



Identification of three independent fern gametophytes and *Hymenophyllum wrightii* f. *serratum* from Korea based on molecular data

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ABSTRACT: Colonies of three independent gametophytes (one that is filamentous and two that are ribbon-like) without sporophytes occur in Gyeonggi-do, Gangwon-do, Gyeongsang-do, and Jeju-do, Korea. They have a moss-like appearance at first sight, with tiny plantlets and gemmae, and grow in cool, shaded, relatively deep dint places of large rocks, such as the small caves in high mountains, close to valleys. The gametophytes were identified based on morphological and molecular data by chloroplast DNA (cpDNA) sequence data (*rbcL*, *rps4* gene and *rps4-trnS* intergenic spacer). Here, *rbcL*, *rps4* gene and *rps4-trnS* intergenic spacer data of one independent gametophyte distributed in Korea have the same morphology, DNA sequence and monophyletic group as *Crepidomanes intricatum* from the eastern United States. They also share the same cpDNA data with *Crepidomanes schmidtianum* recently reported from Korea. The other independent gametophyte should be *Hymenophyllum wrightii* based on cpDNA data. The last one was presumed to be *Pleurosoriopsis makinoi* based on molecular data. The taxonomic status was confirmed to be the forma of *Hymenophyllum wrightii* through a revision of *Hymenophyllum wrightii* f. *serratum* based on molecular data.

Keywords: independent gametophytes, *Hymenophyllum wrightii* f. *serratum*, cpDNA, molecular data

Ferns as seed plants have life cycles of two completely free-living phases: a long-lived sporophytes phase and shorter-lived gametophytes phase. This is in contrast to seed plants, which comprise of a few less visible short-lived gametophytes protected within sporophytes. Although most ferns follow the standard alternation of generations cycle closely, the independence of these two life stages allows for both the gametophyte and sporophyte to explore novel ecological niches (Sato and Sakai, 1981) and life history strategies (Dassler and Farrar, 2001). In some ferns, however, if their environment is poor, the gametophyte phase will mainly be in the asexual stage, and the sporophyte stage, as a diploid sexual stage will be rare or possibly non-existent.

In many epiphytic fern species, the gametophyte is capable of vegetative growth, and some can reproduce asexually via gemmae. An epiphytic habitat presumably provides advantages

to persistence through vegetative growth and branching, often producing bryophyte-like colonies (Ebihara et al., 2008; Farrar et al., 2008). Those noncordiform gametophytes characters producing gemmae without producing sporophyte have increased the independent gametophytes as separation of generation (Pinson et al., 2016).

These independent gemmiferous fern gametophytes in filmy ferns (Hymenophyllaceae), vittarioid ferns (Pteridaceae), and grammatid ferns (Polypodiaceae) are usually found in temperate zones from the eastern United States, northeastern Canada, Europe, and Japan (Farrar, 1967, 1985, 1990, 1992; Rumsey et al., 1998; Krukowski and Swierkoz, 2004; Ebihara et al., 2008; Duffy et al., 2015; Pinson et al., 2016; Ebihara et al., 2019). Such independent gametophytes are limited to temperate zones as a hypothesized stable vegetation that appeared to be less affected by climate change during

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glaciations (Harrison et al., 2001). Nevertheless, these independent gametophytes of two vittarioid ferns were recently discovered in nontemperate regions, subtropical Taiwan (Kuo et al., 2017).

During field work in 2014, new bryophyte-like colonies of independent gametophyte (one filamentous) growing without any sporophytes were firstly found on a deep dint place of large rock in a small cave high in Hwayasan Mt. in Gyeonggi-do province, Korea, on February 2014. They had a moss-like appearance at first sight and possessed gemmae. The colonies were distributed broadly on a relatively deep dint place of a large rock in a small cave in the high mountain, which was around valley. To find other colonies of the independent gametophytes, similar habits of high mountains in Gangwon-do, Gyeonggi-do, Gyeongsang-do, and Jeju-do were surveyed from 2014 until 2019. Another independent gametophyte (ribbon-like) was found on deep dint place of a large rock in a province from Korea. These independent gametophytes in Korea were first recorded by Lee and Lee (2015, 2018) as *Crepidomanes schmidtianum*, *Hymenophyllum wrightii*, and *Pleurosoriopsis makinoi* based on the morphology. Park et al. (2020) found independent gametophyte of *Antrophyum obovatum* only in Jeju Island from Korea.

The Sporophytes of *Crepidomanes schmidtianum* were distributed in Mt. Jirisan, Korea as the first recorded species (Lee et al., 2014a). The genus *Crepidomanes* was treated as a single large genus (Morton, 1968) as *Trichomanes* or split into several smaller genera (Ebihara et al., 2006) as *Crepidomanes*. Ebihara et al. (2008) compared the chloroplast genomes between the sporophyte-less filmy fern *T. intricatum* in the eastern United States and *C. schmidtianum* in Japan to investigate their relationship based on the *rbcL* sequences. As the result, they presumed that *T. intricatum* should be treated as a synonym of *C. schmidtianum* (Ebihara et al., 2008). Some authors identified it as *C. intricatum* (Duffy et al., 2015; Pinson et al., 2016).

