Taxonomic identity of *Crepidiastrum ×nakaii* recorded on Hongdo Island

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ABSTRACT: The plant known as “Hong-do-go-deul-ppae-gi” on Hongdo Island, belonging to the genus *Crepidiastrum*, has been recorded as *C. ×nakaii*, a hybrid between *C. denticulatum* and *C. platyphyllum*. During a survey of the flora of Hongdo Island, we reexamined the taxonomic identity of “Hong-do-go-deul-ppae-gi”. The morphological traits, geographical distribution, and internal transcribed spacer (ITS) sequences of “Hong-do-go-deul-ppae-gi” were compared to related species within the genus. A morphological examination revealed that the plant was similar to *C. lanceolatum* in that it has subshrub stems with caudex stout, 8 inner involucral bracts, and 10 to 12 florets. In contrast, similarities to *C. denticulatum* were observed in terms of radical leaves deciduous in the flowering period, cauline leaves membranous with acute serrations, and beaked achenes. Furthermore, *C. denticulatum* and *C. lanceolatum* grew on Hongdo Island and nearby areas, but *C. platyphyllum* was not distributed in these areas. The results of morphological and distributional investigations revealed that the Hongdo Island plant should be regarded as *C. ×muratagenii*, a hybrid between *C. denticulatum* and *C. lanceolatum*, not *C. ×nakaii*, which is a hybrid between *C. denticulatum* and *C. platyphyllum*. However, the resolution of the ITS sequences was insufficient such that we could not separate the examined species.

Keywords: *Crepidiastrum ×muratagenii*, *Crepidiastrum*, geographical distribution, Hongdo Island, morphological traits

The distribution of Hong-do-go-deul-ppae-gi was first recognized in Korea by identifying the specimen (*Ishidoya, T. & T. H. Chung* 3741 TI) collected by T. Ishidoya on Hongdo Island as *Paraixeris denticulato-platyphylla* (Makino) Nakai, a hybrid of *Paraixeris denticulate* Nakai [= *Crepidiastrum denticulatum* (Houtt.) J. H. Pak & Kawano], and *C. lanceolatum* var. *latifolium* Nakai [= *C. platyphyllum* (Franch. & Sav.) Kitam.]. Later, it was treated as *×Crepidiastrixeris denticulato-platyphylla* (Makino) Kitam. by Kitamura (1937). It seems to have been named Hong-do-go-deul-ppae-gi because it was first collected on Hongdo Island (Lee, 1969; Lee, 1996). However, the above scientific name was illegitimate (ICN H6.2. and H.9.1.). Hence, it was corrected to *Crepidiastrum ×nakaii* H. Ohashi & K. Ohashi, as the two parent species were treated as *Crepidiastrum* by Pak and Kawano (1992) (Ohashi and Ohashi, 2007). Saito et al. (2006) reported that *C. ×nakaii* occurs in the area where the parents occur sympatrically, and *C. ×nakaii* shows repeated frequent hybridization between the parent taxa as a result of

an incomplete breeding barrier between the two taxa. However, there is disagreement as to whether or not *C. platyphyllum* is distributed in Korea (Ohwi, 1965; Koyama, 1995; Kadota et al., 2017). Furthermore, Lee (1996) described Hong-do-go-deul-ppae-gi as a hybrid of *C. denticulatum* and *C. lanceolatum*. Therefore, controversy exists regarding the identity of this plant.

Natural hybridization is a frequent evolutionary phenomenon in flowering plants (Grant, 1981), playing an important role in the formation of new species (Arnold, 1997; Rieseberg, 1997; Seehausen, 2004). Under the limited migration from the continent, species richness of insular flora can be increased via hybridization which may lead to the generation of new species with novel combinations of potentially advantageous parental traits in the new environment (Francisco-Ortega et al., 1996; Brochmann et al., 2000; Jorgensen and Olesen, 2001; Fuertes-Aguilar et al., 2002; Howarth and Baum, 2005). Because of the importance of plant hybridization in evolution, it is of great importance to accurately identify hybrid individuals.

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To determine the taxonomic identity of Hong-do-go-deul-ppae-gi, this study examined its morphological characteristics and distribution, and analyzed nuclear ribosomal DNA internal transcribed spacer (ITS) sequences with its related species in the genus *Crepidiastrum*.

