. hallasanensis) from East Asia form a sister group to Papua New Guinean and Australian species. The biogeographic reconstruction suggests an Australian origin for the East Asian species that arose through long-distance dispersal during the late Oligocene.

Keywords: Isoëtes, East Asia, phylogenetic relationship, biogeographic origin, dispersal

Isoëtes L. is a perennial emergent hydrophyte belonging to Isoëtaceae, most of which grow in submerged places at least once in their lifetime (Taylor and Hickey, 1992). It is known that approximately 150 species, or up to 350 species of Isoëtes, are distributed throughout the world (Taylor and Hickey, 1992; Hickey et al., 2003). Recently, however, Troia et al. (2017) classified Isoëtes plants worldwide into 192 taxa consisting of 183 species, seven subspecies, and two varieties.

Isoëtes has attained a distinct phylogenetic position in vascular plants since it first appeared in the Paleozoic Devonian (Taylor and Hickey, 1992; Pigg, 2001). Lycopodiophyta (Lycopodiaceae, Selaginellaceae, Isoëtaceae), including Isoëtes, are clearly differentiated from other monilophytes and spermatophytes due to their distinct structures of vascular bundles and lycophylls (Pryer et al., 2001). According to the molecular phylogeny of vascular plants, Lycopodiophyta is also considered to be a sister group of all other vascular plants (Smith et al., 2006). In Lycopodiophyta, Isoëtes is distinguished from other lycophytes due to the presence of ligules and heterospory features, and it forms a sister group with Selaginellaceae (Taylor et al., 1993; Yatsentyuk et al., 2001).

The morphologies of Isoëtes species have a very simple characteristic by which elongated sporophylls are arranged in a 2-3-lobed corm (Pfeiffer, 1922; Chung and Choi, 1986; Hickey, 1986) (Fig. 1A, B). The ligule (Fig. 1C) at the top of the sporangium protects the sporangium from the beginning

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of plant growth until the maturation of the spores (Sharma and Singh, 1984). Megasporangium includes megaspores and microsporangia with microspores located inside the megaphyll and microphyll bases, respectively (Fig. 1D). Due to the simple external morphology of *Isoëtes* species, identifying taxonomic limitations or analyzing phylogenetic relationships have remained difficult.

The surface ornamentation of megaspore in *Isoëtes* has traditionally been used as an important trait-delimiting sections (Pfeiffer, 1922; Fuchs-Eckert, 1981; Hickey, 1986). In addition, identification of *Isoëtes* species largely rests on megaspore and microspore ornamentation. Thus, they are used as the main diagnostic characters when describing new species (Wang et al., 2002; Choi et al., 2008; Kim et al., 2010a). Although the surface ornamentation of the spores is useful for identifying species, it does not reflect the phylogeny of *Isoëtes* at all. For example, *I. echinospora* complex that shows echinate ornamentation of megaspore, did not form a monophyletic group including all species of the complex (Kim and Choi, 2016). *Isoëtes japonica* distributed in Japan and *I. durieui* distributed in Turkey along the Mediterranean coast and in France are all the reticulate type. However, in previous molecular phylogenetic analyses, these two species show a distant relationship (Larsén and Rydin, 2016; Kim and Choi, 2016). Thus, the morphological features and similarity of the spores in *Isoëtes* are interpreted as a result of convergent evolution. Recently, a variety of molecular markers have been used to identify *Isoëtes* species and to assess phylogenetic relationships (Taylor et al., 2004; Hoot et al., 2006; Kim et al., 2009a, 2010b).

In East Asia, a total of 12 species of *Isoëtes* were reported, with four species in China (*I. sinensis*, *I. yunguiensis*, *I. hypsophila*, *I. orientalis*) and one in Taiwan (*I. taiwanensis*).