These findings suggest that climate change during Pleistocene resulted in ecophysiological maladaptation of nontemperate in dependent gametophyte ferns, resulting in postzygotic sterility by eliminating their sporophytes (Kuo et al., 2017).

Identifying ferns to genus and species level based on gametophyte morphology is difficult because of the fern gametophyte size and cryptic morphology (Schneider and Schuettpelz, 2006; Watkins et al., 2007; Li et al., 2009). Nevertheless, identifying the genus or subgenus is often possible in taxonomic groups from the gametophyte morphologies (Farrar et al., 2008). Recent studies of

gametophyte ecology applied molecular methods to conserve the new insights (Ebihara et al., 2008; Duffy et al., 2015; Pinson et al., 2016; Kuo et al., 2017).

To identify the independent gametophytes and verify their presence in Korea, it was an analysis of morphological characteristics and molecular data was carried out using the chloroplast DNA (cpDNA) sequence of *rbcL* and *rps4-trnS* (*rps4* gene and *rps4-trnS* intergenic spacer [IGS]) regions of cpDNA.

Hymenophyllum wrightii f. *serratum* C. S. Lee & K. Lee (Hymenophyllaceae) was published as a new taxon of *Hymenophyllum wrightii* based on their smaller leaves, broader basal part of the leaf blade, broad-ovate laminae, larger sori, and serrate margins of lips of involucre (Lee et al., 2014b). The taxonomic status of *Hymenophyllum wrightii* f. *serratum* was inferred based on an analysis of the cpDNA data of *rbcL* and *rps4-trnS* (*rps4* gene and *rps4-trnS* IGS) regions of this taxon with the related taxa.

Materials and Methods

The sources of plant materials for morphology and DNA analysis and GenBank accession numbers are listed in Appendix 1. All leaf materials were obtained from natural populations in Korea, 100–200 gametophyte cells or 1 cm² of sporophyte leaf tissue was used for DNA extraction with a DNease Plant Mini Kit (Ebihara et al., 2008). A total of 14 accessions for *rbcL* sequences and 14 accessions for *rps4-trnS* (*rps4* gene and *rps4-trnS* IGS) sequence data were newly analyzed (Appendix 1), and outgroup and several ingroup data (34 accessions) were obtained from GenBank.

In order to identify three independent gametophytes (*Crepidomanes schmidtianum*, *Hymenophyllum wrightii*, and *Pleurosoriopsis makinoi*), it was added newly 10 accessions from Korea and 3 accessions from DNA bank (Ebihara et al., 2008; Duffy et al., 2015) for cpDNA sequence of *rbcL* and *rps4-trnS* (*rps4* gene and *rps4-trnS* IGS) regions of cpDNA. In comparing the molecular analysis, the sporophytes of three taxa was added newly 4 accessions sampled from Korea, and 10 accessions gotten from DNA bank (Hasebe et al., 1994; Ebihara et al., 2007, 2008; Kim et al., 2013). And we got newly 5 accessions and 16 accessions from DNA bank (Hasebe et al., 1994; Schneider et al., 2004; Kreier and Schneider, 2006; Ebihara et al., 2007, 2010; Wang et al., 2010; Nitta et al., 2011), which examined from one taxon of *Polypodium*, two taxa of *Pyrrhosia*, one taxon of *Colysis*, and two taxa of *Lepisorus* for outgroup.

Moreover, to evaluate the status of *Hymenophyllum wrightii*

f. *serratum*, we sampled 4 accessions from Jeju-do, Korea. In comparing the molecular analysis, we aligned with the related taxa, above mentioned *H. wrightii*, newly 1 accession of *H. polyanthos*, 4 accessions of *H. barbatum* from Korea, and 5 accessions gotten from DNA bank (Hasebe et al., 1994; Kim et al., 2013; Vasques et al., 2019).

Total genomic DNA was extracted using the DNeasy plant mini kit (Qiagen Inc., Valencia, CA, USA). Primers used for amplification and sequencing were *rbcl*-TKT-F1 and *rbcl*-TKT-R3N-2 for the *rbcl* gene (Ebihara et al., 2005), *trnS* (TACCGAGGGTTCGAATC) (Souza-Chies et al., 1997; Smith and Cranfill, 2002), the *rps4F1* (RPSF1 GCCGCTAGACAATT AGTCAATC) (Hennequin et al., 2003) for part of the *rps4* gene and *rps4-trnS* IGS (Schneider et al., 2004). Amplification was conducted using a PTC-100 thermal cycler (MJ Research, Watertown, MA, USA) with the following temperature profile for all regions: a 32 to 37 cycle reaction with denaturalization at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 2 to 3 min. In addition, an initial denaturalization at 94°C for 2.5 min and a final extension at 72°C for 10 min were performed. PCR products were purified with AccuPrep PCR Purification Kit (Bioneer Inc., Daejeon, Korea). Automated sequencing analysis was performed using a sequencer (Base station, MJ Research).

