広島大学学位請求論文

Taxonomic and Phylogenetic revision of Asian *Glossadelphus sensu Brotheri*

(アジア産ヒラツボゴケ属の分類学的および系統学的再検討)

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Taxonomic and Phylogenetic revision of Asian *Glossadelphus sensu Brotheri*

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General introduction

Glossadelphus M. Fleisch. was originally described as a genus of Semtatophyllaceae (Bryophyta) by Fleischer (1923). According to Wijkia *et al*. (1962) and some additional literature, 88 taxa were recorded on this genus throughout the world. They ditstributed in America, Hawaii, Africa and Asia. Especially, 45 taxa were reported from the Southeast Asia and Japan. Unfourtunally, Fleischer did not design a typus of *Glossadelphus*. So that, this genus had been faced with a taxonomical argument, which was uclear diagnostic trait. Therefore, Robinson (1974) performed lectotypification of *Glossadelphus* and designated *Hypnum truncatulum* Müll.Hal. [≡ *Glossadelphus truncatulus* (Müll.Hal.) M.Fleisch.] as the type species. Later, Buck (1987) treated the genus *Glossadelphus* as a synonym of the genus *Phyllodon* Shimp. Despite advanced studies, this genus was poorly understood. Furthermore, East Asian *Glossadelphus* including Japanese species are not yet clarified to their taxonomical positions. Therefore, this study re-examined the East Asian *Glossadelphus*, and verified the phylogenetic relationship among them.

In chapter 1, detailedly introduced the history of *Glossadelphus*, and its taxonomical problems. This genus was consisted of two sections, which were sect. *Anastigma* (Cardot) M.Fleisch., and sect. *Collophyllum* M.Fleisch. Robinson (1974) and Buck (1987) recognized that sect. *Collophyllum* was acceptable to the concept of *Glossadelphus*. Each section has a complicated taxonomical history, and problems of *Glossadelphus* was caused from theirs history.

In chapter 2, preferentially revised the East Asian *Glossadelphus*, excluded from Buck's study (1987). In first, morphological characteristics were examined. And, the phylogenetic analysis was performed to reveal the taxonomical position of East Asian *Glossadelphus* by using *rbc*L sequences. Additionally, a new species was described in the genus from Kyushu, Japan.

In chapter 3, anlayzing genetic variations of East Asian *Glossadelphus* related to the geographical distribution. Sampling took place from 10 regions in Japan and Korea. And, sequences from *rps*4 to *pas*A partial, ITS and *nad*5 sequences were evaluated and used to analyzing the genetic variations. Throughout the result, it was inferred to the speciation or evolution tendency of East Asian *Glossadelphus*.

In chapter 4, anlyzeing the phylogenetic relationship of *Phyllodon* and its related genera. *Phyllodon* was lectotypificated by Buck (1987), and he treated *Glossadelphus* as a synonym of *Phyllodon*. Howevere, it was poorly understood and known to the phylogeny of *Phyllodon*. Therefore, its taxonomical position was verified and its phylogenetic relationship was analysed by using *rbc*L and *rps*4. Additionally, a new species was described in *Bryocrumia* L.E.Anderson from Taiwan.

Chapter 1. Histrory of *Glossadelphus* and its taxonomical problem

1. History of *Glossadelphus*

The genus *Glossadelphus* M.Fleisch. (Sematophyllaceae, Bryophyta) was established by Fleisher (1923), and consisted of two distinct sections, which were sect. *Collophyllum* Fleisch. and sect. *Anastigma* (Cardot) Fleisch. Diagnostic features of each section were heteromorphic. And, each section had own history.

Fristsly, the section *Anastigma* was originally described as *Taxithelium* Mtt. sect. *Anastigma* Cardot (Hypnaceae) by Cardot (1905). He suggested it for *Taxithelium lingulatum* Cardot (≡ *Glossadelphus lingulatus*). So that, this section was a monotypic section at first time. However, 6 species were newly placed in the subgen. (Untergatt.) *Anastigma* (Cardot) Broth. (=sect. *Anastigma* Cardot) by Brotherus (1908) (Table 1). They had distinct morphological features, which were oblong or lingulate leaf form, rounded and notched or roughly denticulate leaf tip. Later, Fleischer (1923) recognized that this subgenus was distinguished from *Taxithelium*, and suggested the new genus *Glossadelphus* for this subgenus (Table 1). In other words, subgen. *Anastigma* of *Taxithelium* was treated to the sect. *Anastigma* of *Glossadelphus* by Fleischer (1923). Moreover, he newly placed two species, wheich were *G. zollingeri* (Müll.Hal.) M.Fleisch. and *G. prostratus* (Dozy & Molk.) M.Fleisch., in this section. However, both species differed from hetromorphic traies, such as obtused at the apex and laceolate leaf form. Later, Brotherus (1925) accepted the concept of Fleischer (1923) and described 27 species in the sect. *Anastigma.* Mostly of them were transferred from the genus *Ectropothecium* Mitt. (Hypnaceae), and resembled to *G. zollingeri.* Consequently, the concept of the section *Anastigma*

by Cardot (1905) was modified by Fleischer (1923), and this section's diagnostic character got heteromorphic.

Secondly, the other section *Collophyllum* had more complex history than sect. *Anastigma*, however the diagnostic characters of sect. *Collophyllum* are clear. This section was firstly described as the subsection *Limnobiella* Müll.Hal. of the genus *Hypnum* Hedw. (Hypnaceae) by Mueller (1875). Then, the subsection *Limnobiella* consisited with three species, *H. acuminatulum* Hornsch., *H. octodiceroides* Müll.Hal., and *H. schweinfurthii* Brid. However Paris (1898) and Brotherus (1908) considered that these species were members of *Taxithelium*. Especially, Brotherus (1908) re-organized sect. *Limnobiella* as two groups, which were Group A and Group B. Later, Fleischer (1923) maintained Group A on *Taxithelium*, and he transferred Group B to to *Glossadelphus*. Fleishcer suggested for Gropu B to be included in *Glossadelphus* sect. *Collophyllum*. Therefore, Group B of sect. *Limnobiella* on the genus *Taxithelium* was treated to the sect. *Collophyllum* of the genus *Glossadelphus*. This section's diagnostic character was clear, so that it was not doubtful after the description. In addition, Robinson (1974) mentioned that the species of the sect. *Collophyllum* form the most distinctive element of *Glossadelphus*, and he designated *G. turncatulus* (Müll.Hal.) M.Fleisch. as a lectoype of the genus.

Table 1. History of *Glossadelphus*

	Brotherus (1908)	Fleischer (1923)	Brotherus (1925)	Buck (1987)
Family	Hypnaceae (Plagiotheciaceae)	Sematophyllaceae	Sematophyllaceae	Hypnaceae
Genus	**Taxithelium**	**Glossadelphus**	**Glossadelphus**	***Phyllodon***
Subgenus	Polystigma			
Section	***Limnobiella***	**Sect. *Collophyllum***	**Sect. *Collophyllum***	
Group B	*T. truncatum*	*G. truncates*	*G. truncates*	*P. truncates*
	T. scutellifolium	*G. scutellifolius*	*G. scutellifolius*	*P. scutellifolius*
	T. truncatulum	*G. truncatulus*	*G. truncatulus*	*P. truncatulus*
	T. choiropyxis		*G. choiropyxis*	
	T. oophyllum	*G. oophyllus*	*G. oophyllus*	*P. oophyllus*
	T. natans	*G. natans*	*G. natans*	*P. natans*
Subgenus	***Anastigma***	**Sect. *Anastigma***	**Sect. *Anastigma***	
			Group B	
	T. perplanicaule	*G. perplanicaulis*	*G. perplanicaulis*	
	T. torrentium	*G. torrentium*	*G. torrentium*	*Taxiphyllum torrentium*
			Group Ca	
	T. subretusum	*G. subretusus*	*G. subretusus*	
	T. similans	*G. similans*	*G. similans*	
	T. glossoides	*G. glossoides*		
	T. linglatum	*G. lingulatus*	*G. lingulatus*	*P. lingulatus*
			Group Cbb.	
	T. ivoreanum	*G. ivoreanus*	G. ivoreanus	
			Group Aa	
		G. prostrates	*G. prostrates*	*T. protratum*
		G. zollingeri	*G. zollingeri*	*Ectropothecium zollingeri*
		G. zollingeri	*G. zollingeri*	
		var. *filicaulis*	var. *filicaulis*	
		var. *molis*	var. *molis*	
		f. *robusta*	f. *robusta*	
		f. *flaccida*	f. *flaccida*	
			Group Ca	
		G. hermaphorditus	*G. hermaphorditus*	
			G. isopterygioides	*T. isopterygioides*
			Group Ab	
		G. amboinensis	*G. amboinensis*,	
			G. kiushiuensis	
			G. ovalifolius	*E. ovalifolium*
			Group Ac	
		G. planifrons	*G. planifrons*,	
			G. vivicolor	*Bryocrumia vivicolor*
			G. angustiretis	*T. angustirete*
			G. divergens	
			G. obscurus	
			G. bornensis	
			G. anisopterus	
			G. malacocladus	
			G. boutani	
			G. scbrifolius	
			G. strictifolius	
			G.serrifolius	*E. ovalifolium*

2. The relationship between *Glossadelphus* and *Phyllodon*

As previously stated, *Glossadelphus* was consisted by two distinct sections, whichi had the heteromorphic traits. Therefore, there was continuously argued to unclear diagnostic characters. Robinson (1974) performed lectotypification of *Glossadelphus*, and designated *G. truncatulus* from sect. *Collophyllum* as the lectotype of this genus. Moreover, he noted that the species of sect. *Anastigma* resembled to *Taxiphyllum* M.Fleisch. (Hypnaceae), and treated American species of sect. *Anastimgma* as species of the genus *Taxiphyllum.* Later, Buck (1987) partially revised *Glossadelphus* and treated this genus as a synonym of *Phyllodon* Bruch & Schimp., which was originally described by Shimper (1851). The type species of this genus was *Hookeria retusa* Wilson, and this species had the distinct morphology, which was strong papillose and the distinctive shape of the peristome teeth. However, unfortunately the genus *Phyllodon* had not been recognized to other bryologists. So that *Phyllodon* had remained a monotypic genus. Then, this genus was redescribed by Buck (1987). He discovered a specimen of *Hookeria retusa* in the herbarium of BM, and considered that this specimen was corresponded to the protologue of the genus *Phyllodon*, as well as it was identified with *Hypnum truncatulum*. Therefore, he did the lectotyfification of *Phyllodon* and designated *H. turunatulum* [≡ *Glossadelphus truncatulus* (Müll.Hal.) M.Fleisch. ≡ *Phyllodon truncatulum* (Müll.Hal.) Buck] as a lectotypus of *Phyllodon*. Consequently, the geunus *Glossadelphus* was treated a snynonym of the genus *Phyllodon*.

3. What is the problem?

Glossadelphus was treated as a synonym of *Phyllodon* by Buck (1987). However, this study was nothing more that a only partial reviewed of the genus *Phyllodon*. So that, many Asian species have been remained in the genus *Glossadelphus*. For example, Iwatsuki (2012) agreed to Buck (1987), and treated *G. lingulatus* as a *Phyllodon* in the checklist of Japanes Mosses. However, he maintained *Glossadelphus* for other Japanese species, which were *G. ogatae* Broth. & Yasuda and *G. yakoushimae* (Cadot) Nog. Because these species were members of sect. *Anastigma*, so that their morphology was not suitable to *Phyllodon*. Therefore, it is necessary to revise the Asian *Glossadelphus* excluded from Buck's study (1987), and verify the taxonoimical position of them. There was no study about the phylogeny of *Phyllodon*. So that, this study revised East Asian *Glossadelphus*, and verified the phylogenetic relationship between *Phyllodon* and its related genera.

Chapter 2. Taxonomy of *Filibryum*

Abstract

Glossadelphus was originally described by Fleischer (1923). However, this genus was known to having unclear diagnostic trait. Buck (1987) treated *Glossadelphus* as a synonym of *Phyllodon*. However, he did not revise all taxa of *Glossadelphus*. In addition, some species of *Glossadelphus* excluded from Buck's study were not suitable to the diagnostic characters of *Phyllodon*. Threfore, this study revised East Asian *Glossadelphus*, which were excluded from Buck's study (1987), and analyzed the molecular phylogenetic relationship amog them. As the result, two East Asian species, which were *G. ogatae* and *G. yakoushimae*, were not shown the diagnostic morphological characters of *Phyllodon*. Additionally, there was not the phylogenetic relationship between these species and *Phyllodon*. Then, a curious moss was newly discovered at the Kyushu, Japan. This curious moss was similar to *G. ogatae*, and the nearest to *G. ogatae* in the phylogenetic tree. Threfore, this study suggested a new gneus *Filibryum* for the East Asian species, *G. ogatae*, *G. yakoushimae* and a curious moss. Additionally, a curious moss newly named *F. deguchianum*. Therefore, *Filibryum* consisted three species, which were *F. ogatae*, *F. yakoushimae* and *F. deguchianum*.