Materials and Methods

Morphological observation and survey of geographical distribution

To compare the morphological characters of Hong-do-go-deul-ppae-gi and its closely related taxa, we collected specimens from Hongdo, Jindo, and Geomundo Islands in Jeollanam-do Province, South Korea, and deposited them in the Herbarium of Inha University (IUI). In addition, we examined

additional specimens throughout the Korea National Biospecies Information System (<http://www.nature.go.kr>). For identification, reference was made to Kitamura (1937, 1955), Pak and Kawano (1992), and Ohashi and Ohashi (2007). Distribution data were collected from the Global Biodiversity Information Facility (GBIF) (<http://www.gbif.org>), the Korea National Biospecies Information System (<http://www.nature.go.kr>), Science Museum Net (<http://science-net.kahaku.go.jp>), and the Chinese Virtual Herbarium (<http://www.cvh.ac.cn>).

DNA extraction and PCR amplification

Total genomic DNA was extracted from dried leaf materials with silica gel (deposited in the herbarium of Inha University, IUI) using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The

Table 1. List of species used for the Internal Transcribed Spacer (ITS) sequences analysis with voucher and GenBank accession numbers.

Taxon/locality	Voucher	GenBank accession No.	Reference
<i>Crepidiastrum denticulatum</i> (Houtt.) J. H. Pak & Kawano 이고들빼기			
Mt. Dongseogsan, Jindo, Jeollanam-do, Korea	<i>D. P. Jin 2010016</i> (IUI)	MW938634	This study
Gitdaebong, Hongdo Island, Sinan-gun, Jeollanam-do, Korea	<i>Y. J. Jang 201028-11</i> (IUI)	MW938645	This study
Nagahama, Kanagawa, Honshu, Japan	<i>N1-PR 1194</i> (TNS)	AY876275	Saito et al. (2006)
Hubei, Shenlongjia, China	<i>Y. L. Peng 989-7</i> (CDBI)	KC968062	Peng et al. (2014)
<i>Crepidiastrum lanceolatum</i> (Houtt.) Nakai 갯고들빼기			
Geomundo Island, Yeosu-si, Jeollanam-do, Korea	<i>Y. J. Jang 201020-3</i> (IUI)	MW938639	This study
Geomundo Island, Yeosu-si, Jeollanam-do, Korea	<i>Y. J. Jang 201020-4</i> (IUI)	MW938640	This study
Bonin Islands, Japan	<i>A. Goto and M. Ito</i>	AB002618	
Yomitan, Okinawa Island, Japan	<i>Kokubugata 2492</i> (HAST)	AB598563	Nakamura et al. (2012)
<i>Crepidiastrum platyphyllum</i> (Franch. & Sav.) Kitam. 절영풀			
Arasaki, Kanagawa, Honshu, Japan	<i>AS-CR 1193</i> (TNS)	AY876259	Saito et al. (2006)
Nagahama, Kanagawa, Honshu, Japan	<i>N2-CR 1197</i> (TNS)	AY876260	Saito et al. (2006)
Bishyamon, Kanagawa, Honshu, Japan	<i>B2-CR 1242</i> (TNS)	AY876262	Saito et al. (2006)
Kurosaki, Kanagawa, Honshu, Japan	<i>K2-CR 1266</i> (TNS)	AY876263	Saito et al. (2006)
<i>Crepidiastrum</i> × <i>muratagenii</i> H. Ohashi & K. Ohashi 홍도고들빼기			
Graves around Cheong-eo Mireuk, Hongdo Island, Sinan-gun, Jeollanam-do, Korea	<i>Y. J. Jang 200918-11</i> (IUI)	MW938638	This study
Yangsangbong ridge, Hongdo Island, Sinan-gun, Jeollanam-do, Korea	<i>Y. J. Jang 201028-4</i> (IUI)	MW938643	This study
Yangsangbong ridge, Hongdo Island, Sinan-gun, Jeollanam-do, Korea	<i>Y. J. Jang 201028-6</i> (IUI)	MW938644	This study
Gitdaebong ridge, Hongdo Island, Sinan-gun, Jeollanam-do, Korea	<i>Y. J. Jang 201029-3</i> (IUI)	MW938646	This study
<i>Youngia japonica</i> (L.) DC. 뿌리쑥이			
Rongjing, Sichuan, China	<i>X. F. Gao 11605</i> (CDBI)	KC968024	Peng et al. (2014)

extracted DNA was electrophoresed on a 1% agarose gel to confirm the presence or absence of DNA, and the concentration and quality of DNA were confirmed using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). We used PCR amplification of the ITS of the nuclear ribosomal DNA. Primer and PCR conditions were as described in Saito et al. (2006). The PCR products were visualized on 1% agarose gels and sequenced using an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). The determined sequences were deposited in the Genbank (accession nos. MW938634–MW938646). Additionally, the nuclear ribosomal ITS sequences of Japanese and Chinese individuals deposited in GenBank were used for the ITS analysis (Table 1).