Nucleotide sequences of cpDNA regions were each aligned with default alignment parameters, using the Clustal X program (Thompson et al., 1997) with final manual adjustment (Gibson et al., 1994; Thompson et al., 1997). The two data matrices were analyzed initially using maximum parsimony (MP) by PAUP* (Swofford, 2002), treating gaps with missing values, and later in separate analyses. Heuristic MP searches were replicated 1000 random stepwise addition of taxa, tree-bisection-reconnection (TBR) branch swapping, and saving multiple trees. Bootstrap values (Felsenstein, 1985) were calculated from 100 replicate analyses using TBR branch swapping and simple stepwise addition of taxa.

Results and Discussion

Morphology and distribution of three independent gametophytes

The natural habits of independent gametophytes of *Crepidomanes schmidtianum*, *Hymenophyllum wrightii*, and *Pleurosoriopsis makinoi* are the relatively deep dint place of large rocks, such as small caves in the high mountains around a valley, as shown in Fig. 1. They have a moss-like appearance at first sight and have gemmae. The filamentous gametophyte type has been observed in *C. schmidtianum*, and ribbon like gametophyte morphology has been observed in *H. wrightii* and *Pleurosoriopsis makinoi* (Table 1). The gemmae shape of *H. wrightii* is linear, *C. schmidtianum* has filamentous linear gemmae. *P. makinoi* has round or oblong gemmae (Fig. 1). Among these three taxa, many rhizoids were observed only in *H. wrightii*.

The filamentous type, gametophytes of *Crepidomanes schmidtianum* were found in the deep dint place of moist rocks, such as small caves of high mountains around valley: Hwayasan Mt. in Gyeonggi-do province and Seolarksan Mt., Taebaeksan Mt. in Gangwon-do from Korea (Fig. 2). This independent gametophyte in Korea has the same morphology as the sporophyte-less, independent gametophytes of *Trichomanes intricatum*, which is treated as the synonym of *C. schmidtianum*, and is distributed widely in the eastern United States (Farrar, 1990; Ebihara et al., 2008). The sporophytes of *C. schmidtianum* were collected from forests in Jirisan Mt., Baegmu-dong, Macheon-myeon, Gyeongsangnam-do from Korea. The sporophytes of this taxon are distributed from Himalaya region to Japan (Iwatsuki, 1985). In the eastern United States, this taxon has been producing sporophytes only as occasional sterile juveniles or not producing sporophytes at all (Ebihara et al., 2008). Interestingly, *C. schmidtianum* is distributed far apart in Korea alone, both in form of independent gametophytes and sporophytes.

Table 1. Morphological characters and distributions of three independent gametophytes.

Characters	<i>Crepidomanes schmidtianum</i>	<i>Hymenophyllum wrightii</i>	<i>Pleurosoriopsis makinoi</i>
Gametophytes morphology	Filamentous	Ribbon like	Ribbon like
Gemmae shape	Filamentous linear	Linear	Round or oblong
Rhizoids	Absent	Many present	A little present
Distribution of independent gametophytes	USA, Korea	Northwestern America (Washington, and Alaska of USA, British Columbia of Canada), Jeju Island of Korea	Japan, Korea
Distribution of sporophytes	East-central Asia	Northwestern America (Haida Gwaii, British Columbia of Canada), East Asia (Jeju Island of Korea; Japan; Far east of Russia)	East Asia