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

| Species         | No. of corm lobes | Megaspore (µm) | Microspore (µm) | Chromosome number | Distribution          | Source            |
|-----------------|-------------------|----------------|-----------------|-------------------|-----------------------|-------------------|
| *I. jejuensis*  | 3                 | Rugulate (325–425) | Echinate (26–32) | 2n = 44           | South Korea           | Choi et al. (2008) |
| *I. hallasanensis* | 3            | Echinate (356–464) | Echinate (26–31) | 2n = 44           | South Korea           | Choi et al. (2008) |
| *I. coreana*    | 3                 | Cristate (355–484) | Echinate (31–38) | 2n = 66           | South Korea           | Chung and Choi (1986) |
| *I. yunguiensis*| 3                 | Cristate-reticulate (340–430) | Levigate (20–25) | 2n = 22           | China                 | Wang et al. (2002) |
| *I. orientalis* | 3                 | Reticulate (350–450) | Tuberculate-echinate (19–29) | 2n = 66           | China                 | Liu et al. (2005) |
| *I. hypsophila* | 3                 | Reticulate (ca. 320) | Perforate (15–18) | 2n = 22           | China                 | Palmer (1927)     |
| *I. sinensis*   | 3                 | Cristate (330–462) | Echinate (19–20) | 2n = 44           | China, Japan          | Palmer (1927)     |
| *I. taiwanensis*| 3 (rarely 4-5)    | Tuberculate (310–390) | Echinate (ca. 25) | 2n = 22           | Taiwan               | DeVol (1972)      |
| *I. asiatica*   | 2                 | Echinate (413–563) | Levigate (21–33) | 2n = 22           | Japan                 | Takamiya et al. (1997) |
| *I. japonica*   | 3 (rarely 2)      | Reticulate (300–563) | Levigate (25–38) | 2n = 66           | Japan                 | Takamiya et al. (1997) |
| *I. ×michinokuana* | 3         | Reticulate (338–539) | Echinate (25–38) | 2n = 77           | Japan                 | Takamiya et al. (1997) |
| *I. pseudojaponica* | 3        | Reticulate (375–600) | Echinate (26–38) | 2n = 88           | Japan                 | Takamiya et al. (1997) |

*Length variation of spores in parenthesis.*
five in Japan (I. asiatica, I. japonica, I. ×michinokuana [=I. japonica × I. pseudojaponica], I. pseudojaponica, I. sinensis), and four in Korea (I. japonica, I. coreana, I. jejensis, I. hallasanensis) (Table 1; Palmer, 1927; DeVol, 1972; Chung and Choi, 1986; Takamiya et al. 2002; Lee, 2003; Liu et al., 2005; Choi et al., 2008). As in other regions, the number of lobes of a corm, the sizes and surface structures of megaspores and microspores, and the number of chromosomes have all been used as traits to identify East Asian Isoëtes species (Table 1) (Jung et al., 2009). For example, I. asiatica, which grows in Hokkaido, Japan, is distinguishable from other Isoëtes species in East Asia through its two lobes on the corm. Korean endemic, I. coreana is similar to I. sinensis in that it has cristate megaspores and echinate microspores but differs from this species in terms of the number of chromosomes (Table 1).

Most of the East Asian Isoëtes species are endangered species, and various studies have been carried out at the population level to establish their conservation plants because they are endemic species. Extensive research has been carried out, including studies on the growth environment for the preservation and restoration of endangered species (Wang et al., 2005) as well as studies involving population genetic diversity analyses using various molecular markers such as random amplification of polymorphic DNA, amplified fragment length polymorphism (AFLP), and nucleotide and chloroplast DNA sequences (Kang et al., 2005; Kim et al., 2008, 2009b; Jung et al., 2014). Some of the studies have also been focused on speciation mechanisms of parental species via polyploidization (Kim et al., 2010b), and addressed their phylogenetic relationships and biogeographical origins (Kim and Choi, 2016).

In this paper, we intend to review the phylogenetic relationships among Isoëtes species in East Asia and present their biogeographical origins. We also propose directions for future research based on the phylogenetic relationships and the results of biogeographical studies of East Asian Isoëtes species that have been conducted so far.

**Phylogeny of East Asian Isoëtes**

Isoëtes species are distributed throughout the world and grow in a variety of habitats, such as aquatic, semi-aquatic, and terrestrial environments. Although they have a long evolutionary history, considerable difficulty has arisen when studying phylogenetic relationships using morphological features due to the simplicity, convergent evolution, and speciation by polyploidization associated with these plants (Hickey et al., 1989; Hoot et al., 2004; Kim et al., 2010b). In addition, the Isoëtes taxa are highly endemic due to limited local distributions and obtaining samples can be difficult because they are endangered plants with small populations (Choi et al., 2008; Jung et al., 2009, 2013). Therefore, studies are underway to delimit Isoëtes species taxonomically using various molecular markers or to analyze the phylogenetic relationships among the species (Taylor et al., 2004; Hoot et al., 2006; Kim et al., 2009a, 2010b). In particular, species-specific molecular markers are useful for identifying the taxonomic boundaries of Isoëtes species that contain many endangered plants because they can be used to identify species or to elucidate phylogenetic relationships among the lineages using young or small samples.