Introduction

Glossadelphus M.Fleisch. is a controversial genus described by Fleischer (1923) who recognised 18 species, two varieties and two forma that were classified into two sections: sect. *Anastigma* (Cardot) M.Fleisch. and sect. *Collophyllum* M.Fleisch. Wijk *et al*. (1962) listed 73 species and 5 varieties of *Glossadelphus*. Most *Glossadelphus* species were transferred from several genera including *Ectropothecium* Mitt., *Homalia* Brid., *Hypnum* Hedw., *Stereodon* (Brid.) Mitt., *Trichosteleum* Mitt., and *Taxithelium* Mitt. Fleischer (1923) did not designate a type species of *Glossadelphus*, so circumscription of the genus has not been properly understood. Robinson (1974) lectotypified *Glossadelphus* and designated *Hypnum truncatulum* Müll. Hal. [= *Glossadelphus truncatulus* (Müll.Hal.) M.Fleisch.]. In addition, he noted that species of sect. *Anastigma* resembled *Taxiphyllum* M.Fleisch. and treated American species in this section as belonging to the genus *Taxiphyllum.* Later, Buck (1987) partially re-examined the genus *Glossadelphus* and treated it as a synonym of *Phyllodon* Bruch & Schimp., a genus described by Shimper (1851) but almost unknown to most bryologists. The genus *Phyllodon* was lectotypified by Buck (1987). Thus, *P. truncatulus* (= *Hypnum truncatulum*) was designated as the lectotype. Distinct characteristics of *Phyllodon* are the lingulate leaf shape and the bifid teeth at the leaf apex. Buck transferred six species of *Glossadelphus* into *Phyllodon* and some species into other genera such as *Bryocrumia* L.E.Anderson, *Ectropothecium*, *Hampeohypnum* W.R.Buck (= *Sclerohypnum* Dixon) and

Taxiphyllum, However, Tixier (1988) continued to recognise *Glossadelphus* in the traditional sense. Buck's concept of *Phyllodon* was recently accepted by Kis (2002), Câmara (2010), and He & Nguyen (2012). However, many Asian species of *Glossadelphus* have not been re-examined. For Japanese *Glossadelphus*, Iwatsuki (2004) agreed with Buck (1987) and treated Japanese *G. lingulatus* as *Phyllodon*, but did not recognise *G. ogatae* Broth. & Yasuda and *G. yakoushimae* (Cardot) Nog. as *Phyllodon.* Moreover, the distinct morphological characteristics of *Phyllodon* were not observed in *G. ogatae* and *G. yakoushimae*. Previous phylogenetic studies suggested that *G. ogatae* and *P. lingulatus* divided to each clade (Olsson *et al.* 2009) and the former was related to the *Taxiphyllum* group (Tsubota *et al.* 2001, 2002; Olsson *et al.* 2009). So, it is necessary to re-examine the taxonomy and phylogeny of the Japanese *Glossadelphus* species (Table 2). Additionally, we collected a curious moss from Yakushima Island, Prov. Kagoshima, Misato, Prov. Miyazaki, and Chojabaru, Prov. Oita, Japan and . After careful examination, we found that the unknown moss showed morphological characteristics that differed from those of other known species of *Glossadelphus*. Therefore, we re-examined morphological characters of Japanese *Glossadelphus* and analysis the molecular phylogeny of them.

Table 2. History of Japanes *Glossadelphus*

wijkia et al. (1963)	Basionym	Type Local.	Currently accepted names
Glossadelphus zolligeri (C. Muell.) Broth.	*Hypnum zollingeri* C. Muell. (1851)	Java	*Ectropothecium zollingeri* (C. Muell.) Jaeg.
G planifrons (Broth. & Paris) Fleisch.	*Stereodon planifrons* Broth. & Paris (1902)	Kagoshima, Kyushu	*E. zollingeri* (C. Muell.) Jaeg.
G yakoushimae (Cardot) Nog.	*Taxithelium yakoushimae* Cardot (1913)	Liou-Kiou, Yakushima	*G yakoushimae* (Cardot) Nog.
G subfulvu (Broth.) Broth.	*Isoterygium subfulvum* Broth. (1921)	Prov. Ise, Honshu	*E. obtusulum* (Card.) Iwats.
G kiushiuensis (Broth.) Broth.	*I. kiushiuense* Broth. (1921)	Mt. Kirisima, Kyushu	*E. zollingeri* (C. Muell.) A. Jaeg.
G ogatae Broth. & Yas.	*G ogatae* Broth. & Yas. (1926)	Miyazaki Pref., Kyushu	*G ogatae* Broth. & Yas.
G nipponicus Reim. & Sak.	*G nipponicus* Reim. & Sak. (1931)		*G ogatae* Broth. & Yas.
G sakuraii Reim.	*G sakuraii* Reim. (1931)	Higane, Izu. Prov.	*G ogatae* Broth. & Yas.
G nanophyllus Sak.	*G nanophyllus* Sak. (1932)	Tanegashima, Prov. Ohsumi	*E. zollingeri* (C. Muell.) A. Jaeg.
G glossoides var. *japonicus* Sak.	*G glossoides* var. *japonicus* Sak. (1933)	Yakushima, Kagoshima Pref., Kyushu	*E. zollingeri* (C. Muell.) A. Jaeg.
G percymbifolius Sak.	*G percymbifolius* Sak. (1934)	Prov. Higo, Kunimi	*G ogatae* Broth. & Yas.
G pernitens Sak.	*G pernitens* Sak. (1934)	Mt. Takakuma, Prov. Ohumi, Kyushu	*Entodon luridus* (Griff.) Jaeg.
G doii Sak.	*G doii* Sak. (1935)	Prov. Ohsumi, Kyushu	*E. macropodus* (Hedw.) C. Muell.
G recurvo-marinatus Dix. et Sak.	*G recurvo-marinatus* Dix. et Sak. (1936)	Mt. Takakuma, Prov. Ohumi, Kyushu	*Symphyodon perrottetii* Mont.
G yasudae Dix. et Sak.	*G yasudae* Dixon & Sakurai (1939)	Prov. Inaba, Tottori	*Entodon macropodus* (Hedw.) Müll.Hal.

Material and Method

Morphological observations

Morphological studies were based on herbaria specimens from Hiroshima University (HIRO), the National Museum of Nature and Science, Tokyo (TNS), the Makino Herbarium (MAK), Kochi University (KOCH), the Hattori Botanical Laboratory, Miyazaki (NICH), Paris (PC), and Leiden (L), as well as on numerous specimens collected by the authors in Japan and Korea (deposited at HIRO), with duplicates in the National Institute of Biological Resources (KB) and the National Museum of Nature and Science, Tokyo (TNS).

Microscopic examinations and measurements were performed using an Olympus-BX52 light microscope. Specimens were examined in 1% potassium hydroxide. Descriptions and illustrations of median, alar, and apical leaf cells were generated from leaves obtained from the middle of the stems and branches. Leaf width was measured at the widest part.

Table 3. List of the voucher specimen information and Genebank assession numbers for *rbc*L and ITS sequenses of *Filibryum* and *Phyllodon*.

Species	Voucher No.	Site	Accession No.	
			*rbc*L	ITS
Filibryum ogatae	W. Kim 1357	Japan, Yakushima Aigodake	KT804664	KT804686
F. ogatae	W. Kim 1260	Japan, Yakushima maedake	KT804662	KT804680
F. ogatae	W. Kim 1267	Japan, Yakushima maedake	KT804663	KT804681
F. ogatae	W. Kim 1400	Japan, Yakushima jomonsugi	KT80665	KT804687
F. ogatae	W. Kim 1443	Japan, Yakushima jomonsugi	KT804668	KT 804688
F. ogatae	W. Kim 1431	Japan, Yakushima yakusugirando	KT804666	KT 804689
F. ogatae	W. Kim 1441	Japan, Yakushima yakusugirando	KT804667	KT 804690
F. ogatae	W. Kim 1041	Japan, Okayama Iwaya Dani	KT804661	KT 804684
F. ogatae	W. Kim 1472	Korea, Seoul Bukhansan	KT804670	KT 804682
F. ogatae	W. Kim 1468	Korea, Seoul Bukhansan	KT804669	KT 804683
F. deguchianum	W. Kim 1336	Japan, Yakushima Aigodake	KT804658	KT 804675
F. deguchianum	W. Kim 1331	Japan, Yakushima Aigodake	KT804657	KT 804676
F. deguchianum	W. Kim 1346	Japan, Yakushima Aigodake	KT804659	KT 804677
F. deguchianum	W. Kim 1353	Japan, Yakushima Aigodake	KT804660	KT 804678
F. deguchianum	W. Kim 1263	Japan, Yakushima Maedake	KT804655	KT 804672
F. deguchianum	W. Kim 1265	Japan, Yakushima Maedake	KT804656	KT 804673
F. deguchianum	W. Kim 529	Japan, Yakushima Onagawa	KT804654	KT 804679
F. deguchianum	W.Kim 1069	Japan, Oitaken Jojabaru	KT804653	KT 804674
Phyllodon lingulatus	W.Kim 522	Japan, Yakushima Onoaida	KT804671	KT 804691

Molecular protocols

We performed a phylogenetic analysis by using fresh samples collected in Japan and South Korea (Table 3). Additionally, some species of representative genera of Hypnales were assembled into a data set of 56 *rbc*L (ribulose bisphosphate carboxylase large-subunit gene) sequences on chloroplast, including data from Tsubota *et al.* (2002) and Arikawa *et al.* (2008) (Appendix 1). Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, USA). Polymerase chain reactions were performed in an Eppendorf Mastercycler using the following programme for *rbc*L: an initial cycle at 95℃ for 2 min followed by 35 cycles of 98℃ for 20 s, 58℃ for 60 s, and 68℃ for 1.5 min. A final cycle at 68℃ for 10 min was included to terminate amplification. The primers have been described by Tsubota *et al.* (1999, 2000). For internal transcribed spacer (ITS) sequences, an initial cycle was performed at 95℃ for 2 min followed by 40 cycles of 98℃ for 20 s, 60℃ for 60 s, and 68℃ for 1.5 min, and a final cycle at 68℃ for 10 min to terminate amplification. The primers used have been described by Oguri *et al.* (2003). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, USA) and were sequenced by Macrogen Inc., South Korea (www.macrogen.com).

Phylogenetic analyses

For each taxon and sequenced DNA region, forward (5'–3') and reverse (3'–5') sequences were checked for inaccurate base calling using GeneDoc ver. 2.7.0 software (Nicholas & Nicholas 1997). Consensus sequences were aligned using ClustalX ver. 2.1 software (Larkin *et al.* 2007, Thompson *et al.* 1997), and MEGA 6 software (Tamura *et al.* 2013) was used for some manual adjustments of the alignment. Phylogenetic analysis using chloroplast DNA *rbc*L sequences was performed using maximum likelihood criteria (Tsubota *et al*. 2002, Arikawa *et al*. 2008) with some differences as follows. Prior to phylogenetic reconstruction, the Kakusan4 script (Tanabe 2011) was implemented in the bias-corrected version of the Akaike information criterion (AIC, Sugira 1978) to facilitate informed decisions regarding which nucleotide-based substitution model best fitted our data, and an approximately unbiased (AU) test (Shimodaira 2002, 2004) was implemented in the final stage of the analysis scheme. Phylogenetic trees were constructed using the following four software programs: (1) RAxML ver. 8.1.5 (Stamatakis 2014) with the maximum likelihood (ML) method (Felsenstein 1981) using a general time-reversible (GTR)+ gamma model; (2) PAUPRat (Sikes & Lewis 2001) over PAUP* ver. 4.0b10 (Swofford 2002) with the maximum parsimony (MP) method (Fitch 1971) using the Parsimony Ratchet search strategy (Nixon 1999) with random weighting of each character in fifty 200-iteration runs; and (3) MrBayes ver.3.1.2 (Ronquist & Huelsenbeck 2003) with the Bayesian inference (BI) method using the GTR + gamma model with 1,000,000 generations. Topologies from these analysis were sampled to obtain the candidate topologies for the AU test (Shimodaira 2002, 2004).

Alternative topology and branch tests were performed using the *p*-value of the AU test (AU, Shimodaira 2002, 2004), bootstrap probability calculated using the same theory as for AU (NP, non-scaled bootstrap probability) tests, and Bayesian posterior probabilities (PP) calculated using CONSEL ver. 020 software (Shimodaira & Hasegawa 2001) from Bayesian information criterion (BIC) approximation (Schwarz 1978; Hasegawa & Kishino 1989). AU tests, bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch of the the phylogenetic tree (Fig. 3).