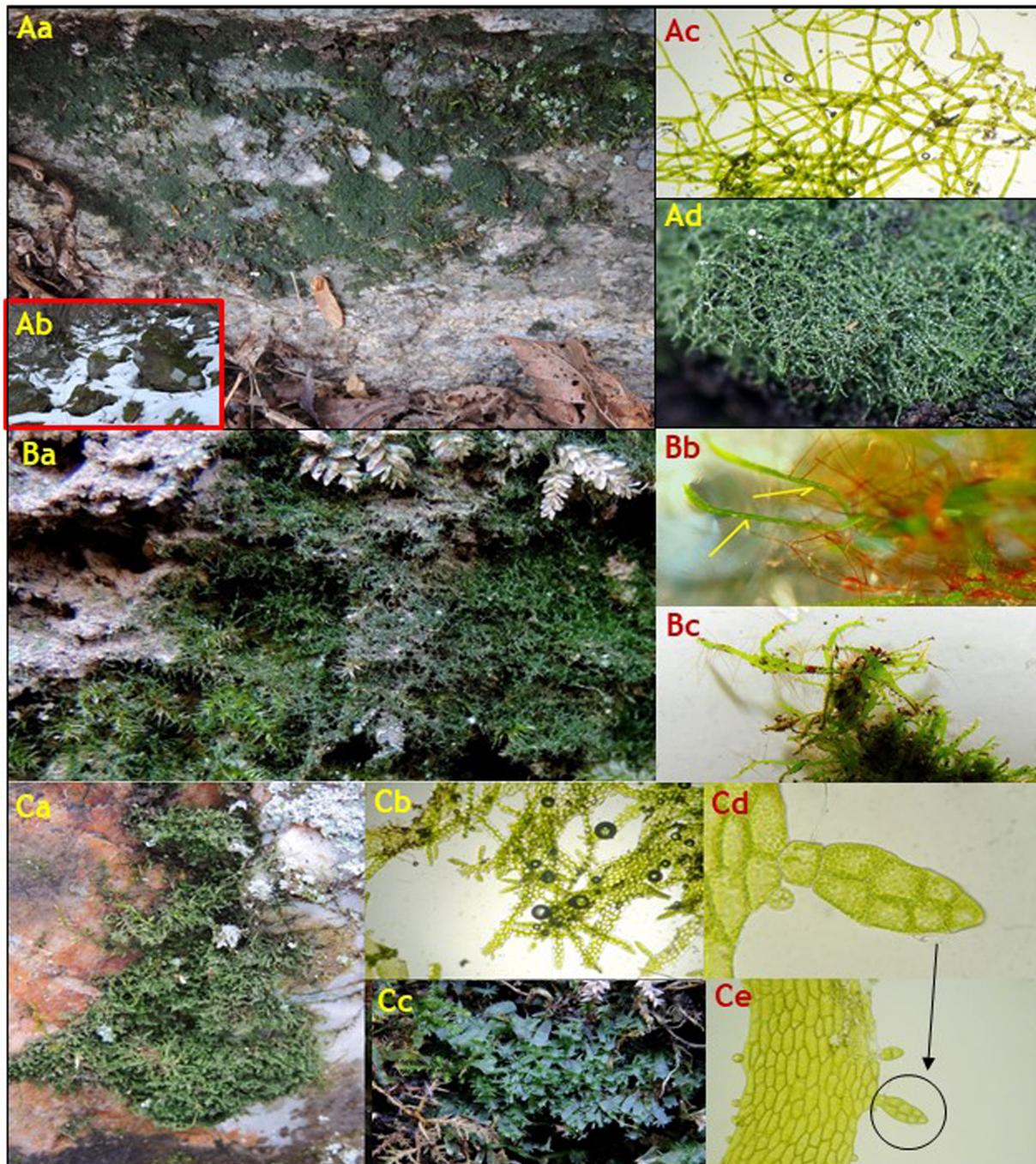


Fig. 1. Photographs of colonies of ribbon like or filamentous independent gametophytes. **A.** *Crepidomanes schmidtianum* distributed on the rock at the high mountain, the site 500 m height of Gangwon-do in Korea. a, b. Habit. c, d. Filamentous independent gametophytes. **B.** *Hymenophyllum wrightii* distributed on the rock of Jeju-do. a. Habit. b, c. Ribbon like independent gametophytes. **C.** *Pleurosoriopsis makinoi* distributed on the rock of Gyeonggi-do. a, c. Habit. b. Ribbon like independent gametophytes. d, e. Microscopic view of gametophyte with gemmae.

Ribbon like gametophytes of *Hymenophyllum wrightii* were collected in Jeju Island in this study (Fig. 2). Park et al. (2020) collected them from seven sites in Jeju Island located at altitudes ranging from 200 m to 700 m. The sampling in

the present study contained this species. The gametophytes of *H. wrightii* were not present, and sporophytes were found only in Japan (Ebihara, 2016; Park et al., 2020). On the other hand, the species in Northwestern America have been reported to

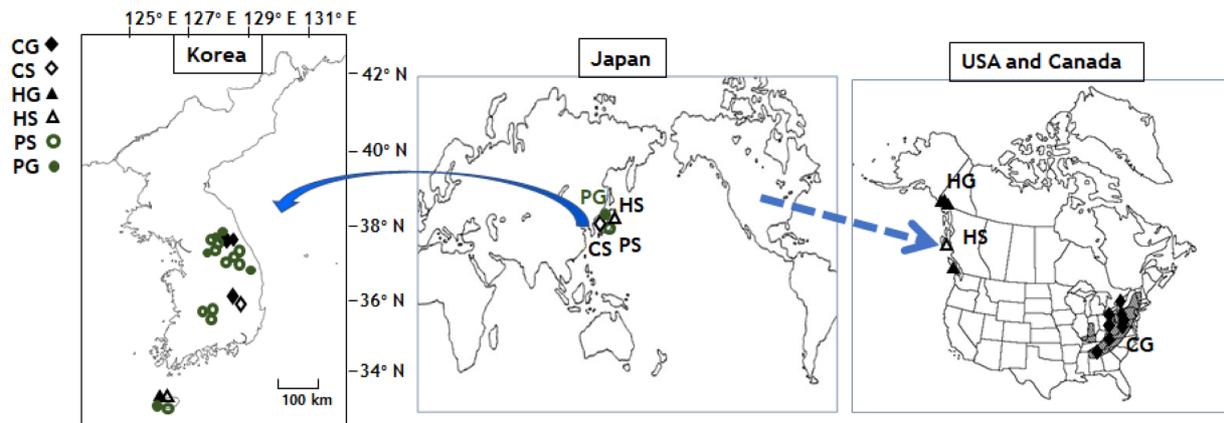


Fig. 2. Map of three independent gametophytes and sporophytes, *Crepidomanes schmidtianum* (= *C. intricatum*) (C), *Hymenophyllum wrightii* (H), and *Pleurosoriopsis makinoi* (P). G, independent gametophytes; S, sporophyte.