Molecular phylogenetic studies using nuclear ribosomal internal transcribed spacer (ITS) sequences have shown that the Isoëtes species divided into three major clades (Fig. 2): (1) The South African species (I. toximontana, I. capensis, I. stellenbosiensis, I. stephansenii) form a monophyletic group (clade I in Fig. 2) and sister to the group consisting of the rest of the Isoëtes species. (2) Species distributed in South America (I. panamensis, I. cubana, I. jamaicensis) form a clade with Indian species (I. coromandelina var. coromandelina) and some species in Australia and Southeast Asia (I. australis, I. coroneliana var. macrotuberculata, I. laosensis) (clade II). (3) The remaining Isoëtes species also form a clade (clade III). Within Clade III, African species form a clade with some species in southern Europe, India (clade IIIa). In particular, the East Asian taxa are closely related to the species in eastern Australia (clade IIIa), forming a sister group with North American species (clade IIIb).

Studies of the Isoëtes in East Asia have been relatively active. Nuclear and chloroplast DNA sequences and AFLP markers have been used to reconstruct the phylogenetic relationships and for parental species identification of the polyploid species in the East Asian Isoëtes species (Taylor et al., 2004; Kim et al., 2009a, 2009b, 2010b, Kim and Choi, 2016). The East Asian Isoëtes species are divided into two clades: a clade containing I. asiatica and a clade containing the remaining East Asian Isoëtes species (Kim et al., 2009a, 2010a). In other words, I. asiatica is closely related to Isoëtes species (I. echinospora, I. maritima) in the Russian Far East and in North America, specifically in Alaska (Kim et al., 2009a), forming a sister group with the Isoëtes species distributed in Papua New Guinea and Australia (Kim et al., 2010b). Therefore, East Asian Isoëtes species can be considered to have at least two independent evolutionary histories.

Meanwhile, a phylogenetic analysis using the nuclear LEAFY gene revealed a parental species of the East Asian Isoëtes
Fig. 2. Reconstruction of phylogeny and historical biogeography of *Isoëtes*. A maximum likelihood tree was generated using 91 nuclear ribosomal ITS regions ($-\text{lnL} = 6368.7$). Ancestral states and distribution patterns were estimated by the Bayesian binary method of S-DIVA. The bar and pie graphs on each node indicate the distribution pattern and probability of the ancestral state, respectively.
species excluding *I. asiatica* (Kim et al., 2010b). In this phylogenetic tree (Fig. 3), East Asian *Isoëtes* plants were divided into two clades. Of these, *I. coreana*, *I. japonica*, and *I. sinensis* were divided into two groups (A and B), respectively, and none of the groups formed a monophyletic group. The first clade included *I. taiwanensis*, *I. coreana* (A, Fig. 3. Bayesian consensus phylogram obtained using the TreeAnnotator from the Bayesian analysis of 72 LEAFY types from 10 *Isoëtes* species in East Asia and Australia. Numbers above nodes indicate support values (maximum parsimony bootstrap/Bayesian posterior probability); – indicates that a node was not retrieved with bootstrap value greater than 50% by MP analysis. (Kim et al., 2010b)
B), *I. jejuensis*, *I. hallasanensis*, *I. coreana* (A), and *I. sinensis* (A). The second clade contained *I. yunguiensis*, *I. coreana* (B), and *I. sinensis* (B). Diploid species, *I. taiwanensis* and *I. yunguiensis*, were located in both clades. In the first clade, *I. coreana* (A) and *I. coreana* (B) were clustered with *I. taiwanensis* and *I. hallasanensis*, respectively. *Isoëtes japonica* (A) showed a close relationship with *I. taiwanensis*, whereas *I. japonica* (B) showed a close relationship with *I. sinensis* (B). This relationship is supported by phylogenetic analyses using chloroplast DNA (Kim et al., 2010b). Therefore, the parental species of *I. coreana* (6 ×) in South Korea were *I. taiwanensis* (2 ×) and *I. hallasanensis* (4 ×), while those of *I. japonica* (6 ×) in Japan were *I. taiwanensis* and *I. sinensis* (4 ×) according to the tree (Fig. 4).