Phylogenetic analysis

The analyzed nucleotide sequences were determined after checking the chromatogram using Geneious R 7.1.9 (Biomatters Ltd., Auckland, New Zealand). All sequences were aligned using the MUSCLE software (Edgar, 2004). Maximum

likelihood trees based on ITS data were constructed using the W-IQ-TREE Server (<http://iqtree.cibiv.univie.ac.at/>) (Trifinopoulos et al., 2016), based on user-friendly web servers for IQ-TREE 1.5 (Nguyen et al., 2015). Each aligned sequence dataset was tested to determine the best-fit model by using W-IQ-TREE with the Akaike criterion, and new model selection procedures. TIM3e was confirmed as the best-fit model for the ITS. We evaluated the node supported by 1,000 ultrafast bootstrap replicates (UFBS) (Minh et al., 2013). As an outgroup, *Youngia japonica* (L.) DC., which is most closely related to the genus *Crepidiastrum*, was used (Kilian et al., 2009).

Results and Discussion

Observation of morphological traits

According to Pak and Kawano (1992), *C. denticulatum* is a herb, lacking radical leaves during the flowering season, membranous cauline leaves with acute serrations, 13–15 florets, and short and stout beaked achenes. In contrast, *C.*

Fig. 1. *Crepidiastrum x muratagenii* from Hongdo Island. **A.** Specimen (29 Aug 1919, Ishidoya, T. & T. H. Chung 3741 TI). **B.** Habit. **C.** Stem and rhizome. **D, E.** Cauline leaf. **F.** Involucral bracts. **G.** Flowers. **H.** Achene.

Table 2. Comparison of the morphological characters among *Crepidiastrum denticulatum*, *C. lanceolatum*, *C. platyphyllum* and the Hongdo Island plants.

	<i>C. denticulatum</i>	<i>C. lanceolatum</i>	<i>C. platyphyllum</i>	Hongdo plants
Habit	Herbs	Subshrubs	Subshrubs	Subshrubs
Stem	Solitary, erect, branched	Caudex stout, short, rhizomelike	Caudex stout, short, rhizomelike	Caudex stout, short, erect, branched
Radical leaf	Radical leaves withered during flowering season	Rosulate leaves ovate to narrowly so, rarely elliptic	Rosulate leaves obovate to obovate, elliptic	Radical leaves withered during flowering season
Cauline leaf	Membranous texture with acute serrations	Thicker texture without serration	Thicker texture without serration	Membranous texture with acute serrations
No. of involucre bracts	8	8	5	8
No. of florets	13–15	8–12	5	10–12
Achene	With short and stout beak	Beakless	Beakless	With short and stout beak

lanceolatum and *C. platyphyllum* are subshrubs, persisting radical leaves, and thicker cauline leaves with entire and beakless achenes. In addition, *C. lanceolatum* has 8 inner involucre bracts, 8–12 florets, and *C. platyphyllum* has 5 inner involucre bracts, and 5 florets. As a result of observations, Hongdo Island individuals showed mixed characteristics of *C. denticulatum* and *C. lanceolatum* (Fig. 1, Table 2). Hongdo Island individuals were similar to *C. lanceolatum* in a shrub with caudex stout, 8 inner involucre bracts, florets less 10–12 (13–15 in *C. denticulatum*), but differed from *C. lanceolatum* by having radical leaves withered during the flowering season, membranous cauline leaves with acute serrations, and short and stout beaked achenes as *C. denticulatum*.

