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 *Isoetes* species, identifying taxonomic limitations or analyzing phylogenetic relationships have remained difficult.

Fig. 1. Habit of *Isoetes coreana* Chung & Choi (Cham-mul-bu-chu) from Korea: (A) Sporophylls, (B) Comb lobes, (C) Ligule, and (D) Megasporangia (Chung and Choi, 1986; Choi et al., 2008).

The surface ornamentation of megaspore in *Isoetes* has traditionally been used as an important trait-delimiting sections (Pfeiffer, 1922; Fuchs-Eckert, 1981; Hickey, 1986). In addition, identification of *Isoetes* 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 *Isoetes* 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). *Isoetes japonica* distributed in Japan and *I. duriei* 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 *Isoetes* are interpreted as a result of convergent evolution. Recently, a variety of molecular markers have been used to identify *Isoetes* 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 *Isoetes* 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 *Isoetes* species from East Asia.

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

^aLength 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. jejuensis*, *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. coromandelina* var. *macrotuberculata*, *I. laosiensis*) (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 IIIc). 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 *Isoetes*. A maximum likelihood tree was generated using 91 nuclear ribosomal ITS regions ($-lnL = 6368.7$). 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.

Fig. 3. Bayesian consensus phylogram obtained using the TreeAnnotator from the Bayesian analysis of 72 *LEAFY* types from 10 *Isoetes* 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)

species excluding *I. asiatica* (Kim et al., 2010b). In this phylogenetic tree (Fig. 3), East Asian *Isoetes* 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. 4. A model of speciation of seven East Asian *Isoetes* species involving autopolyploid and allopolyploid speciation based on phylogenetic analyses of the sequences of nuclear and chloroplast genome (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. *Isoetes 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 *Isoetes* 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 *Isoetes* species in Southeast Asia. Therefore, there is almost no phylogenetic relationship to *Isoetes* in these areas. Recently, new species of *Isoetes* 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 *Isoetes*

Isoetes 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 *Isoetes* diverged from Selaginellaceae during the Devonian period (mean=375 million years ago [mya]), and the main crown group of *Isoetes* 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 *Isoetes* 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 *Isoetes* 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 *Isoetes* plants, one 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 *Isoetes* 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 *Isoetes* 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 *Isoetes* using more taxa and high-resolution molecular markers. Recently, Kim and Choi (2016) undertook phylogenetic studies and biogeographical studies of *Isoetes* 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*) sequences. As mentioned above, the East Asian *Isoetes* species are divided into two major lineages: (1) northern Asian species and (2) the remaining East Asian *Isoetes* (Clade III a and b in Fig. 2, respectively). The northern Asian *Isoetes* in the Russian Far East and in Siberia are closely related to *Isoetes* plants in western North America and Alaska. Therefore, it has been suggested that *I. asiatica* 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 *Isoetes* species were closely related to the *Isoetes* 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 *Isoetes* 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; McLoughlin, 2001; Morley, 2003; Nie et al., 2012). However, biogeographic studies of the East Asian *Isoetes* 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 *Isoetes* 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 *Isoetes*

Isoetes 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 phylogeographical analyses. In the case of *Isoetes* 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 *Isoetes* 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 biogeographic histories (Firetti et al., 2017). However, the entire chloroplast genome of *Isoetes* 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 *Isoetes* 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 biogeographic history of each clade through a molecular clock analysis (Fig. 5).

The classification system of *Isoetes* is based on the surfaces ornamentation of megaspores (Pfeiffer, 1922). Pfeiffer (1922) classified megaspores in *Isoetes* into four types, and Hickey (1986) reorganized these further into 12 categories. Although the molecular phylogeny of *Isoetes* does not form monophyletic groups according to the shape of each megaspore

Fig. 5. (A) Ancestral area reconstruction of the North Pacific *Isoetes* 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 *Isoetes* 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 *Isoëtes*, 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.

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동아시아 물부추속 식물의 분자계통 및 식물지리학적 기원에 대한 고찰

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아주대학교 생명과학과, ¹동북아생물다양성연구소, ²한국수자원공사, ³가천대학교 생명과학과

적 요: 물부추속(*Isoetes* L.)은 물부추과(Isoëteaceae)에 속하는 이형포자성을 보이는 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 멸종위기종으로 보호되고 있다. 오랜 종분화 과정에서 극도의 수렴진화와 자가배수체 형성과정을 거치면서 형태적으로 매우 단순화되었다. 이로 인하여 이 식물군의 형태적인 형질을 이용한 계통학적 연구와 유연관계의 규명에 많은 어려움을 보여주고 있다. 본 연구에서는 분자계통학적 마커를 이용하여 극동아시아에 분포하는 물부추속의 계통학적 유연관계를 파악하고, 분자시계를 이용하여 이들의 식물지리학적 기원 및 분화시기 등에 대해서 알아보려고 하였다. 분자마커로서 핵과 엽록체 DNA의 염기서열을 이용한 분자계통학적 연구결과, 동아시아 물부추속은 크게 두 개의 분계군으로 구분된다: 일본 홋카이도에 분포하는 북방계분계군과 나머지 물부추속 식물을 포함하는 동아시아 분계군으로 구분이 된다. 북방계인 아시아물부추(*Isoetes asiatica*)는 극동러시아와 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(*Isoetes sinensis*, *I. yunguiensis*, *I. hypsophila*, *I. orientalis*, *I. japonica*, *I. coreana*, *I. taiwanensis*, *I. jejuensis*, *I. hallasanensis*)은 파푸아뉴기니아와 호주의 물부추속 식물과 밀접한 유연관계를 보인다. 이들은, 점진세 후기(late Oligocene)에 호주 대륙의 동부 지역으로부터 원거리 산포과정(long-distance dispersal)을 통해 이동되어진 것으로 추론되었다. 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, 생물지리적 기원, 산포