Thus far, the phylogeny of *Isoëtes* species in East Asia has been studied in detail, but there has been little research on taxa in Southeast Asia, India and Australia. Hoot et al. (2006) included only two of the taxa (*I. coromandelina*, *I. australis*) distributed in India and Western Australia in their phylogenetic study, and they did not include *Isoëtes* species in Southeast Asia. Therefore, there is almost no phylogenetic relationship to *Isoëtes* in these areas. Recently, new species of *Isoëtes* have been described among the flora of Southeast Asia and India (Shukla et al., 2005; Kim et al., 2010a; Jung et al., 2013). Among the taxa growing in the Southeast Asian region, *I. philippinensis* growing in the Philippines is closely related to the species in East Asia, and *I. laosiensis* reported in Laos is linked to species distributed in India and in Australia (Fig. 2). Southeast Asia appears to have species from different lineages, and additional phylogenetic studies, including those focusing on taxa from India, Southeast Asia, and northwest Australia, may be necessary.

**Phytogeographical origin of the East Asian *Isoëtes***

*Isoëtes* plants are among the most widely distributed species in the world and have the highest species diversity (45 species) in many areas, including the western parts of Brazil and other areas in South America (Troia et al., 2017). In addition, it was found that species diversity is relatively high in eastern North America (26 species), Southern Europe (19 species), Australia (16 species) and South Africa (14 species). Molecular clocks and phytogeographical analyses using DNA sequences have revealed that *Isoëtes* diverged from Selaginellaceae during the Devonian period (mean=375 million years ago [mya]), and the main crown group of *Isoëtes* diverged during the Jurassic period (mean=147 mya) (Larsén and Rydin, 2016; Pereira et al., 2017). Vicariance has been found to play an important role in the initial diversification of *Isoëtes* species, as the taxa from Australia, South America, India, and Africa included on the Gondwana landmass formed a monophyletic group (Pereira et al., 2017). On the other hand, it has been suggested that *Isoëtes* species distributed in North America and East Asia were formed by long-distance dispersal by means of migratory birds after the Cenozoic period (Taylor and Hickey, 1992; Liu et al., 2004).

Two hypotheses have been proposed regarding the biogeographic history of East Asian *Isoëtes* plants, based on an analysis of the cytological characteristics and the other based on molecular phylogenetic research. Liu et al. (2004), based on the number of chromosomes and on fossil data, suggested that Chinese *Isoëtes* plants migrated eastward from the Qinghai-Tibet region of China (*I. hypsophila* [2 ×] - *I. yunguiensis* [2 ×] - *I. taiwanensis* [2 ×] - *I. sinensis* [4 ×]) and migrated from the highlands to the lowlands through the water system. On the other hand, Hoot et al. (2006) suggested that the *Isoëtes* plants distributed in East Asia/Australia form a monophyletic group based on their nucleotide and chloroplast DNA sequences and that they were spread to East Asia through Australia and New Guinea by migratory birds. However, this study not only performed a DNA analysis with limited samples...
but also had a very low resolution of the phylogenetic relationships among the lineages in the East Asia/Australia clades. They also noted that it is necessary to carry out a more in-depth phylogenetic analysis of East Asian *Isoëtes* using more taxa and high-resolution molecular markers. Recently, Kim and Choi (2016) undertook phylogenetic studies and biogeographical studies of *Isoëtes* plants in East Asia, Australia, Papua New Guinea, the Russian Far East, and North America using nucleotide ITS and three chloroplast DNA ([atpB-rbcL, trnL, trnS-psbC](Clade III a and b in Fig. 2, respectively)). The northern Asian *Isoëtes* species are divided into two major lineages: (1) northern Asian species and (2) the remaining East Asian *Isoëtes* (Clade III a and b in Fig. 2, respectively). The northern Asian *Isoëtes* distributed in Hokkaido migrated through the Bering land bridge from the Alaska region of North America during late Miocene (mean = 11.2 mya). The rest of the East Asian *Isoëtes* species were closely related to the *Isoëtes* plants in Papua New Guinea and Australia suggesting that they have migrated through long-distance dispersal up to the late Oligocene (mean = 25.2 mya). Therefore, East Asian *Isoëtes* species have differentiated more recently than the Gondwana species (Figs. 2 and 5). The disjunction distribution pattern between the northern hemisphere and the southern hemisphere was largely accounted for by migrations between North America and South America and between Europe and Africa at various times during the Cenozoic Tertiary, while the migration path between Australia and East Asia was relatively less supported (Raven and Axelrod, 1974; Iturralde-Vinent and MacPhee, 1999; McLaughlin, 2001; Morley, 2003; Nie et al., 2012). However, biogeographic studies of the East Asian *Isoëtes* suggest the importance of migration routes between Australia and East Asia (Hoot et al., 2006; Kim and Choi, 2016). It will be necessary to confirm the dispersion mechanism of East Asian *Isoëtes* by comparing the results of biogeographical studies between Australia and East Asia with the migration routes of migratory birds (Fig. 5).