Survey of geographical distribution

To confirm the distribution of Hong-do-go-deul-ppae-gi and its related taxa, Hong-do-go-deul-ppae-gi was collected from Hongdo and Geomundo Islands in Korea. *Crepidiastrum lanceolatum* occurs in rocky areas along the seashore of Jeju and the southern coast of Korea, while *C. denticulatum* occurs on sunny slopes and roadsides in the mountains of all Korean provinces. According to recent literature, *C. platyphyllum* is known as a Japanese endemic plant distributed in Chiba Prefecture, Kanagawa Prefecture, and Shizuoka Prefecture in Japan (Pak and Kawano, 1992; Koyama, 1995; Kadota et al., 2017). Therefore, it is highly likely that a hybrid of *C. lanceolatum* and *C. denticulatum*, rather than *C. platyphyllum* and *C. denticulatum*, would occur on Hongdo Island (Fig. 2). In addition, the T. Ishidoya specimen (Ishidoya, T. & T. H. Chung 3741 TI) was re-identified as a hybrid of ×*Crepidiastrixeris denticulato-lanceolata* Kitam. (nom. illeg.) [= *C. ×muratagenii* H. Ohashi & K. Ohashi] by Kitamura (1955).

Fig. 2. Distribution of *Crepidiastrum* 5 taxa in East Asia (Source: GBIF [http://www.gbif.org], The Korea National Biospecies Information System [http://www.nature.go.kr], Science Museum Net [http://science-net.kahaku.go.jp], the Chinese Virtual Herbarium [http://www.cvh.ac.cn]).

Phylogenetic analysis of ITS sequences

In the ITS phylogenetic tree, the 4 taxa of the genus *Crepidiastrum* were divided into two clades; *C. lanceolatum* and *C. platyphyllum*, *C. denticulatum*, and Hongdo Island individuals, but each taxon did not form a monophyletic group. Significant differences did not exist between the nucleotide sequences of the Hongdo Island individuals and the Korean *C. denticulatum* (Fig. 3). For that reason, Saito et al. (2006) reported that *C. ×nakaii* in F_1 status showed an additivity of parent sequences at the nrDNA locus, but in F_2 status showed

Fig. 3. Maximum likelihood (ML) tree based on nuclear ribosomal ITS sequences of *Crepidiastrum* 4 taxa. Bootstrap values (>50%) are indicated above branches.

either the ITS sequences of one parent only, or one predominant ITS type, despite the intermediate morphology between *C. platyphyllum* and *C. denticulatum*. These results can be explained through a backcross to *C. denticulatum* or segregating of F_2 . Further studies are required to understand the taxonomic identity of this species.

Origin and scientific name of the plant

Based on our investigations on morphological traits and geographical distribution, the origin of “Hong-do-go-deul-pae-gi” recorded in Hongdo Island should be regarded as a hybrid between *C. denticulatum* and *C. lanceolatum*, and not a hybrid between *C. denticulatum* and *C. platyphyllum*. Therefore, that the scientific name of the plant should be assigned as *C. ×muratagenii*, a hybrid between *C. denticulatum* and *C. lanceolatum*.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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홍도고들빼기의 분류학적 실체

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적 요: 고들빼기속에 속하는 홍도산 홍도고들빼기의 학명은 이고들빼기와 절영풀의 잡종인 *Crepidiastrum ×nakaii*로 기록되어 왔다. 홍도 식물상을 조사하는 과정에서 홍도고들빼기의 분류학적 실체를 재검토하였다. 이를 위해 홍도고들빼기의 형태적 특징과 지리적 분포 및 internal transcribed spacer (ITS) 염기서열을 고들빼기속 근연종들과 비교하였다. 형태적 특성 조사에서, 홍도고들빼기는 짧고 굵은 아관목성 줄기와 8개의 내충포편 및 10 내지 12개의 소화수를 갖는 점에서 갯고들빼기와 유사한 반면, 개화기에 시드는 뿌리잎과 막질로 예저치인 줄기잎 및 부리가 있는 수과 형태 등에서는 이고들빼기와 유사하였다. 한편 이고들빼기와 갯고들빼기는 홍도와 인근 섬 지역에 자라지만, 절영풀은 분포하지 않는다. 이러한 형태 및 분포 조사결과, 홍도고들빼기의 학명은 이고들빼기와 절영풀의 잡종인 *C. ×nakaii*가 아니라, 이고들빼기와 갯고들빼기의 잡종인 *C. ×muratagenii*로 판단된다. 하지만, ITS 염기서열은 조사된 종들을 구별할 수 있는 해상력을 보여주지 못하였다.

주요어: 홍도고들빼기, 홍도, 고들빼기속, 형태적 특징, 지리적 분포