Result and Discussion

1) Comparative morphology of Japanese *Glossadelphus* and related species

There are 14 species of Japaneae *Glossadelphus*, which were usually discovered on Miyazaki and Kagoshima, Kyushu, Japan. As the result of this study, 8 species have morophological characterisitics of *Ectoropothecium* and *Entodon*. This result agrees to the advanced studies (Iwastuski 1967, 2004). It is known that species of *Ectropothecium* has a distinctly inflated basal cornor cell in the alar region. 5 taxa, which are *G. planiforns*, *G. subfulvu*, *G. kiushiuensis*, *G. nanophyllus* and *G. glossodies* var. *japonicas* have this diagnostic characters of *Ectropothecium*. Also, *Entodon* differed from *Glossadelphus* in the alar region developed. As the morphological characters, *G. pernitens* was identified to *Entodon luridus* (Griff.) Jaeg., and *G. doii* Sak. was identified to *E. macropodus* (Hedw.) Müll.Hal. Additionally, a Hawaiian moss, *G. chrysobasilaris* Broth., is treated to the genus *Symphyodon*. This species was treated to a synonym of *G. ogatae* by Tixier (1988). However, this species differs from some morphological characers, which are deccrent at the leaf basal angles, acute at the leaf apex, and serrulate leaf margin. Therefore, it is considered that *G. chrosobasilaris* is close to the genus *Symphyodon*. And, East Asian species, *G. rivicola* Broth. Was described from Taiwan (Brotherus 1929). This species was only known to the type specimens until now. As the result of this study, *G. rivicola* has a distinctly inflated basal corner cell, and slightly prorate leaf median cells. Therefore, it is juged that *G. rivicola* is a member of the genus

Ectropothecium. Finally, Iwastuski (2004) considered that three species, which are *G. nipponicus* Reim. & Sak., *G. sakuraii* Reim., and *G. percymbifolius* Sak., among Japanes *Glossadelphus* were a synonym of *G. ogatae*. This study agrees that dered that two species, *G. nipponicus* and *G. sakuraii*, are same to *G. ogatae*. However, *G. percymbifolius* has slightly different. This sepcies has the traits, which are contracted leaf basal, distinct prorate leaf median cells, and shorter leaf length than *G. ogatae*.

2) Morphological characteristics and habitat of *Filibryum*

As previous result, Japanese Glossadelphus is consisded two species, *G. ogatae* and *G. yakoushimae*. Then, this study discovered a crurious moss from Kyushu, Japan. This newly discovered moss shares the following morphological characteristics with both species: a heteroblastic stem leaf sequence; smaller stem leaves than branch ones; stem leaves that are deciduous on the aged stems; papillose median leaf cells; and an undifferentiated alar region – cells of the alar region are sub-quadrate and non-inflated. Moreover, these species grow on wet or humid boulders near streams in forests. On the other hands, the newly identified moss differs from both *G. ogatae* and *G. yakoushimae* in terms of its branch leaves. *G. ogatae* and *G. yakoushimae* have oblong- or obovate-lingulate branch leaves, which are widest at their upper half (Fig. 3-4). By contrast, this curious moss bears oblong-lanceolate or lanceolate branch leaves that are widest at their lower half. In addition, the apex of each leaf gradually and narrowly acuminates (Fig. 1). Moreover, the unknown species is similar to *Ectropothecium* in terms of its lanceolate leaf form and prorate median cells. However, *Ectropothecium* is classified according to a specific diagnostic characteristic. This genus has very inflated and pellucid alar cells, while the new species has no inflated basal cells. Therefore, we considered that this curious moss was a new species, and closely related to *G. ogatae* and *G. yakoushimae.* In sequence, we compared three species – the new species and both former of *Glossadelphus* – with *Phyllodon* and its related genera. From this comparison we deduced, firstly, that the new species and both of *Glossadelphus* did

not belong to any genera recognised by Buck (1987), or to the genus *Phyllodon*. An important characteristic of *Phyllodon* spp. is the truncate leaf apex and bifid apex cells (Buck 1987). However, members of this genus have obtuse, shortly acute or gradually acuminate leaf apexes, and their apex cells are never bifid. Secondly, Buck (1987) transferred some species from *Glossadelphus* to the genera *Bryocrumia*, *Ectropothecium* and *Sclerohypnum*. The diagnostic feature of *Bryocrumia* is a broadly rounded leaf apex, and *Ectropothecium* and *Sclerohypnum* are distinguished by very inflated basal corner cells. These morphological characteristics were not observed in the three species under discussion. Finally, these species are distinguished from members of the genus *Taxiphyllum* (having the closest phylogenetic relationship, Fig. 5) by the absence of a central strand in cross-sections of the stem and by having smaller stem leaves than branch leaves. However, the stem leaves of *Taxiphyllum* are usually similar to their branch leaves. Furthermore, the three species differ from *Taxiphyllum* in leaf arrangement. Their branch leaves are imbricated, while those of *Taxiphyllum* are almost distichously arranged. Additionally, *Taxiphyllum* is distinguished by their habitat growing – below tree trunks or on rather dry boulders. However, both species of *Glossadelphus* and the new species usually grow on wet or humid boulders, and never on tree trunks. Therefore, the three species may be distinguished from species of *Phyllodon* and related genera, such as *Bryocrumia, Ectropothecium* and *Taxiphyllum*, by morphological characteristics. In terms of nomenclature, *Glossadelphus* was regarded as a synonym of *Phyllodon* by Buck (1987). Although Tixier (1988) maintained the genus *Glossadelphus*, he recognised that Japanese

species of *Glossadelphus* differ from other members of *Glossadelphus*, so he suggested the subgenus *Ogatae* Tixier. However, this study considered that this Tixier's study was not suitable. Because we believe that his interpretation of the diagnostic morphological characteristics of this genus is unclear. For example, he treated *G. lingulatus* to a synonym of *G. laevifolius* (Mitt.) Bartr., however we think that both species differ from the leaf apex shape each other. Furthermore, Buck (1987) recognised that *G. laevifolius* was similar to species of *Taxiphyllum*. Consequently, we concur with Buck's concept of the genus *Phyllodon* (1987). However, this suty considers that the three species under discussion cannot be classified within the genus *Phyllodon* because they do not share the diagnostic morphological characteristics with the species of *Phyllodon*. Thus, we propose a new genus – *Filibryum* – for these species. In addition, the new genus *Filibryum* is well supported by phylogenetic analyses (Fig. 5).

Taxonomic Treatment

Filibryum **W.Kim & T.Yamag., gen. nov.**

Plant slender, creeping and flattened, growing in turf on moist or wet boulders and rocks, green to deep-green or yellowish-green, rarely becoming reddish-orange, not or somewhat glossy when dry. Stems sparsely and irregularly sympodially branched, primary and secondary stems prostrate and reddish- or orange-brown, in cross-section with outer cortical cells of 2–3 rows of small, thick-walled cells, and loosely areolated inner cortical cells that are large and thick-walled; pseudoparaphyllia foliose or filamentous. Stem leaves arranged in a loosely heteroblastic sequence, triangular to lanceolate, deciduous, smaller than branch leaves; branch leaves imbricate, lanceolate, oblong-lanceolate, ovate or oblong-ovate, non-decurrent, acuminate and acute or obtuse at apex; margins plane, crenulate or entirely below, slightly serrulate near the leaf apex; costa short and double, somewhat indistinct. Leaf cells vermiculate or linear-vermiculate, prorate at the end of the cell; alar region not differentiated, cells small, rectangular, becoming quadrate or subquadrate at the basal corner.

Type: *Filibryum deguchianum* W.Kim & T.Yamag Etymology: *Filibryum* means slender moss and thread-like

Filibryum includes three species: *F. deguchianum*, *F. yakoushimae*, and *F. ogatae*. *Filibryum* spp. are closely related to *Taxiphyllum* and *Ectropothecium* spp., however, *Filibryum* spp. clearly differ from *Taxiphyllum* spp. in leaf arrangement. Leaves of *Filibryum* spp. are imbricate, while those of *Taxipyllum* are usually distichous-like. Moreover, *Ectropothecium* is distinguished by large basal corner cells.

Key to the species fo *Filibryum*

1. Branch dorsal leaves oblong-lanceolate or lanceolate ..... 1. *F. deguchianum*

1. Branch dorsal leaves oblong-ovate or oblong 2

2. Branches flagelliform, branch dorsal leaves shortly acute at apex .....2. *F. yakoushimae*

2. Branches not flagelliform, obtuse, branch dorsal leaves obtuse or broadly obtuse at apex..... 3. *F. ogatae*

1. ***Filibryum deguchianum*** **W.Kim & T.Yamag.** ***sp. nov.*** (Figs. 1, 2)

Type: Japan, Kagoshima Pref.: Yakushima Island, along the road on Mt. Aigodake, on boulders, *W. Kim 1336* (holotype KB; isotypes HIRO, TNS).

Plant small, slender, creeping, flattened, growing in turf on moist stones or boulders, green to deep-green, not glossy. Primary and secondary stems prostrate, reddish or orange-brown, loosely and irregularly sympodially branched; in cross-sections with an epidermis of 2–3 rows of small, thick-walled cells; cortical cells loosely areolated, thick-walled, central strand absent. Branches short, ascending, 2–5 mm long, loosely leaved. Stem with a heteroblastic leaf sequence; leaves 0.30–0.52 mm long, 0.13–0.20 mm wide, deciduous in aged stems, lanceolate, oblong-lanceolate or rarely triangular, slightly narrowed at the base, gradually acuminate; costa double, short; margin crenulate, or somewhat serrulate above. Pseudoparaphyllia foliose to filamentous. Branch leaves slightly homomallous, larger than stem leaves; 0.4–0.7 mm long, 0.15–0.30 mm wide; dorsal leaves asymmetrically lanceolate; lateral leaves oblong-lanceolate or ovate; costa double, less than 1/3 of leaf length; margin serrulate upper 1/2; median laminal cells vermiculate, 20–37 μm long, ± 3 μm wide, prorate at both cell ends, apical laminal cells small; alar cells subquadrate, small, forming indistinct groups.

Sporophytes unknown.

Etymology: The new species is named in honour of Prof. Hironori Deguchi in recognition of his outstanding contributions to our understanding of the Japanese moss flora and his extensive involvement in training new researchers.

Other specimens examined (paratypes): JAPAN. Kagoshima Pref., Yakushima Island, Onnagawa River, *W. Kim 529* (KB, HIRO); Mt. Maedake, *W. Kim 1263, 1265* (KB, HIRO); Mt Aigodake, *W. Kim 1321*, *1322*, *1323*, *1325*, *1326*, *1327*, *1329*, *1330*, *1332*, *1333*, *1334*, *1335*, *1337*, *1338*, *1340*, *1342*, *1343*, *1344*, *1345*, *1346*, *1347*, *1348*, *1351*, *1353*, *1354*, *1356*, *1385*, and *1387* (KB, HIRO); *Yamaguchi 34374, 34383,* and *34384* (all HIRO). Oita Pref., Chojabaru, *W. Kim 1069* (KB, HIRO), *Deguchi 23590* (HIRO). Miyazaki Pref. Misato, Ogawa, *W. Kim 1731* (KB, HIRO).

Habitat: Growing on moist boulders and rocks on an inclined plane around a valley and forest stream. So far found at three sites on Yakushima Island: Mt. Aigodake, Mt. Maedake, and the Onnagawa River. At Mt. Aigodake, the species is found from the lower parts to around 1,000 m altitude.

Distribution: Endemic to Japan.

Diagnostic characteristics of this species are: (1) lanceolate or oblong-lanceolate leaf form, (2) lamina cells prorate at both ends, and (3) a heteroblastic stem leaf sequence.

This species is closely related to *Filibryum ogatae* and *F. yakoushimae*, but distinguished by the leaf shape. Leaves of *F. ogatae* are oblong or obovate-lingulate, widest at 3/4 distance from the base. Leaves of *F. yakoushimae* are oblong and short, abruptly acuminate at the apex, while leaves of *F. deguchianum* are oblong-lanceolate or lanceolate, gradually and narrowly acuminate at the apex, and widest at 1/4 distance from the base. These three species share common characteristics: (1) alar cells of leaves are more or less differentiated, sub-quadrate, or rectangular; (2) in cross-section of the stem, a central strand is absent; the inner

cortical cells are loosely areolated, enlarged, and thick-walled; the outer cortical cells are small, in 2–3 rows, and reddish-orange thick-walled; and (3) stems are typically reddish-brown or orange-brown with deciduous stem leaves. *Glossadelphus anomalus* Thér. and *Ectropothecium zollingeri* (Müll. Hal.) A.Jaeger are similar to *Filibryum* species; however, *G. anomalus* is distinguished by two swollen hyaline cells at the basal corner of leaves, and *E. zollingeri* by one very inflated and pellucid cell at the extreme basal corners of leaves.