have a wide distribution from Washington State up to parts of British Columbia and Alaska (Duffy et al., 2015). Sporophytes of *H. wrightii* is distributed on rocks or tree trunks in wet forests in Korea, Japan, and Russia (Sakhalin) of east Asia, and are found on dark, shady, wet cliffs near sea level of coast in of Northwestern Canada (Duffy et al., 2015; Lee and Lee, 2015, 2018; Ebihara, 2016). Unlike the wide distribution of gametophytes, the sporophytes of *H. wrightii* were rarely found in Gyeongsangnam-do and Jeju Island of Korea as in Haida Gwaii of Northwest Canada (Duffy et al., 2015; Lee and Lee, 2018). In Japan, the sporophytes of *H. wrightii* were distributed widely without independent gametophytes (Ebihara, 2016). The gametophytes of *H. wrightii* were distributed in Jeju Island, Korea, located near sporophytes. In North America, however, the more widely distributed gametophytes of this taxon in the lowland coast of Northwest America were located far from sporophytes (Duffy et al., 2015). Park et al. (2020) suggested that *H. wrightii* spreads from Japan to northeast Asia and North America, but environmental changes, such as aridity, temperature, or light conditions (Makgomol and Sheffield, 2001; Ebihara et al., 2019) suppress the growth of sporophytes. Although this explanation appears reasonable, a detailed study based on environmental factors or biogeographic level will be needed to confirm the rapid disappearance of sporophytes and only the presence of gametophytes.

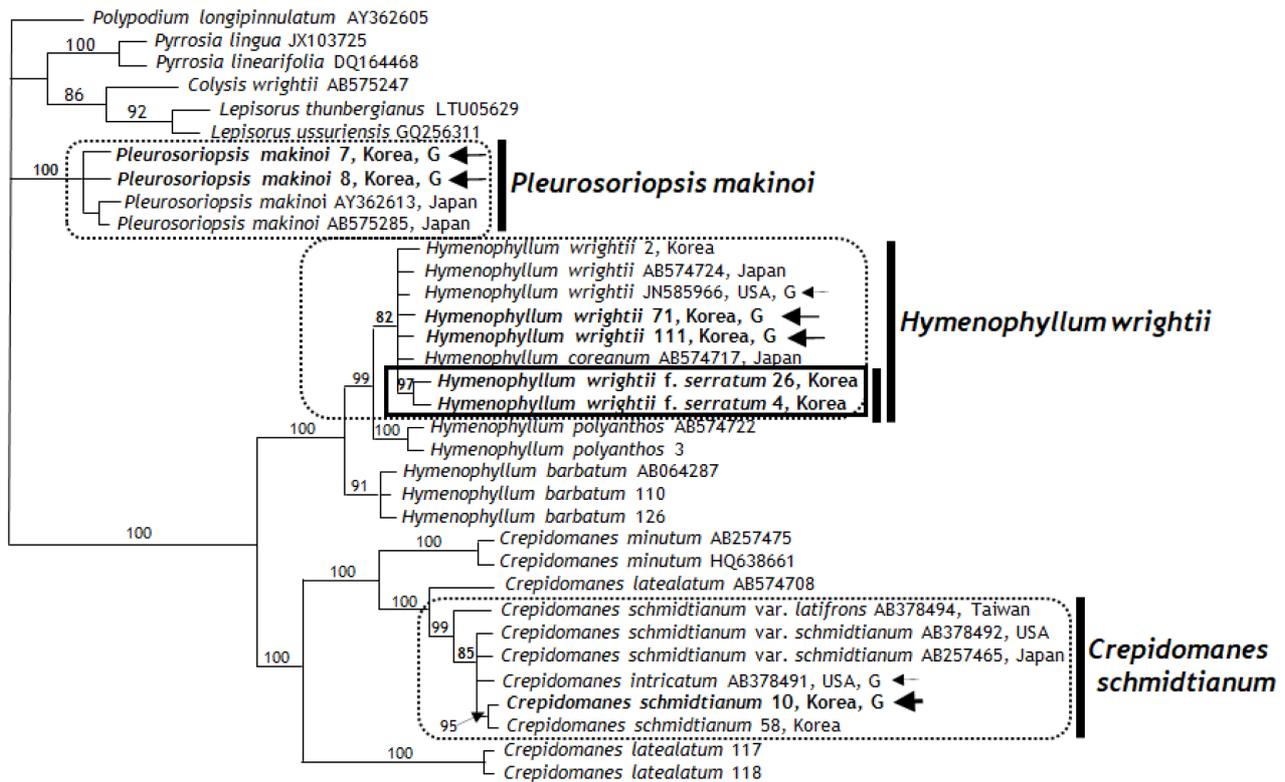
Ribbon like gametophytes of *Pleurosoriopsis makinoi* were found in on deep dint place of moist rock, such as small caves in humid mountains around a valley in Sangsan Mt., Kwangdeoksan Mt. in Gyeonggi-do province and Seolarksan Mt., Taebaekwsan Mt. in Gangwon-do, and Jeju-do from Korea (Fig. 2). Ebihara et al. (2019) reported that the gametophytes of *P. makinoi* were found occasionally in Unazawa Valley,

Okutama-machi, Tokyo, Japan without sporophytes. Sporophytes of *P. makinoi* form small populations on the Korean Peninsula, but they are widely spread (Fig. 2). In Japan, the sporophytes of *P. makinoi* are difficult to find (Ebihara et al., 2019). A more detailed examination of the distribution will be needed to explain the biogeographic evolution.