### Suggestions for future research on *Isoëtes*

*Isoëtes* species are mostly endangered aquatic plants with limited distributions and small populations, and the establishment of conservation and restoration plans is crucial to secure their biodiversity and genetic diversity. In order to establish a conservation and restoration plan, a clear taxonomic identification of the taxa to be preserved and restored is essential. In addition, with regard to rare plants that are in danger of extinction, it is necessary to develop species-specific molecular markers that can identify species even when only very few samples exist. It is also necessary to grasp evolutionary patterns and migration routes by comparing and analyzing the relationships and geographical distributions among species or of individual species through phylogenetic and biogeographical studies. Phylogenetic studies should be preceded by the establishment of conservation and restoration plans because different conservation and rehabilitation plans are required between taxa representing independent lineages (Fig. 4).

Molecular markers are generally selected by taking into account the resolution of the target taxon and the marker to be analyzed. However, there may be differences in resolutions depending on the phylogeny of each taxonomic group. Therefore, it is of primary importance to select useful molecular markers for phylogenetic and phyllogeographical analyses. In the case of *Isoëtes* species of East Asia and North America, which are known to have undergone relative differentiation more recently, it is necessary to analyze the phylogeny and biogeographical histories with molecular markers at high resolutions. Thus far, phylogenetic and biogeographical studies of *Isoëtes* have been performed using a small number of nuclear and chloroplast DNA sequences (e.g., Hoot et al., 2006; Pereira et al., 2017). The next generation of sequencing (NGS) technology continues to evolve, and analysis costs are becoming lower. Therefore, studies to develop high-resolution molecular markers by comparing nucleotide sequences of whole chloroplast genomes have been active (Park et al., 2016), as have those to elucidate phylogenetic relationships and biogeographical histories (Firetti et al., 2017). However, the entire chloroplast genome of *Isoëtes* plants is only reported for the North American species *I. flaccida* (Karol et al., 2010). In order to understand the phylogeny and evolutionary histories of *Isoëtes* plants distributed around the world, it is necessary to select representative taxa from each clade and analyze the structures and complete nucleotide sequences of the chloroplast genomes using the NGS technique. It is also necessary to understand the precise divergence time and biogeographical history of each clade through a molecular clock analysis (Fig. 5).

The classification system of *Isoëtes* is based on the surfaces ornamentation of megaspores (Pfeiffer, 1922). Pfeiffer (1922) classified megaspores in *Isoëtes* into four types, and Hickey (1986) reorganized these further into 12 categories. Although the molecular phylogeny of *Isoëtes* does not form monophyletic groups according to the shape of each megaspore...
Fig. 5. (A) Ancestral area reconstruction of the North Pacific *Isoëtes* species based on the Bayesian Binary Method (BBM) and on S-DIVA analyses. The BBM ancestral area reconstructions with the highest likelihood levels are denoted by the large circles for each clade and subclade. S-DIVA ancestral area reconstructions are indicated by the boxes at the nodes; two boxes separated by a branch indicate the ancestral ranges inherited by each of the daughter lineages arising from the node. An asterisk indicates a branch with a maximum parsimony bootstrap value of >75% and posterior probability of >0.90. (B) Migration pathway of *Isoëtes* species in the North Pacific region based on BBM and S-DIVA. Biogeographical regions used in BBM and S-DIVA: A, West Beringia; B, western North America; C, East Asia; D, South East Asia; E, Australia-Papua New Guinea; F, eastern North America; G, Mediterranean. Divergence times for each clade and subclade and color key ancestral ranges at different nodes are provided in the figure (Modified from Kim and Choi, 2016).
type (Cox and Hickey, 1984), it would be interesting to determine the evolutionary direction of megaspores. In addition, in *Isoetes*, speciation by polyploidization often occurs after hybridization. It is necessary to resolve the controversy as to whether the evolution of megaspores can be interpreted as a result of speciation by hybridization or whether it is associated with the habitat environment. Furthermore, it is necessary to estimate the timing of the evolution of spores.

**Conflict of Interest**

The authors declare that there are no conflicts of interest.