2. *Filibryum yakoushimae* (Cardot) W.Kim & T.Yamag., comb. nov. (Fig. 3)

Basionym: *Taxithelium yakoushimae* Cardot, Bull. Soc. Bot. Genève sér. 2, 5: 318. 1913. Type: JAPAN. Isl. Yakushima, *Faurie 1142* (holotype PC, isotypes, HIRO!, TNS!)

Plant medium-sized, very slender, creeping, growing in turfs; green to deep green, not glossy. Primary and second stems prostrate, reddish or orange-brown; cross section with epidermis of 2–3 rows of small, thick-walled cells; cortical cells loosely areolated, thin-walled; central strand absent; loosely and sparsely branched. Branches thin, flagelliform, loosely imbricate leaves. Stem leaves ovate; costa double, short, somewhat indistinct; margin crenulate or serrulate above. Branch leaves larger than stem leaves, dorsal oblong or oblong-ovate, acute at the apex; costa double, more than less 1/3 leaf long; margin slightly serrulate near the apex; 0.9–1.2 mm long, 0.4–0.7 mm width; median leaf cells linear vermiculate, 43–68 μm long; weakly prorate at the end of cells; alar cells subquadarate, small, slightly indistinct.

Sporphytes unknown.

Specimens examined: JAPAN. Mt. Aigodake, *W.Kim 1379*, *Yamaguchi 34376, 34381* (all HIRO); Yakusugi Land, *Ohgue 2268* (KB, KYO).

Distribution: Endemic to Japan.

3. *Filibryum ogatae* (Broth. & Yas.) W.Kim & T.Yamag., comb. nov. (Fig. 4)

Basionym: *Glossadelphus ogatae* Broth. & Yas., Rev. Bryol. 53: 4. 1926. Type: JAPAN. Mt. Kano, Miyazaki Pref. M. Ogatae, in hb. *Sasaoka, no. B. 1979* (isotype PC, cotype TNS!)

Glossadelphus nipponicus Reim. & Sak., Bot. Jahrb. 64: 556, t. 22. 1931. Type: JAPAN. Higane, Prov. Izu, in hb. Sakurai, no. 2012 (isotype MAK).

Glossadelphus sakuraii Reim., Bot. Jahrb. 64: 556, t. 22. 1931. Type: JAPAN. Higane, Prov. Izu, in hb. Sakurai, no. 2028 (isotype, MAK).

Glossadelphus percymbifolius Sak., Bot. Mag. Tokyo 53: 290, f. 6.1936. Type: JAPAN. Mt. Kunimi, Prov. Higo, in hb. Sakurai, no. 8998 (isotype MAK).

Plant robust, creeping, growing in turfs and like a curtain; yellowish-green to green, glossy when dry. Primary and second stems prostrate, reddish or orange-brown; cross section with epidermis of 2–3 rows of small, thick-walled cells; cortical cells loosely areolated, thin-walled; central strand absent; loosely and irregularly branched. Branches, obutse, loosely imbricate leaves. Stem leaves ovate; costa double, short; margin crenulate or serrulate above. Branch leaves larger than stem leaves, dorsal oblong or ovate, obutes at the apex; costa double, more than less 1/3 leaf long, asymmetric; margin serrulate near the apex; 0.9–1.5 mm long, 0.4–0.6 mm width; median leaf cells vermiculate, 70–85 mm long; papillose at the end of cells; alar cells subquadarate, small, slightly distinct.

Specimems examined: JAPAN, Kagoshima Pref., Yakushima Island,

Onnagawa, *W.Kim529* (KB, HIRO); Maedake, *W.Kim 1251, 1254, 1258, 1261, 1262, 1267* (KB, HIRO); Aigodake, *W.Kim 1355, 1357, 1359, 1360, 1361, 1364, 1365, 1366, 1367, 1368, 1369, 1373, 1374, 1375, 1378* (KB, HIRO); Ohkabu-hodo Trail, *W.Kim 1381, 1384, 1394, 1395, 1398, 1399, 1400, 1404, 1406, 1409, 1410, 1412, 1415, 1419* (KB, HIRO); Yakusugirando, *W.Kim 1434, 1437, 1438, 1442, 1443, 1444* (KB, HIRO); Ibaraki Pref., Mt. Tsukuba, *M. Takaoka s.n., H. Sasaoka s.n.* (TNS 185475); Mie Pref., Kinomoto terrestrial, *M. Tagawa 2372*, Kizu, *E. Sakuma 3495*; Wakayama Pref., Mt. Nati, *M. Tagawa 2387*; Hiroshima Pref., Iwaidani, *Watanabe 23594*; Saitama Pref., Mt. Kumakura, *T. Iwata 936, 967, 1261*; Yamagata Pref., Momizi-kyo, *Y. Sato 23*; Izu Islands, Mikura-jima, *M. Higuchi 37463, 37487, 37533*; Tochigi Pref., Senjogahara Moor, *H. Inoue 25463*, Aomori Pref., Mt. Kinashi-dake, *N. Saito 498* (all TNS); Korea: Gyeongi Prov., Bukhansan, *W.Kim 1454, 1456, 1460, 1466, 1468, 1472* (KB, HIRO); Gangwon Prov., Chiaksan, *W.Kime 1514, 1515, 1516, 1517, 1526* (KB, HIRO).

Distribution: Japan (Honshu, Kyushu, Sikoku) and Korea (Gyeonggi-do, Gangwon-do)

Tixier (1988) regarded *Glossadelphus chrysobasilaris* Broth. as a synonym of *Filibryum ogatae* (= *G. ogatae*). However, we recognised that the former was similar to *Symphyodon* spp. Although we re-examined the isotype specimens of the former, we did not recognise any common morphological characteristics between both species. Furthermore, this species was distinguished from *F. ogatae* by having decurrent leaf bases. Therefore, we recognised that *G. chrysobasilaris* was a species of genus *Symphyodon*, and could not therefore be regarded as a synonym of *F. ogatae*.

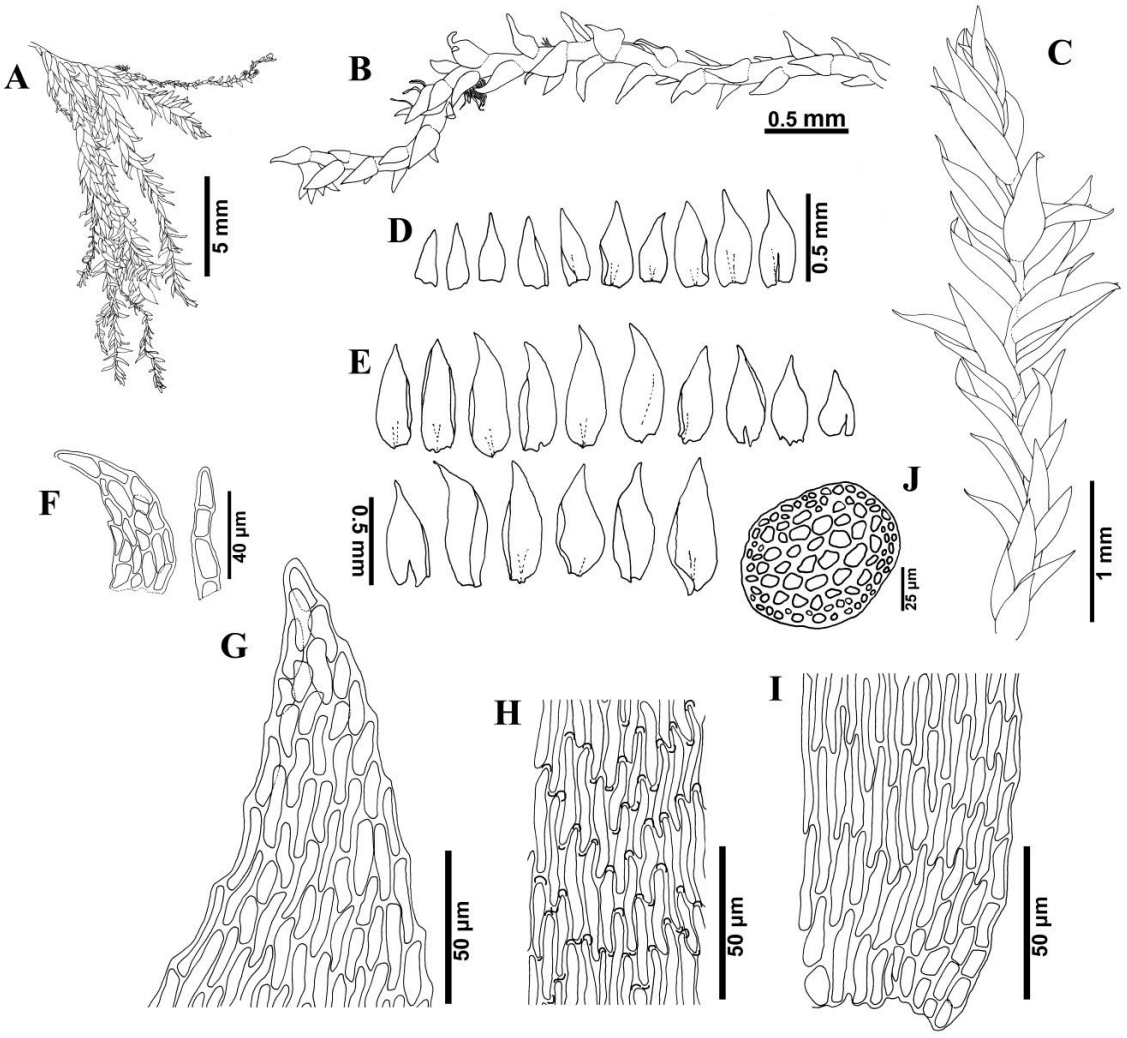

Figure 1. *Filibryum deguchianum* W.Kim and T.Yamag. (all from the holotype, *W.Kim 1336*, KB). A. Plant. B. Stem. C. Branch. D. Stem leaves. E. Branch leaves. F. Pseudoparaphyllia. G Apex of a branch leaf. H. Median cells of a branch leaf. I. Basal angle of a branch leaf. J. Portion of cross-section of stem.

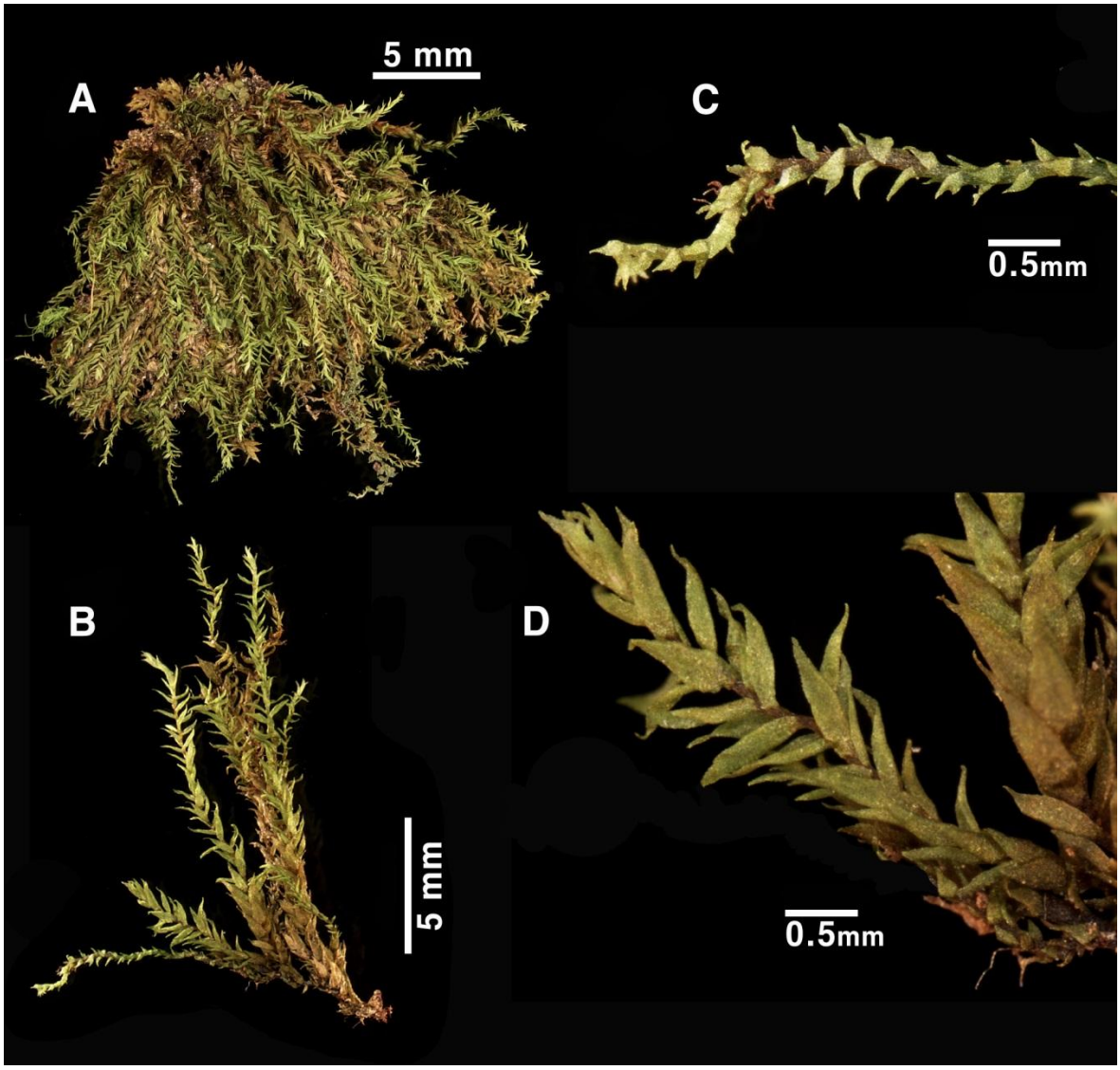

Figure 2. *Filibryum deguchianum* W.Kim and T.Yamag. (all from the holotype, *W.Kim 1336,* KB); A. Habit B. Plant. C. Stem. D. Branch.