Molecular data analysis of three independent gametophytes and *Hymenophyllum wrightii* f. *serratum*

The aligned length of *rbcL* region is 1,200 bp, and aligned length of the *rps4* gene and *rps4-trnS* IGS region is 804 bp. Of the 1,200 aligned *rbcL* region, 845 sites (70.4%) were identical, 38 sites (3.17%) are parsimony uninformative, and 317 sites (26.4%) provide phylogenetic information. MP analysis of the entire *rbcL* sequences revealed two retained trees with a tree length (TL) of 591, a consistency index (CI) of 0.7191 (0.6998 excluding uninformative characters), and a retention index (RI) of 0.9363. Fig. 3 presents one of the two equally most parsimonious trees. Because all the parsimony trees and a strict consensus tree have similar topologies on positions and relationships of ingroups, one of parsimony tree is presented.

In the 804 bp aligned *rps4* gene and *rps4-trnS* IGS region, 244 sites (33%) were identical, 59 sites (7.3%) were parsimony uninformative, and 501 sites (62.3%) provide phylogenetic information. MP analysis of the entire *rps4* gene and *rps4-trnS* IGS sequence found 1,034 equally most parsimonious trees with 1 TL, with a high CI (0.8211 or 0.8097 excluding uninformative characters) and RI (0.9434). Fig. 4 presents one of the equally most parsimonious tree. Because all the parsimony trees and a strict consensus tree have similar



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Fig. 3. Phylogenetic tree of three independent gametophytes, *Hymenophyllum wrightii* f. *serratum*, and related taxa deduced from *rbcL* data. Bootstrap values (>50%) by maximum parsimony method are shown above branches. Arrow and G, independent gametophytes.

topologies on positions and relationships of ingroups, one of parsimony tree is presented.

rbcL region analysis showed that the filamentous gametophyte and sporophyte of *Crepidomanes schmidtianum* from Korea and sporophyte of *C. schmidtianum* from Japan (GenBank AB257465, AB378492) (Ebihara et al., 2008) with the gametophyte of *C. intricatum* from United States (GenBank AB378491) (Ebihara et al., 2008) formed a monophyletic clade with an 85% bootstrap value (Fig. 3). In contrast, *C. schmidtianum* exhibits two haplotypes refracting the geographic patterns: Japan (accession no. AB257426) and Korea types (accessions no. 10, 58) (Fig. 3). Among the aligned 1,205 bp, only two nucleotide positions (0.1%) had different sequences. In an analysis of the *rps4* genes and *rps4-trnS* IGS region (aligned 804 bp), the gametophytes and sporophytes of *C. schmidtianum* from Korea and sporophyte from Japan (GenBank AB378497) (Ebihara et al., 2008) formed a clade with the strong support of a 100% bootstrap value. Among them, three different nucleotide positions (0.37%) were detected. Based on the strong bootstrap value and 99% sequences, the independent gametophytes of *C. schmidtianum*

from Korea are present in Korea. These results are supported by morphological features, such as filamentous gametophyte, filamentous linear gemmae, and rhizoid absence (Ebihara et al., 2008). The cpDNA data of one independent gametophyte distributed from Korea has the same sequence and monophyletic group as *Crepidomanes intricatum* from eastern United States. Moreover, it shares the same cpDNA data of *Crepidomanes schmidtianum* newly reported from Korea (Lee et al., 2014a). This result strongly suggests that it should be same species, even although there may have been the same female parent between two taxa (Ebihara et al., 2008). Interestingly, two newly obtained accessions (117, 118) and one accession gotten from GenBank (AB574708) of *C. latealatum*, did not form a group according to their taxonomic species. Big difference of sequences shows the possibility of mis-identification.

rbcL region analysis showed that the gametophytes and sporophytes of *Hymenophyllum wrightii* from Korea, sporophyte from Japan (GenBank AB574724) (Ebihara et al., 2010), and gametophytes of United States (GenBank JN585966) (Duffy et al., 2015) with the sporophytes of *H.*