**Literature Cited**

Choi, H.-K., J. Jung and C. Kim. 2008. Two new species of *Isoetes* (Isoetaceae) from Jeju Island, South Korea. Journal of Plant Biology 51: 354–358.

Chung, Y. H. and H. K. Choi. 1986. *Isoetes coreana*, a new species from Korea. Korean Journal of Plant Taxonomy 16: 1–12. (in Korean with English abstract)

Cox, P. A. and R. J. Hickey. 1984. Convergent megaspore evolution and *Isoetes*. American Naturalist 124: 437–441.

DeVol, C. E. 1972. *Isoetes* found on Taiwan. Taiwania 17: 1–7.

FIRETTI, F., A. R. ZUNTINI, J. W. GAIRARS, R. S. OLIVEIRA, L. G. DE VOL, C. E. 1972. *Gymnosperms and seed plants* of the world: a guide to cultivated and other economically important taxa. Elsevier, Amsterdam.

DeVol, C. E. 1972. *Isoetes* found on Taiwan. Taiwania 17: 1–7.

Firetti, F., A. R. Zuntini, J. W. Gaiarsa, R. S. Oliveira, L. G. DeVol, C. E. 1972. *Gymnosperms and seed plants* of the world: a guide to cultivated and other economically important taxa. Elsevier, Amsterdam.

Hickey, R. J. 1986. *Isoetes* megaspore surface morphology: nomenclature, variation, and systematic importance. American Fern Journal 76: 1–16.

Hickey, R. J., C. Macluf and W. C. Taylor. 2003. A re-evaluation of *Isoetes*. American Fern Journal 93: 126–136.

Hickey, R. J., W. C. Taylor and N. T. Luebke. 1989. The species concept in Pteridophyta with special reference to *Isoetes*. American Fern Journal 79: 78–89.

Hoot, S. B., N. S. Napier and W. C. Taylor. 2004. Revealing unknown or extinct lineages within *Isoetes* (Isoetaceae) using DNA sequences from hybrids. American Journal of Botany 91: 899–904.

Hoot, S. B., W. C. Taylor and N. S. Napier. 2006. Phylogeny and biogeography of *Isoetes* (Isoetaceae) based on nuclear and chloroplast DNA sequence data. Systematic Botany 31: 449–460.

Iturralde-Vincent, M. A. and R. D. E. MacPhee. 1999. Paleogeography of the Caribbean region: implications for cenozoic biogeography. Bulletin of the American Museum of Natural History 238: 1–95.

Jung, J., C. Kim, H. Kim and H.-K. Choi. 2009. Taxonomic examination of *Isoetes* L. (Isoetaceae) in South Korea. Korean Journal of Plant Taxonomy 39: 63–73.

Jung, J., Y. Ryu, H. Won and H.-K. Choi. 2013. Morphological and molecular characterization of a new record of *Isoetes coromandelina* subsp. *coromandelina* from Cambodia. Plant Systematics and Evolution 300: 43–50.

Jung, J., S. K. Singh, H. C. Pande, G. K. Srivastava and H.-K. Choi. 2014. Genetic diversity and population structure of Indian *Isoetes dixtiei* Shende based on amplified fragment length polymorphisms and intron sequences of LEAFY. Aquatic Botany 113: 1–7.

Kang, M., Q. Ye and H. Huang. 2005. Genetic consequence of restricted habitat and population decline in endangered *Isoetes sinensis* (Isoetaceae). Annals of Botany 96: 1265–1274.

Karol, K. G., K. Arumuganathan, J. L. Boone, A. M. Duffy, K. D. E. Everett, J. D. Hall, S. K. Hansen, J. V. Kuehl, D. F. Mandoli, B. D. Mishler, R. G. Olmstead, K. S. Renzaglia and P. G. Wolf. 2010. Complete plastome sequences of *Equisetum arvense* and *Isoetes flaccida*: implications for phylogeny and plastid genome evolution of early land plant lineages. BMC Evolutionary Biology 10: 321.

Kim, C., S. Bounphanmy, B.-Y. Sun and H.-K. Choi. 2010a. *Isoetes laosiensis*, a new species from Lao PDR. American Fern Journal 100: 45–53.

Kim, C. and H.-K. Choi. 2016. Biogeography of North Pacific *Isoetes* (Isoetaceae) inferred from nuclear and chloroplast DNA sequence data. Journal of Plant Biology 59: 386–396.

Kim, C., H. R. Na and H.-K. Choi. 2008. Genetic diversity and population structure of endangered *Isoetes coreana* in South Korea based on RAPD analysis. Aquatic Botany 89: 43–49.