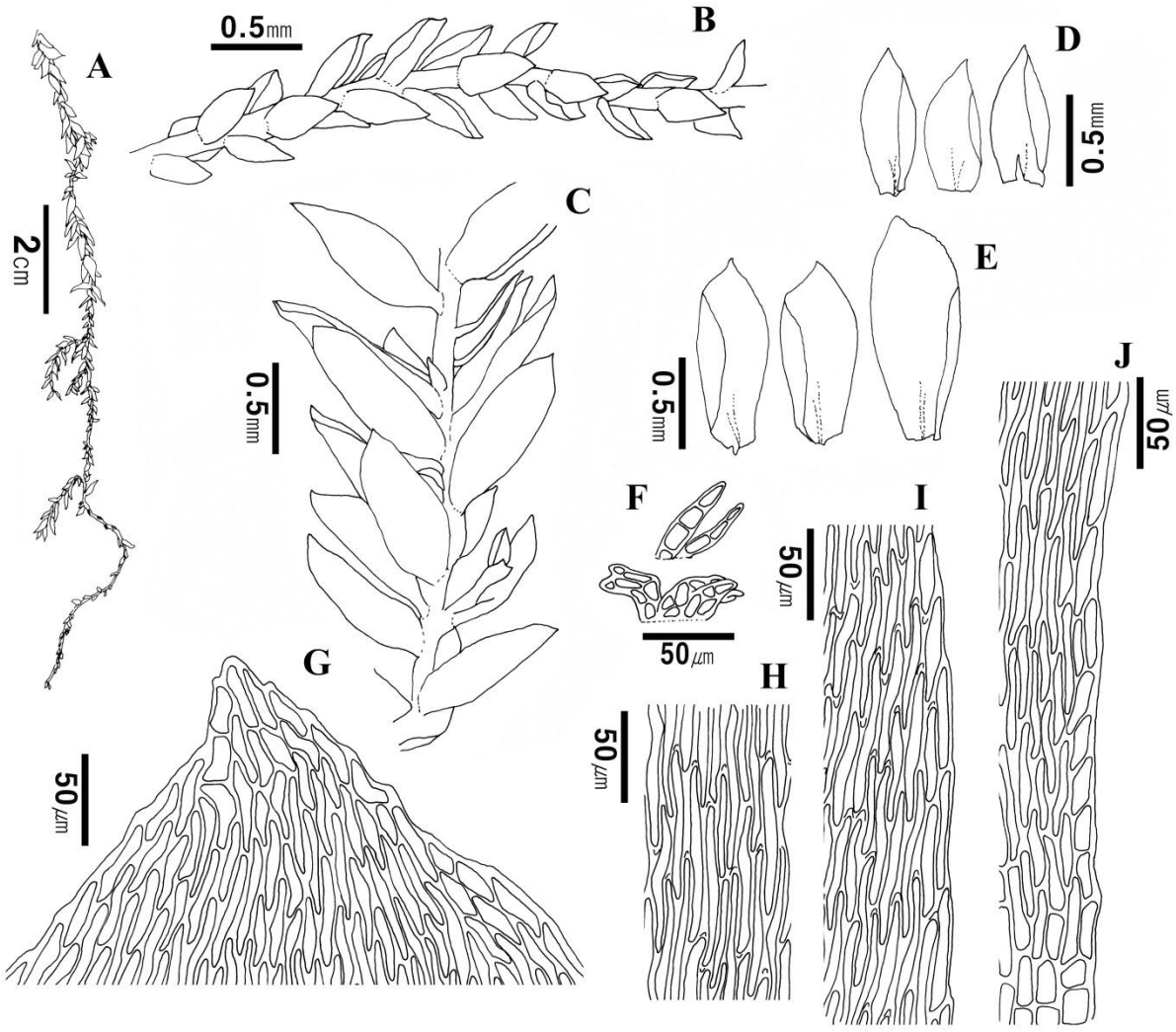

Figure 3. *Filibryum yakoushimae* (all from Isotype). A. Plant. B. Stem. C. Branch. D. Stem leaves. E. Branch leaves. F. Pseudoparaphyllia. G. Apex of branch leaf. H. Median cells of branch leaf. I. Basal angle of branch leaf.

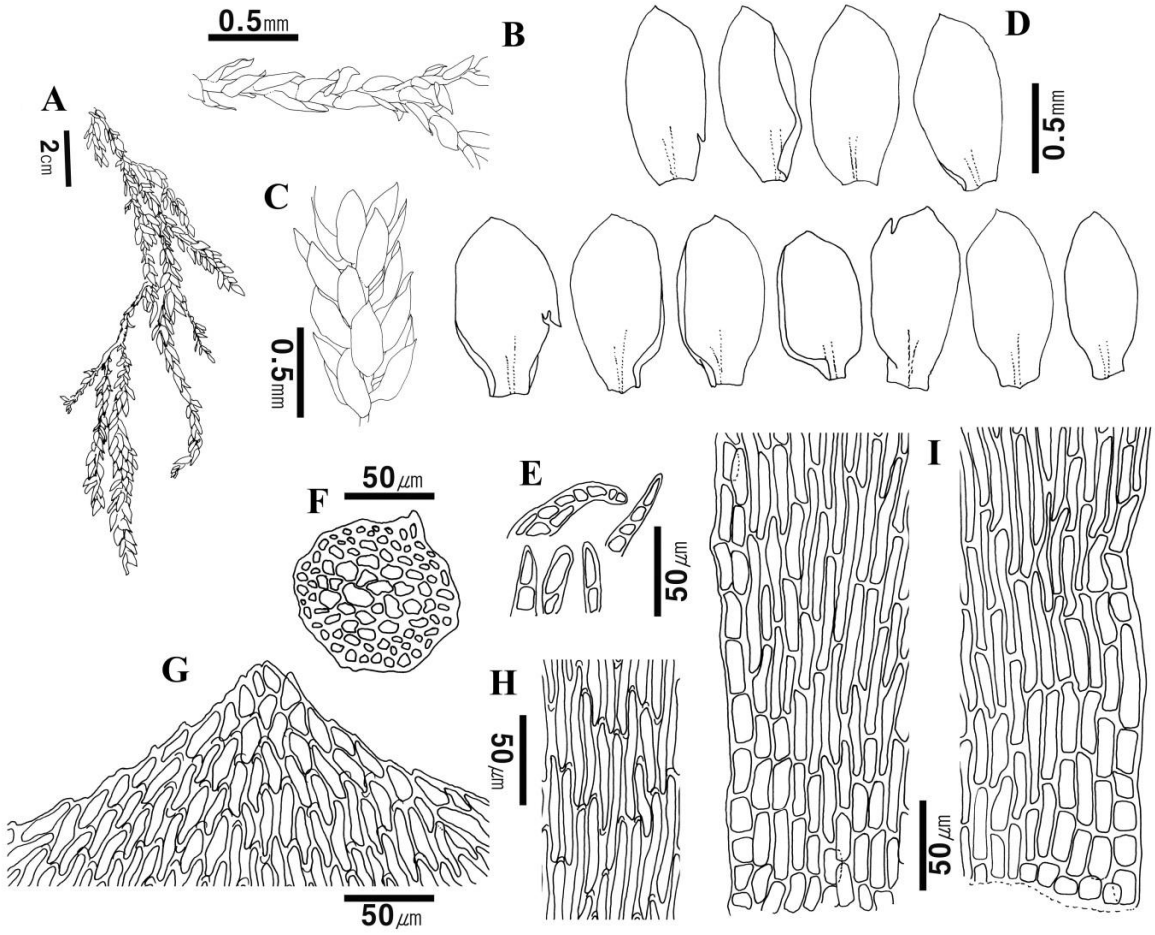

Figure 4. *Filibryum ogatae* (all from Isotype). A. Plant. B. Stem. C. Branch. D. Stem leaves. E. Pseudoparaphyllia. F. Portion of cross-section of stem. G. Apex of branch leaf. H. Median cells of branch leaf. I. Basal angle of branch leaf.

3) Phylogenetic position

Based on DNA sequence analysis of *Filibryum deguchianum* and *F. ogatae* genes, there are 30 parsimony informative sites in chloroplast ribulose bisphosphate carboxylase large subunit (*rbc*L) gene sequences (1,428 bp) between both species. Additionally, both species differed by 14 bp in internal transcribed spacer (ITS) region sequences (563 bp) from the ITS 1 & 2 regions. From intraspecific genetic variation analysis using ITS sequences, a 1-bp variation was observed among eight operational taxonomic units (OTUs) of *F. deguchianum* (Table 1).The phylogenetic analysis based on *rbc*L data resulted total 15,131 topologies, 1079 from the ML analysis, 10050 from the MP analysis, and 4002 from BI analysis. After removing tree with the same topology, 5,711 trees remained, of which 4,174 topologies passed the AU test. *p*-value derived from the AU test (AU), bootstrap probability (NP), and Bayesian posterior probability (PP) values analyzed by using these topologies are shown on the best ML tree (Fig. 5). The original ML, MP and BI analysis confirmed that *F. deguchianum* is the closest relative to *F. ogatae* which is also strongly supported by AU test (AU), bootstrap probability (NP), and Bayesian posterior probability (PP) values of 98, 100, and 100%, respectively (Fig. 5). The phylogenetic tree contains three major clades: Clade I, which includes Sematophyllaceae and *Symphyodon* Mont., and Clade II, which includes Hypnaceae. Then, *Filibryum* was placed in the last clade, Clade III. Clade III is heterogeneous and comprises five main lineages in an unresolved relationship: *Filibryum* group, *Taxiphyllum-Hondaella* group, *Miyabea-Bissetia* group, *Eurohypnum-Hypnum cupressiforme* group, and *Thamnobryum-Neckera* groups. *Filibryum* was found to

be most closely related to *Taxiphyllum*, as suggested by Tsubota *et al*. (2002) and Arikawa *et al*. (2008). Furthermore, *P. lingulatus* was shown to have closer relationship together with *Symphyodon* than *Filibryum*. This result supports the advanced research (Pokorny *et al*., 2012). Consequently, we considered *P. lingulatus* - *Symphyodon* spp. to be closely related to the Sematophyllaceae group in Clade I (Fig. 5). Then, *Ectropothecium* – similar in terms of morphology – was placed within Clade II. Therefore, we verify that neither of these *Filibryum* species shares diagnostic features of *Phyllodon* and yet they share a significant molecular phylogenetic relationship with *Phyllodon* spp. Moreover, they have no phylogenetic relationship with *Ectropothecium* (Fig. 5).

In summary, *Filibryum* spp. differ from *Phyllodon* spp., and with related *Ectropothecium* spp., in the present study involving molecular phylogenetic and morphological analysis. Yet, they have a very close phylogenetic relationship with *Taxiphyllum* spp., although distinguished by morphological characteristics. Therefore, a new genus – *Filibryum* – is included tentatively within the family Hypnaceae because of the sister relationship that exists between members of *Filibryum* and *Taxiphyllum*.