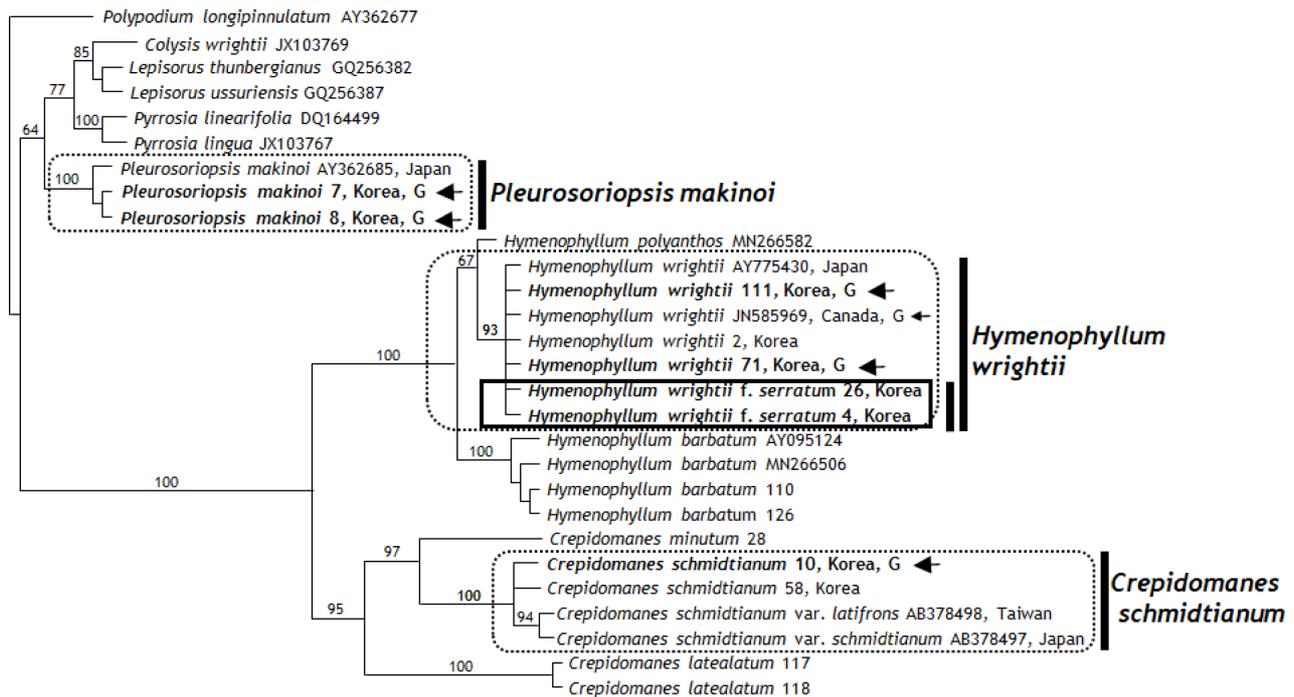


Fig. 4. Phylogenetic tree of three independent gametophytes, *Hymenophyllum wrightii* f. *serratum*, and related taxa deduced from *rps4* gene and *rps4-trnS* intergenic spacer data. Bootstrap values (>50%) by maximum parsimony method are shown above branches. Arrow and G, independent gametophytes.

coreanum (GenBank AB574717) and *H. wrightii* f. *serratum* formed the same clade with 82% bootstrap value (Fig. 3). Further study on Japanese and Korean populations of *H. coreanum* will be needed to confirm it. The same 100% same nucleotide positions among them were detected without *H. wrightii* f. *serratum*. In an analysis of the *rps4* genes and *rps4-trnS* IGS region (aligned 804 bp), the gametophytes and sporophytes of *H. wrightii* from Korea and the sporophyte from Japan (GenBank AY775430) (Hennequin et al, 2006) and the gametophytes of United States (GenBank JN585969) (Duffy et al., 2015) with *H. wrightii* f. *serratum* formed a clade with the strong support of a 93% bootstrap value. Among them, four different nucleotide positions (0.49%) were detected. Based on the strong bootstrap value and 99% sequences, we confirm that the independent gametophytes of *H. wrightii* exist in Korea. These results are supported by morphological features, such as ribbon like gametophytes, linear gemmae, and many rhizoids.

Hymenophyllum wrightii f. *serratum* C. S. Lee & K. Lee from Korea is distinguished from *H. wrightii* f. *wrightii* by its smaller height and broader basal leaf blades, broad-ovate lamina, larger sori, and serrated margins of involucre lips as a new taxon at the infraspecific level (Lee et al., 2014b). *Hymenophyllum wrightii* f. *serratum* were monophyletic within

a *Hymenophyllum wrightii* clade based on the *rbcL* region, and formed same clade within *H. wrightii* clade based on an analysis of the *rps4* genes and *rps4-trnS* IGS region. Three unique genes were detected at the 320 position (G), 357 position (T), and 465 position (T) in the *rbcL* region. These molecular results were supported by the morphological characteristics as an intraspecific level of *Hymenophyllum*. *Hymenophyllum wrightii* f. *serratum* is a new taxon (Lee et al., 2014b) that should be treated as forma based on a similar cpDNA sequence to *Hymenophyllum wrightii*.