Kim, C., H. R. Na, H. Shin and H.-K. Choi. 2009a. Systematic evaluation of *Isoetes asiatica* Makino (Isoetaceae) based on AFLP, nrITS, and chloroplast DNA sequences. Journal of Plant Biology 52: 501–510.

Kim, C., H. Shin, Y.-T. Chang and H.-K. Choi. 2010b. Speciation pathway of *Isoetes* (Isoetaceae) in East Asia inferred from molecular phylogenetic relationships. American Journal of Botany 97: 958–969.

Kim, C., H. Shin and H.-K. Choi. 2009b. Genetic diversity and population structure of diploid and polyploid species of *Isoetes* in East Asia based on amplified fragment length polymorphism markers. International Journal of Plant Sciences 170: 496–504.
Larsén, E. and C. Rydin. 2016. Disentangling the phylogeny of *Isoëtes* (Isoetales) using nuclear and plastid data. *International Journal of Plant Sciences* 177: 157–174.

Lee, T. B. 2003. *Coloured Flora of Korea*. Hyangmunsa, Seoul, Vol. 1, 914 pp, Vol. 2, 910 pp.

Liu, H., Q.-F. Wang and W. C. Taylor. 2005. *Isoëtes orientalis* (Isoëtaceae), a new hexaploid quillwort from China. *Novon* 15: 164–167.

Liu, X., W. R. Gituru and Q.-F. Wang. 2004. Distribution of basic diploid and polyploidy species of *Isoetes* in East Asia. *Journal of Biogeography* 31: 1239–1250.

McLoughlin, S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* 49: 271–300.

Morley, R. J. 2003. Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 5–20.

Nie, Z.-L., H. Sun, S. R. Manchester, Y. Meng, Q. Luke and J. Wen. 2012. Evolution of the intercontinental disjunctions in six continents in the *Ampelopsis* clade of the grape family (Vitaceae). *BMC Evolutionary Biology* 12: 17.

Palmer, T. C. 1927. A Chinese *Isoëtes*. *American Fern Journal* 17: 111–113.

Park, H., C. Kim, Y.-M. Lee and J.-H. Kim. 2016. Development of chloroplast microsatellite markers for the endangered *Maianthemum bicolor* (Asparagaceae s.l.). *Applications in Plant Sciences* 4: 160032.

Pereira, J. B. S., P. H. Labiak, T. Stützel and C. Schulz. 2017. Origin and biogeography of the ancient genus *Isoëtes* with focus on the Neotropics. *Botanical Journal of the Linnean Society* 185: 253–271.

Pfeiffer, N. E. 1922. *Monograph of the Isoetaceae*. *Annals of the Missouri Botanical Garden* 9: 79–233.

Pigg, K. B. 2001. Isoetalean lycopsid evolution: from the Devonian to the present. *American Fern Journal* 91: 59–114.

Pryer, K. M., H. Schneider, A. R. Smith, R. Cranfill, P. G. Wolf, J. S. Hunt and S. D. Sipes. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* 409: 618–622.

Raven, P. H. and D. I. Axelrod. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* 61: 539–673.

Sharma, B. D. and R. Singh. 1984. The ligule in *Isoëtes*. *American Fern Journal* 74: 22–28.

Shukla, P. K., G. K. Srivastava, S. K. Shukla and P. K. Rajagopal. 2005. Two new species of the genus *Isoetes* L. (Isoetaceae-Lycopsida) from India. *Taxon* 54: 109–116.

Smith, A. R., K. M. Pryer, E. Schuettelz, P. Korall, H. Schneider and P. G. Wolf. 2006. A classification for extant ferns. *Taxon* 55: 705–731.

Takamiya, M. 2001. *Isoëtes sinensis var. sinensis* in Korea (Isoëtaceae: Pteridophyta). *Fern Gazette* 16: 169–170.

Takamiya, M., M. Watanabe and K. Ono. 1997. Biosystematic studies on the genus *Isoëtes* (Isoëtaceae) in Japan. IV. Morphology and anatomy of sporophytes, phytogeography and taxonomy. *Acta Phytotaxonomica et Geobotanica* 48: 89–122.

Taylor, W. C. and R. J. Hickey. 1992. Habitat, evolution, and speciation in *Isoetes*. *Annals of the Missouri Botanical Garden* 79: 613–622.