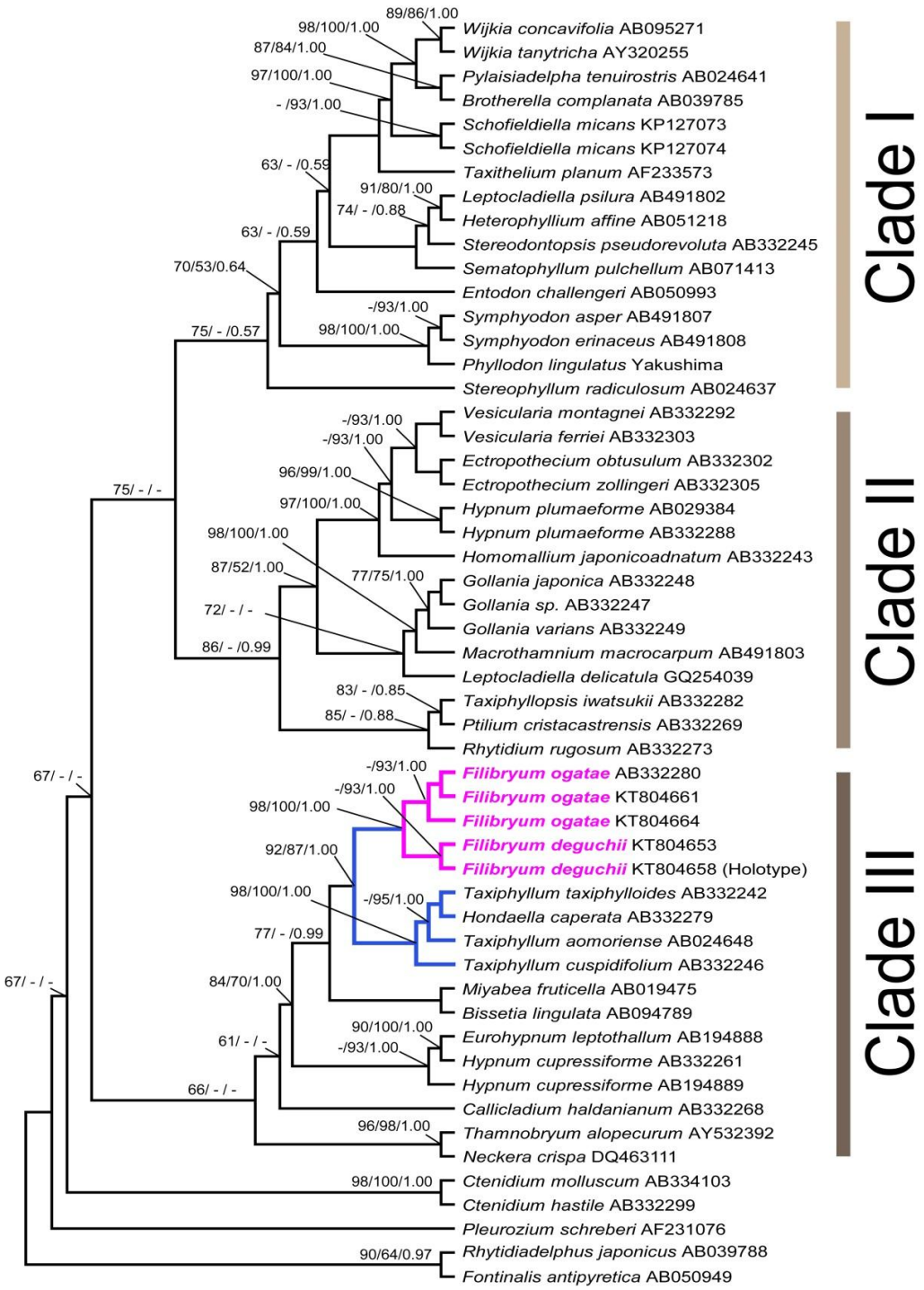

Figure 5. Phylogenetic tree based on analysis of the chloroplast *rbc*L sequence (RAxML 8.1.5, GTR + Gamma model; -ln*L* = 5940.175494). Supporting values more than 50% obtained by the program CONSEL were overlaid: the values by the AU test (AU), bootstrap probabilities (NP), and Bayesian posterior probabilities (PP) are shown on or near each branch (AU/NP/PP; in %).

Chapter 3. Cryptic sepciation of *Filibryum*

Abstract

Filibryum consists three species, and they are distributed in East Asia. However the circumscription of the interspecific morphological difference between *F. ogatae* and *F. yakoushimae* are till unclear. So, the present study anlayzed the genetic variation between both species in order to solve the problem. Furthermore, this study examined the genetic variation of *F. ogatae* related to the geographical distribution. *F. ogatae* is widely distributed in Japan and Korea. On the other hands, *F. yakoushima* is an endemic species of Yakushima Island, Japan. This study analyzed nrDNA ITS, cpDNA *psaA-rps*4 region and mtDNA *nad*5 sequences. In first, the *ycf*3 Intron 1 region was only an interspecific variation site between *F. ogatae* and *F. yakoushimae*. The other regions were not shown to any clear interspecific variation. Secondly, the samples of *F. ogatae* were grouping into three population, which were Japan, Korea and Yakushima population, in order to analyze the geographical genetic variation. As the result, Yakushima population has distinct genetic differences presented on ITS 1 region, *pasA-ycf*3 IGS, *yacf*3 Intron 1 and *rps*4. Therefore, Yakushima population was clustered at the independent clade, and the Japan and Korea population were close on the phylogeny tree. However, it was not shown to any morphological differentiation related to the genetic variation. Therefore, it is possible to assume that Yakushima population of *F. ogatae* is a potential cryptic species undergoing the cryptic speciation.

Introduction

Today, it is possible to quickly get a lot of genetic information for diverse organisms through developing the molecular biological technology. Then, DNA barcoding, a new method in order to identifying large number of species, is brought this increasing availability of DNA sequence data. DNA barcoding enables quick and accurate identification of diverse organism (Xiwen et al., 2015). Also, the application of molecular methods such as DNA barcoding to species delimitation brings about recognizing cryptic diversity (Pérez-Porela et al., 2013). Cryptic species is recognized that two or more distinct species are erroneously classified (and hidden) by morphological characters under one species name (Bickford et al., 2007). Traditional method of species recognition was based on morphological characters, either typoloical or quantitative. Then, which still are considered primary evidence by most of biologists (Bauer et al., 2010; Ahmadzadeh et al., 2013). However, recently developed various methods, such as DNA barcoding, for species delimitation offer the possibility to supplement each other (Templeton, 2001; Morando et al., 2003; Pons et al., 2006; O'Meara, 2010; Fujita et al., 2012, Ahmadzadeh et al., 2013). Recently, cryptic species is received attention against increasing species extinction by destruction and disturbance of natural ecosystem (Bickford et al., 2006). Research on cryptic species has significantly increased over the past two decades, and identifying them challenged taxonomists and biologists even before the Linnaean classification system was adopted (Ezaz et al, 2006;

Bickford et al., 2007). In the case of bryophyte research, Shaw (2001) argued that morphological uniformity masks underlying genetic complexity. It is also acknowledged that most cryptic species result from recent speciation so that morphological or other diagnosable traits have not yet evolved or become manifested (Saez and Lozano, 2005). Furthermore, there is lots of argument as to the morphological difference for delimitating species. Extreme environmental conditions might impose stabilizing selection on morphology, reducing or eliminating morphological change that can accompany speciation (Bickford *et al*., 2007; Schröngge *et al*., 2002). Nevo (2001) argued that evolving under severe environmental extremes can also limit changes in morphology, because there are a limited number of ways in which an organism can adapt to harsh conditions. Therefore, there is a problem of the lack of unequivocal correspondence between morphotype and genotype (Kaliontzopoulo et al., 2012). Moreover, even though some bryophyte species somewhat differe from related species in morphology, those are not shown to the disticint genetic difference. Of course, it means that the genetic difference is not on the whole genome but on the generally using phylogenetic analysis site or genes, such as *rbc*L, *rps*4, ITS and etc. Then, the morphological difference is very subtle, so that it is not easy to express into the description. Morphology of bryophytes is simple compared with vascular plants, therefore, it is very hard to select the diagnostic character among species. Especially, pleurocarpous mosses are growing on flattened and creeping, and branches are spread. Therefore, pleurocarpous mosses usually prefer to grow gametophytes for life time.

Sporophytes is somewhat rare. Consequently, there is lower genetic variation than morphological variation in pleurocarpous mosses. So, it is known to bring about the problem in phylogenetic analysis of them. There is the new pleurocarpous moss genus *Filibryum* from East Asia. This genus is suggested by Kim & Yamaguchi (2016). *Filibryum* is recently consisted to three species, *F. ogatae* (Broth. & Yasda) W.Kim & T.Yamagu., *F. yakoushimae* (Card.) W.Kim & T.Yamagu. and *F. deguchianum* W.Kim & T.Yamagu., and *F. deguchianum* is distinct species from other species as a morphology and genetic information. However, *F. ogatae* and *F. yakoushimae* are not clear to the delimitation of species. These two species are very similar to each other. Then, *F. yakoushimae* is known to distribute in the only type locality, Yakushima Island, Kagoshima Prefecture, and this species is very rare. On the other hands, *F. ogatae* is widely distributed in Japan and Korea. So that, this study firstly verified the genetic variation between *F. ogatae* and *F. yakoushimae*. Nextly, this study also examined the geographical genetic variation of *F. ogatae*, and checked the possibility of cryptic species related to geographical distribution of this species.

Material and Method

Material

Morphological studies were based on herbarium specimens from Hiroshima University (HIRO), the National Museum of Nature and Science, Tokyo (TNS), the Makino Herbarium (MAK), Kochi University (KOCH), the Hattori Botanical Laboratory, Miyazaki (NICH), as well as numerous specimens collected by the authors in Japan and Korea (deposited at HIRO), with duplicates in the National Institute of Biological Resources (KB) and the National Science Museum, Tokyo (TNS).

Morphological observations

Microscopic examinations and measurements were performed using Nikon Eclipse 80i light microscope. Specimens were examined in 1% potassium hydroxide. Descriptions and illustrations of median, alar, and apical cells were made from leaves obtained from the middle of the stem and branch. Leaf width was measured at the widest part.

Molecular taxon sampling, DNA amplification and sequencing

We sampled total 36 samples, 33 vouchers of *Filibryum* and 3 related species vouchers, from 10 regions, 7 regions in Japan Archipelago and 3 regions in Southern Korean Peninsula (Table 5). Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, USA). Polymerase chain reactions were performed in an Eppendorf Mastercycler using the following program: an initial cycle at 95°C for 2 min followed by 35 cycles of 98°C for 20 s, 58°C for 60 s, and 68°C for 1.5 min. A final cycle at 68°C for 10 min was included to terminate amplification. Primers (Table 4) used to amplify and sequence the *nad*5, *rps*4, *rbc*L and ITS1&2 regions are described in Buck *et al.* (2005), Tusbota *et al.* (1999, 2000), Oguri *et al.* (2003) and Inoue *et al.* (2012). PCR products were purified using a Qiaquick PCR Purification Kit (Qiagen, USA) and were sequenced by Macrogen Inc., South Korea (www.macrogen.com). For each sequenced DNA region, forward (5'-3') and reverse (3'-5') sequences were assembled and checked for inaccurate base calling using Sequencher (vers. 5.1, Gene Codes Corp.). Consensus sequences were aligned using CLUSTALX ver. 2.1 software (Larkin *et al.* 2007, Thompson *et al.* 1997) and MEGA 7 software (Kumar *et al.*, 2015) was used for some manual adjustments of the alignment.

Evolutionary analyses

For each OTU and sequenced DNA region, forward (5'-3') and reverse (3'-5') sequences were checked for inaccurate base calling using Sequencher 5.2 (Gene Code Corporation, Ann Arbor, Michigan, USA). Consensus sequences were aligned using CLUSTALX (Thompson et al., 1997). MEGA ver. 7.0.14 (Kumar *et al*., 2015) was used for minor manual adjustments of the alignment. For each data set, phylogenetic reconstruction with Neighbor-Joining method (Saitou and Nei, 1987) by using MEGA7. The evoulationary distances were computed using Kimura 2-parameter method (Felsenstein 1985), and 1 000 replicates of bootstrap test. The substitution pattern and rates were estimated under the Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985). A tree topology for the substitution rates was automatically computed by MEGA7.

Table 4. Primers used in amplification and sequencing of this study.