rbcL region analysis indicated that the gametophytes of *Pleurosoriopsis makinoi* from Korea and sporophytes of *P. makinoi* from Japan (GenBank AY362613: Schneider et al., 2004; AB575285: Ebihara et al., 2010) form a clade with the strong support of a 100% bootstrap value (Fig. 3). On the other hand, *Pleurosoriopsis makinoi* exhibits two haplotypes refracting the geographic patterns: Japan and Korea types. Among the aligned 1,205 bp, only two nucleotide positions (0.1%) were different sequences. In an analysis of the *rps4* genes and *rps4-trnS* IGS region (aligned 804 bp), the gametophytes of *Pleurosoriopsis makinoi* from Korea and sporophyte of *P. makinoi* from Japan (GenBank AY362685) (Schneider et al., 2004) form a clade with the strong support of a 100% bootstrap value. This cpDNA marker was more

quickly evolved than *rbcL* region as the previous paper pointed out (Ebihara et al., 2008). Among them, five different nucleotide positions (0.6%) were detected. Based on 100% bootstrap value and 99% sequences, the independent gametophytes of *P. makinoi* are present in Korea. These results are supported by the morphological features, such as ribbon like gametophyte, gemmae shape, and a slight rhizoid presence. To prove the geographical variation between Japan, China, and Korea, more detailed research on the taxonomical revision of *P. makinoi* in the three countries will be needed.

An independent gametophyte was observed in the deep dirt place of a moist rock, such as a small cave of a humid mountain around a valley in Gyeonggi-do, Gangwon-do, and Jeju-do province, Korea. Sporophytes of *P. makinoi* were rarely founded under the light condition of humid mountains throughout the country. Ebihara et al. (2019) examined the growth environment of the independent gametophytes of *P. makinoi*. They suggested that low light conditions in winter due to the planting of evergreen conifers caused the independence of gametophytes without growing sporophytes by detaching gemmae in winter. The gametophytes and independence gametophytes of *P. makinoi* were found together in same places. Therefore, further monitoring of sporophyte and gametophytes on a nationwide scale is recommended. Additional research with more detailed microenvironment conditions and habitats in Korea will be needed to clarify the independent gametophytes growth factors.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix 1. Collection data of three independent gametophytes and *Hymenophyllum wrightii* f. *serratum*, and related taxa studied in molecular analyses (*DNA accession No. used from GenBank). Voucher specimens were deposited at Ewha Womans University Herbarium (EWH). Numbers in bold after each accession number are the same as in Figs. 3, 4. GenBank accession numbers listed in the following order: *rbcL*, **rps4-trnS*, and *rps4-trnS* intergenic spacer.

Hymenophyllum wrightii Bosch: [**2**], KOREA. Jeju-do: Hallasan Mt., 30 Mar 2014, C. S. Lee & K. Lee 1403010 (EWH), MT989370, *MW020609; [**71**], Gametophyte. Jeju-do: Hallasan Mt., 9 Nov 2014, C. S. Lee & K. Lee 1411005 (EWH), MT989371, *MW020610; [**111**], Gametophyte. Jeju-do: Hallasan Mt., 14 Sep 2017, C. S. Lee & K. Lee 1709004 (EWH), MT989372, *MW020611.

H. wrightii Bosch f. *serratum* C. S. Lee & K. Lee: [**4**], KOREA, Jeju-do: Hallasan Mt., 31 Mar 2014, C. S. Lee & K. Lee 1403015 (EWH), MT989373, *MT989375; [**26**], Jeju-do: Hallasan Mt., 2 Jun 2014, C. S. Lee & K. Lee 1406015 (EWH), MT989374, *MT989376.

H. barbatum (Bosch) Baker: [**110**, **126**], KOREA. Jeju-do: Hallasan Mt., 14 Sep 2017, C. S. Lee & K. Lee 1709017-18 (EWH), MT98377, MT989378, *MT989379, *MT989380.

H. polyanthos (Sw.) Sw.: [**3**], KOREA. Jeju-do: Hallasan Mt., 31 Mar 2014, C. S. Lee & K. Lee 1403020 (EWH), MT989381.

Crepidomanes schmidtianum (Zenker ex Tascher) K. Iwats.: [**10**], Gametophyte. KOREA. Gyeonggi-do: Hayasan Mt., 22 Feb 2014, C. S. Lee 1402002 (EWH), MT989382, * MT989384; [**58**], Geongsang-do: Jirisan Mt., 21 Oct 2014, C. S. Lee & K. Lee 1410018 (EWH), MT9893833, *MT989385.

C. minutum (Blume) K. Iwats.: [**28**], KOREA. Jeju-do: Hallasan Mt., 1 Jun 2014, C. S. & K. Lee 140617 (EWH), *MT989388.

C. latealatum (Bosch) Copel.: [**117**, **118**], KOREA. Jeju-do: Hallasan Mt., 10 Oct 2017, C. S. & K. Lee 1710012-3 (EWH), MT989393, MT989394, *MT989386, *MT989387.

Pleurosopsis makinoi (Maxim. ex Makino) Formin: [**7**, **8**], Gametophyte. KOREA. Gyeonggi-do: Hwayansan Mt., 22 Feb 2014, C. S. Lee 1402008-9 (EWH), MT989389, MT989390, *MT989391, *MT989392.