Taylor, W. C., A. R. Lekschas, Q. F. Wang, X. Liu, N. S. Napier and S. B. Hoot. 2004. Phylogenetic relationships of *Isoëtes* (Isoëtaceae) in China as revealed by nucleotide sequences of the nuclear ribosomal ITS region and the second intron of a *LEAFY* homolog. *American Fern Journal* 94: 196–205.

Taylor, W. C., N. T. Luebke, D. M. Britton, R. J. Hickey and D. F. Brunton. 1993. *Isoetaceae Reichenbach*. In *Flora of North America*, Vol. 2. Pteridophytes and Gymnosperms. Morin, N. R. (eds.), Oxford University Press, New York. Pp. 64–75.

Troia, A., J. B. Pereira, C. Kim and W. C. Taylor. 2017. The genus *Isoëtes* (Isoetaceae): a provisional checklist of the accepted and unresolved taxa. *Phytotaxa* 277: 101–145.

Wang, J.-Y., R. W. Gituru and Q.-F. Wang. 2005. Ecology and conservation of the endangered quillwort *Isoëtes sinensis* in China. *Journal of Natural History* 39: 4096–4079.

Wang, Q.-F., X. Liu, W. C. Taylor and Z.-R. He. 2002. *Isoëtes yan-guensis* (Isoëtaceae), a new basic diploid quillwort from China. *Novon* 12: 587–591.

Yatsentyuk, S. P., K. M. Valiejo-Roman, T. H. Samigullin, N. Wilkström and A. V. Troitsky. 2001. Evolution of Lycopodiaceae inferred from spacer sequencing of chloroplast rRNA genes. *Russian Journal of Genetics* 37: 1068–1073.
동아시아 물부추속 식물의 분자계통 및 식물지리학적 기원에 대한 고찰

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적 요: 물부추속(Isoëtes L.)은 물부추과(Isoëteaceae)에 속하는 이형포자성을 보이고 다년생 정수성 수생식물로, 전 세계에 200여종이 분포하는 것으로 알려져 있다. 물부추속 식물은 고생대 말기에 출현하여 오랜 진화적인 역사를 지닌다. 다양한 생육환경에서 광범위하게 분포하고, 분포 지역에서는 많은 종들이 높은 고유성을 보임으로서 발생위기종으로 보호되고 있다. 오랜 진화과정에서 극도의 수렴화학과 자가배수체 형성과 경을 거치면서 고유적으로 매우 단순화되었다. 이로 인하여 이 식물의 형태학적 특성을 이용한 계통학적 연구와 유연관계의 규명에 많은 어려움을 보여주고 있다. 본 연구에서는 분자계통학적 마커를 이용하여 동아시아에 분포하는 물부추속의 계통학적 유연관계를 파악하고, 분자계통을 이용하여 이들의 식물지리학적 기원 및 분화시기를 조사한다. 분자마커로서 핵과 염색체 DNA의 염기서열을 이용한 분자계통학적 연구결과, 동아시아 물부추속은 크게 두 개의 분계군으로 구분된다. 일본 홋카이도에 분포하는 북방계분계군이 나머지 물부추속 식물과는 동아시아 분계군으로 구분된다. 북방계인 아시아물부추(Isoëtes asiatica)는 북동러시아와 북미의 북서부지역의 물부추속 식물과 깊은 유연관계를 보인다. 이 분계군은 북미의 알래스카 지역에서 베링육교(Bering land bridge)를 통해 중신세후기(late Miocene)에 시베리아로 전파된 것으로 분석되었다. 나머지 동아시아 물부추속 식물분계군(Isoëtes sinensis, I. yunguensis, I. hypsophila, I. orientalis, I. japonica, I. coreana, I. taiwanensis, I. jejuensis, I. hallasanensis)은 파푸아뉴기니와 호주의 물부추속 식물들과 밀접한 유연관계를 보인다. 이들은, 점신세 후기(late Oligocene)에 호주 대륙의 동부 지역으로부터 원거리 산포과정(long-distance dispersal)을 통해 이동되어진 것으로 추정되었다. 향후에 차세대 염기서열 분석(next generation sequencing)과 같은 대규모 유전자 분석법을 이용하여 유용한 분자마커들을 개발하게 되면 전 세계에 분포하는 물부추속 식물에 대한 전반적인 계통지리학적 분석과 각 대륙에 고유종으로 분포하고 있는 이들의 진화적인 역사를 규명할 수 있을 것으로 보인다.

주요어: 물부추속, 동아시아, 계통유연관계, 생물지리학적 기원, 산포