Primer name	Sequence	
rbcL		
HrL1	5'-ATG TCA CCA CAA GAG ACT AAA GCA GG-3'	
rbcL600F	5'-GTG AAA TCA AGT CCA CCA CG-3'	This study
rbcL919F	5'-CAT GGT ATG CAT TTC CGT GTA-3'	
rbcL557F	5'-GGT AGA GCT GTA TAT GAA TGT CTT CGT GGT GG-3'	
rbcL-130	5'-ACA ATG ATA CTG TTT GTT ATA G-3'	
rbcL-100	5'-CCA AAG ATG TTT TTT TAT AAG A-3'	This study
rbcL270R	5'-GCA ATA TAT TGA TTT TCT TCT CCA G-3'	
rbcL1098R	5'-AAC ACC TGG TAA AGA AACC-3'	
rbcL1346hR	5'-GCA GCT AAT TCA GGA CTC C-3'	
tmRn	5'-GGG TTA GAA GGG ATT CGA ACC CTT GAC-3'	
rps4		
rps4_1R	5'-ATG TCC CGT TAT CGA GGA CCT CGT GTA-3'	
rps4_602F	5'-TGA CGA GAA TAA TAT TCT ACA ACT A-3'	
psaA_340F	5'-CTT GAG CAC TAG GTT TAA TAT GAG TAG GAT CA-3'	
tmT36R	5'-GTA ATG CGA TGG TCA TCG GTT CGA CTC CGA TA-3'	
rps4_830R	5'-TTT GAT TAT GCC TCG AAATCT-3'	This study
psaA_700F	5'-CCT CAT ACG GCT CAC CGATTTAA-3'	This study
ycf3_56F	5'-AAG CGA GAT TGC TTG TTTCGAATT-3'	This study
ycf3_1069R	5'-TTA TTA TGA AGC TAT GCAGAAATCTCGTATT-3'	This study
nad5		
nad5_4F	5'-GAA GGA GTA GGT CTC GCT CA-3'	Buck *et al.* 2005
nad5_3R	5'-AAA ACG CCT GCT GTT ACC AT-3'	Buck *et al.* 2005
ITS		
18S1659B	5'-CGT CGC TCC TAC CGA TTG-3'	
18S1764B	5'-AGA GGA AGG AGA AGT CGT AAC-3'	
26S166BR	5'-GAG GAC GCT TCT CCA GAC TAC-3'	
26S102BR	5'-CCG GTT CGC TCG CCG-3'	
5.8S10B	5'-CTC AGC AAC GGA TAT CTT GG-3'	

Table 5. List of voucher specimens and nucleotide sequence informations.

Species	Voucher No.	Coll. Site	Accession No.		
			ITS	nad5	rps4-psaA
Filibryum ogatae	Higuchi 52158	Mt. Tsukuba, Ibaraki-ken	O	O	O
	W. Kim 1041	Japan, Okayama	O	O	O
	T. Ohgue 2543	Nara	O	O	O
	W. Kim 227	Hiroshima	O	O	O
	W. Kim 1015	Kochi	O	O	O
	W. Kim 1017	Kochi	O	O	O
	W. Kim 1074	Japan, Oita Jojabaru	O	O	O
	Yamaguchi 34310	Maetake	O	O	O
	Yamaguchi 34376	Mt. Aiko, 950m	O	O	O
	Yamaguchi 34404	Ohkabu trail, Jomonsugi	-	-	O
	T. Ohgue 2268	around theYakusugirando	O	O	O
	W. Kim 1260	Japan, Yakushima maedake	O	O	O
	W. Kim 1267	Japan, Yakushima maedake	O	O	O
	W. Kim 1357	Japan, Yakushima Aigodake	O	O	O
	W. Kim 1400	Japan, Yakushima jomonsugi	O	O	O
	W. Kim 1401	Yakushima Jomonsugi 920m	O	O	O
	W. Kim 1410	Jomonsugi 998m	O	O	-
	W. Kim 1443	Jomonsugi 1030m	O	O	O
	W. Kim 1431	Japan, Yakushima yakusugirando	O	O	O
	W. Kim 1441	Japan, Yakushima yakusugirando	O	O	O
	W. Kim 535	Onnagawa	O	O	O
	W. Kim 541	Onnagawa	O	O	O
	W. Kim 1456	Korea, Seoul Bukhansan	O	O	O
	W. Kim 1466	Korea, Seoul Bukhansan	O	O	O
	W. Kim 1468	Korea, Seoul Bukhansan	O	O	O
	W. Kim 1472	Korea, Seoul Bukhansan	O	O	O
	W. Kim 1514	Mt. Chiak, Gangwon-do	O	O	O
	W. Kim 1526	Mt. Chiak, Gangwon-do	O	O	O
	W. Kim 2405	Sundol valley, Jeju-do	O	O	O
F. yakoushimae	Yamaguchi 34381	Mt. Aiko, 950m	O	O	O
	W. Kim 1379	Japan, Yakushima Aigodake 940m	O	O	O
Filibryum deguchianum	W. Kim1336	Mt. Aiko, Yakushima	O	-	O
Taxiphyllum aomorience		Gangwondo, Korea	O	O	O
Pylaisialdepha yokohamae	W. Kim 1673	Korea	O	O	O

Result

1. Geographical distribution and Habitat

I checked the specimens of herbaria from Japan, China and Taiwan, such as the National Museum of Narture and Science (TNS), Makino herbarium (MAK), Kochi University (Kochi), the Hattori Botanical Laboratory (NICH), the Hiroshima University (HIRO), China Academy Science (PE), and the National Museum of Science and Nature, Taiwan (TMN). Then, I surveyed many places to verify growing of this species in Japan and Korea. Therefore, we collected numerous specimens from 10 sites, which are 7 in Japan and 3 in Korea (Table 5). As the result, *F. ogatae* is distributed on Honshu, Kyushu and Sikoku in Japan and on Gyeonggi, Gangwond and Jeju Island in in Korea. Then, this study could not verified to distribute in China and Taiwan. Then, *F. yakoushimae* was known to the Japanese endemic species, and this species was only verified in growing on Yakushima Island, Japan as this study. The habitat of both species, *F. ogatae* and *F. yakoushimae*, are similar, and the habitat environmental traits are as follow.

F. ogatae and *F. yakoushimae*, are growing on wet boulder near to a stream or a valley. And, there is a partial shade or full shade. However, sometimes habitat was somewhat dried (Fig. 6). Then, both species was grown from lowland to about 1,000 m altitude.

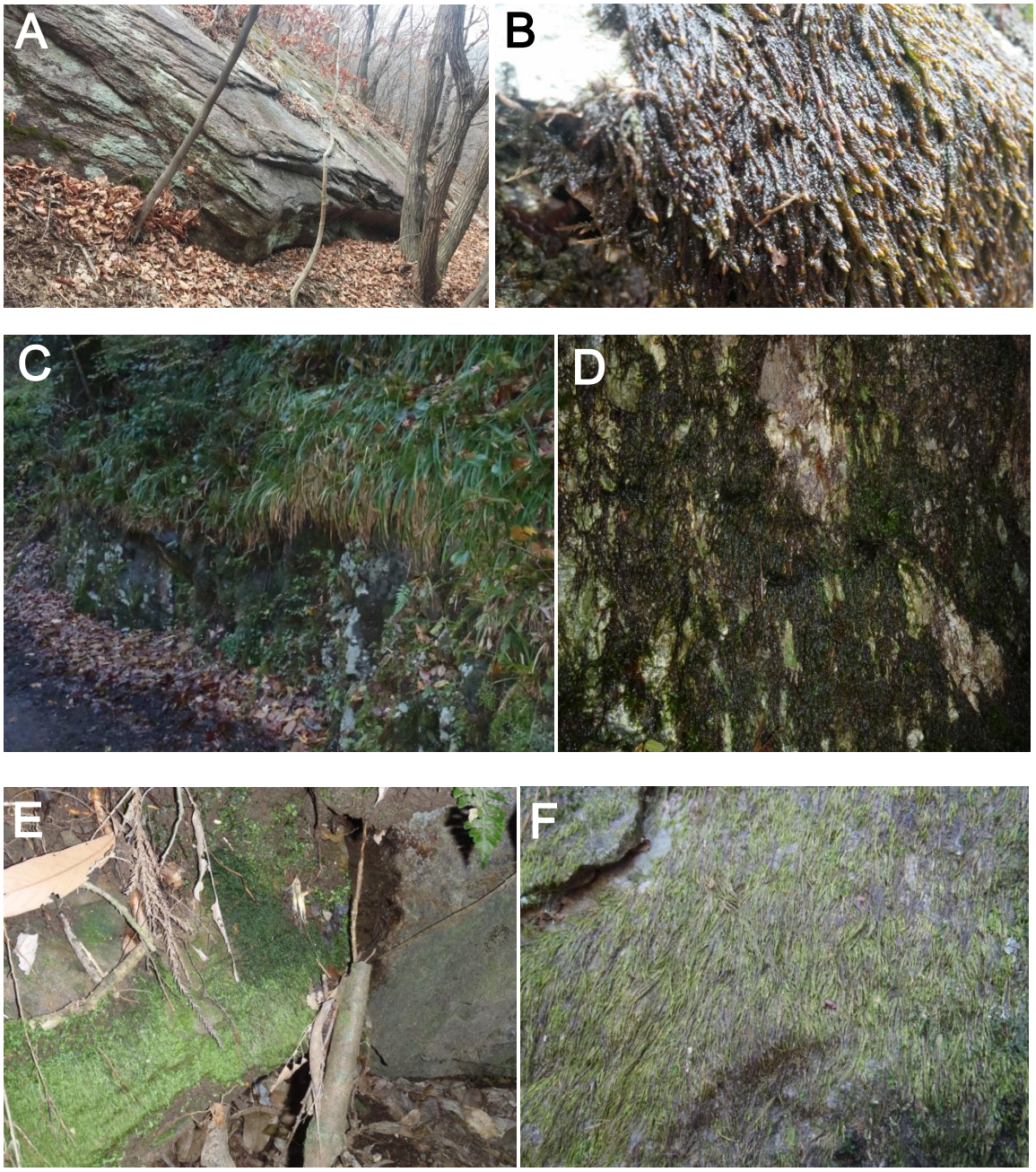

Figure 6. Habitat of *Filibryum ogatae*. A-B. Gyeonggi, Korea. C-D. Hiroshima, Japan. E. Onnagawa, Yakushima. F. Jeju Island, Korea.

2. Molecular data

Sequence size and variation for the different genomic and subgenomic regions are summarized in Table 6. I obtained sequences from 28 individuals of *F. ogatae*, 2 individuals of *F. yakoushimae* and 3 sepcies for outgroups. And, this study reconized that these species were three populations, which are Yakushima, Japan and Korea population, and analyzed the evolutionary relationship among them by using MEGA 7.

Especially, *rps*4 is shown to similar patterns to mitochondrial gene nad5. It is known that the transition/transversion ratio (R) is usually 0.5 to 2.0, and transitions usually occur more frequently than transverstion (Nei and Kumar, 2000).

Table 6. Analysis sequence size of genes.

	Aligned L	% Variable site	Informative	Ti/Tv
ITS	**709**	**5bp**	**1bp**	**1.91**
ITS1	254	0.8	0	
5.8S	160	0.6	0	
ITS2	295	20.7	1	
***rps*4**	**603**	**0.7**	**2**	
*rps*4/*tranS* Spacer	57	0	0	
trnS	**87**	0	0	
tranS/ycf Spacer	199	1	0	
ycf3	**1 930**			**0.14**
*ycf*3 Intron	325	2.2	7	
*ycf*3Exon	1 605	0.1	0	
ycf3/*psaA* Spacer	263	1.1	3	
***psaA** partial*	**181**	**0.6**	**0**	
***nad*5**	757	0.4	2	

2. 1. ITS

ITS sequences were obtained from total 28 OTU of *F. ogatae* and 2 OTU of F. *yakoushimae*. The sequeces length is 709 bp and covers ITS-1 (1-255), 5.8S gene (256-424), ITS-2 (425-667), and 26S gene partial (668-709) (Table 7). Variable sites are 5 sites, and 4 sites among them are singleton variation. Then, ITS-2 region has a substation site, which is a parsinoy informative site. The cytosine type is founded in Yakushima population, and while tymine types with Japan population. Then, both copy types are found in Korea. Reseult of the Neighor-Joining method of this ITS datasets is shown in Fig. 7 (Saitou and Nei, 1987). This tree indicates that the genetic variation of the Yakuhsima population is distinct. There is no interspecific variation between *F. ogatae* and *F. yakoushimae*.

Table 7. Variable and parsimony informative sites on ITS.

Sample No.	Species	Vocher. No.	Population	Subpopulation	ITS1		5.8S	ITS2	26S
					123	136	398	518	682
1	*Filibryum ogatae*	Higuchi52158	Japan	Ibaraki	T	A	A	T	G
2	*F. ogatae*	Ohgue2268	Yakushima	Yakusugirando	·	·	G	C	·
3	*F. ogatae*	Ohgue2543	Japan	Nara	·	·	·	·	·
4	*F. ogatae*	WKim1015	Japan	Kochi	·	·	·	·	·
5	*F. ogatae*	WKim1017	Japan	Kochi	·	·	·	·	·
6	*F. ogatae*	WKim1041	Japan	Okayama	C	·	·	·	·
7	*F. ogatae*	WKim1074	Japan	Oita	·	·	·	·	·
8	*F. ogatae*	WKim1260	Yakushima	Maedake	·	·	·	C	·
9	*F. ogatae*	WKim1267	Yakushima	Maedake	·	·	·	C	·
10	*F. ogatae*	WKim1357	Yakushima	Aikodake	·	·	·	C	·
11	*F. ogatae*	WKim1400	Yakushima	Jomonsugi	·	·	·	C	·
12	*F. ogatae*	WKim1401	Yakushima	Jomonsugi	·	·	·	C	·
13	*F. ogatae*	WKim1410	Yakushima	Jomonsugi	·	·	·	C	·
14	*F. ogatae*	WKim1431	Yakushima	Yakusugirando	·	·	·	C	·
15	*F. ogatae*	WKim1441	Yakushima	Yakusugirando	·	G	·	C	·
16	*F. ogatae*	WKim1443	Yakushima	Jomonsugi	·	·	·	C	·
17	*F. ogatae*	WKim1456	Korea	Gyeonggi	·	·	·	C	·
18	*F. ogatae*	WKim1466	Korea	Gyeonggi	·	·	·	·	·
19	*F. ogatae*	WKim1468	Korea	Gyeonggi	·	·	·	C	·
20	*F. ogatae*	WKim1472	Korea	Gyeonggi	·	·	·	·	·
21	*F. ogatae*	WKim1514	Korea	Gangwon	·	·	·	C	·
22	*F. ogatae*	WKim1526	Korea	Gangwon	·	·	·	C	·
23	*F. ogatae*	WKim227	Japan	Hiroshima	·	·	·	·	·
24	*F. ogatae*	WKim2405	Korea	Jeju	·	·	·	·	·
25	*F. ogatae*	WKim535	Yakushima	Onnagawa	·	·	·	C	·
26	*F. ogatae*	WKim541	Yakushima	Onnagawa	·	·	·	C	·
27	*F. ogatae*	Yamaguchi34310	Yakushima	Aikodake	·	·	·	C	·
28	*F. ogatae*	Yamaguchi34376	Yakushima	Aikodake	·	·	·	C	·
29	*F. yakoushimae*	WKim1379	Yakushima	Yakushima	·	·	·	C	·
30	*F. yakoushimae*	Yamaguchi34381	Yakushima	Yakushima	·	·	·	C	T

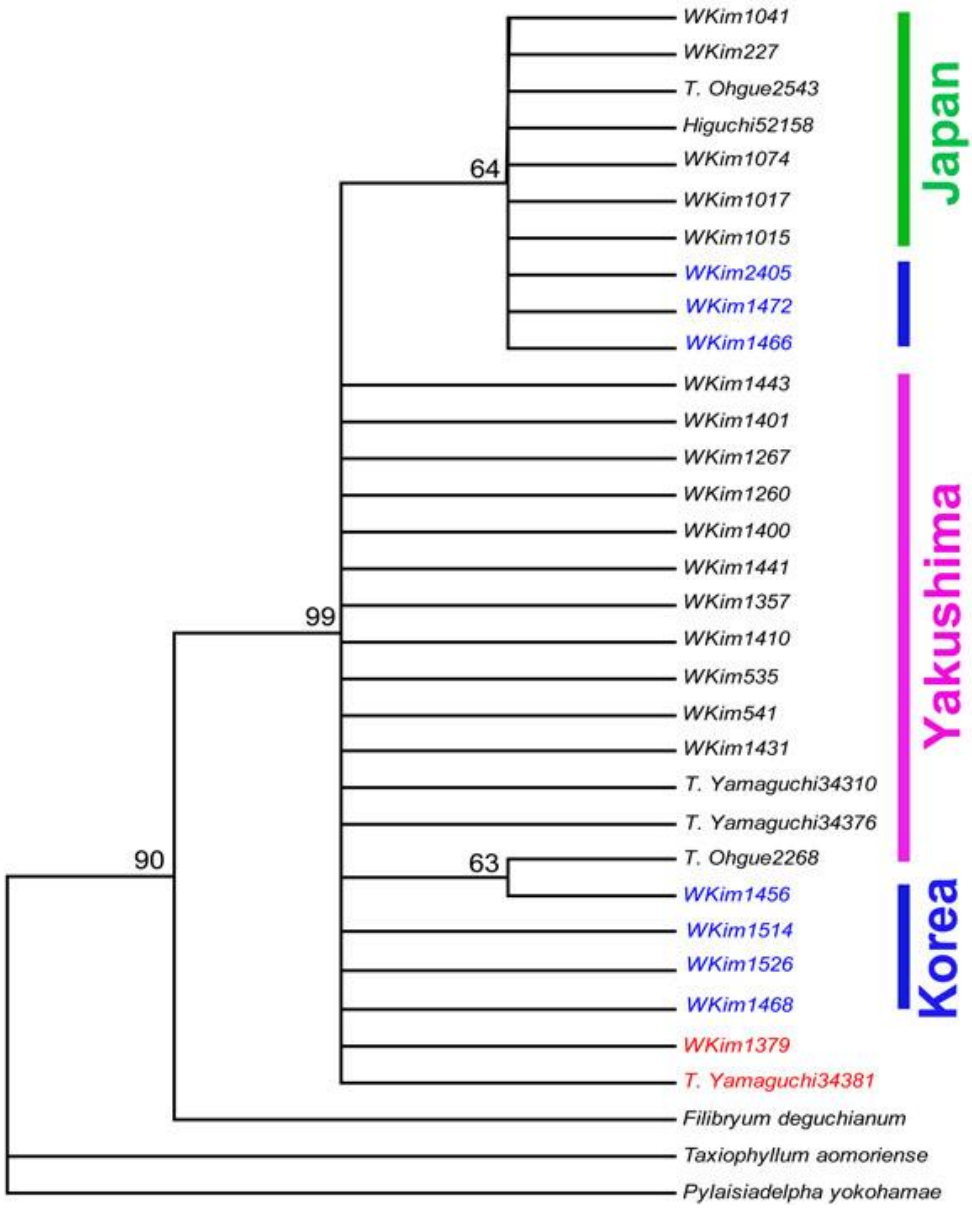

Figure 7. 50% majority rule consensus trees from the Neighbor-Joining method of ITS. The optimal tree with the sum of branch length = 0.18089454 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site.

2.2. *rps*4 – *trn*S - *ycf*3 - partial *psa*A

Alignment of 30 individuals for the three genomic regions plus the coded gaps yielded 3 233 nucleotide sites (603 rps4, 1,930 ycf3, 181 partial pasA, 519 IGS), of which 17 variable sites (0.5%), 12 parsimony-informative (Table 8). The estimated transition/transversion ratio (R) is 0.90. There are four substitution sites. Two of them are on rps4 gene, and the ohters are on *psa*A/*ycf*3 spacer and on *ycf3* Intron 1.

In first, the substitution sites are transition mutation on *rps*4 gene. And, the transversion mutations are on *psa*A/*ycf*3 spacer and *ycf*3 intron 1. The Yakushima population has cytonine at the *psa*A/*ycf*3 spacer (427 site), while Japan and Korea population have adenine. In the case of *ycf*3 intron 1, the adenine type is founded in Yakushima, and while cytosine type with Japan and Korea. Furthermore, there is interspecific variation on *ycf*3 intron1 region. This region has 5 transversion sites between *F. ogatae* and *F. yakoushimae*. The genetic distinct of Yakushima population is more clearly shown on the NJ tree analyzed by using *pas*A-*rps*4 sequences (Fig. 8).

Table 8. Variable and parsimony informative sites from *psa*A partial sequence to *rps*4 complete sequence.

No.	Voucher. No.	*psaA*	*psaA*/*ycf*3 space			*ycf*3 intron1							exon3	intron2	*rps*4			
		81	297	300	427	766	828	829	830	831	1783	2153	2263	2391	2774	3069	3263	3291
1	HG52158	C	G	G	A	A	A	A	A	G	T	C	A	G	G	A	T	C
2	Ohgue2268	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	T
3	Ohgue2543	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
4	wkim1015	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
5	wkim1017	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•
6	wkim1041	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•
7	wkim1074	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
8	wkim1260	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
9	wkim1267	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
10	wkim1357	•	•	•	C	•	•	•	•	•	•	A	•	•	A	G	C	•
11	wkim1400	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
12	wkim1401	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
13	wkim1431	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
14	wkim1441	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
15	wkim1443	•	A	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
16	wkim1456	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
17	wkim1466	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•
18	wkim1468	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
19	wkim1472	•	•	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•
20	wkim1514	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
21	wkim1526	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
22	WKim227	•	A	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•
23	wkim2405	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•
24	wkim535	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
25	wkim541	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
26	YG34310	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
27	YG34376	•	•	•	C	•	•	•	•	•	•	A	•	•	A	•	C	•
28	YG34404	•	•	•	C	•	•	•	•	••	•	A	•	T	A	•	C	•
29	YG34381	•	•	A	C	C	C	T	T	T	•	A	•	•	A	•	C	•
30	wkim1379	•	•	A	C	C	C	T	T	T	•	A	•	•	A	•	C	•

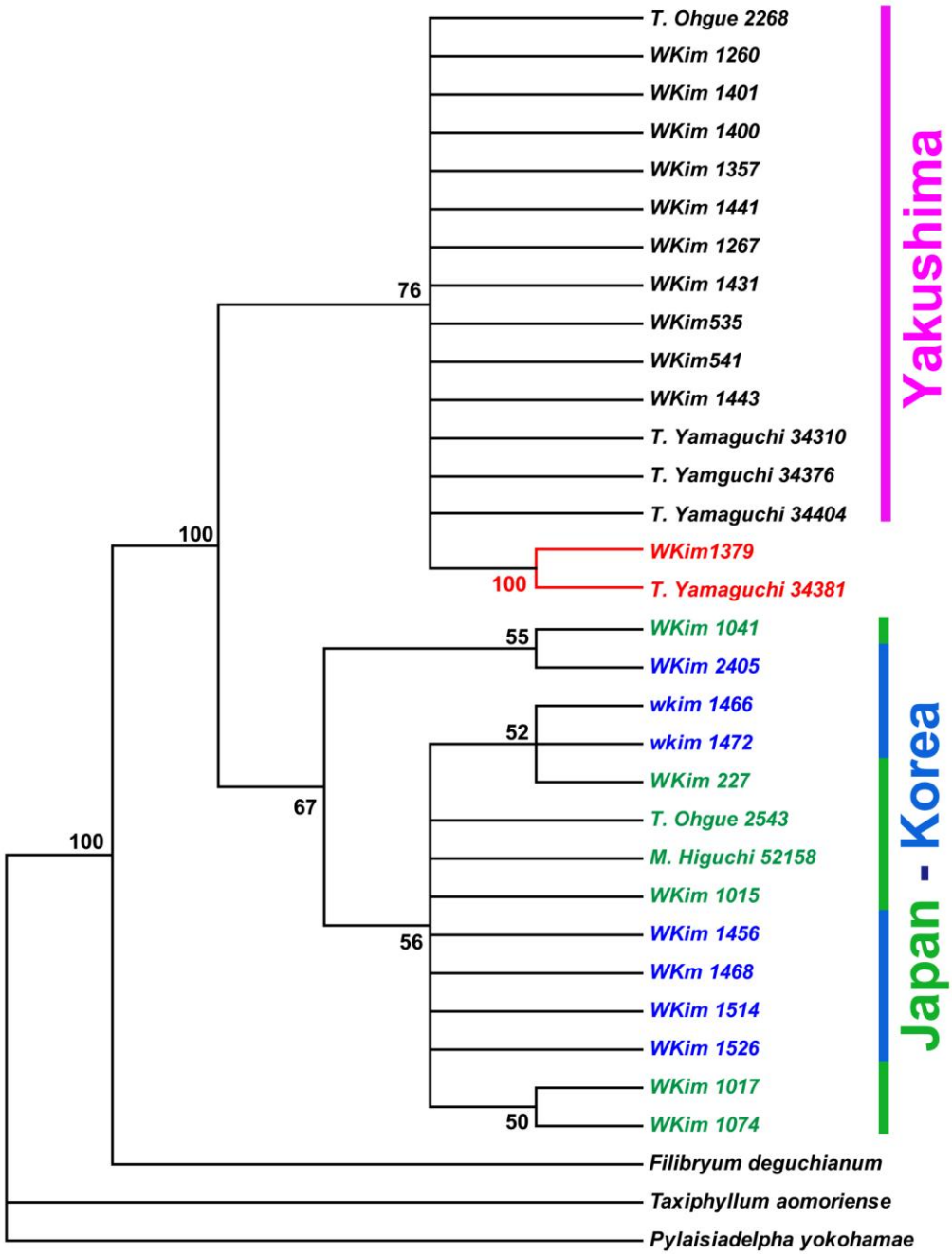

Figure 8. The Neighbor-Joining Tree analyzed by using *pas*A-*rps*4 sequence. The optimal tree with the sum of branch length = 0.09296714 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The analysis involved 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 3238 positions in the final dataset.

2.3 Combine data analysis

This study analyzes that the evolutionary relationship by using combine sequences, which are nuclear DNA ITS sequences and chloroplast DNA *psa*A partial to *rps*4 complete sequences. It is total 4,169 nucleotide sites. As the result, Yakushima population of *F. ogatae* is founded indepently "Yakushima Clade" on the NJ tree (Fig. 8). And, Korea and Japan population are consisted in the same clade "Japan - Korea Clade". There is no genetic variation related to geographical distribution on the Japan-Korea Clade.

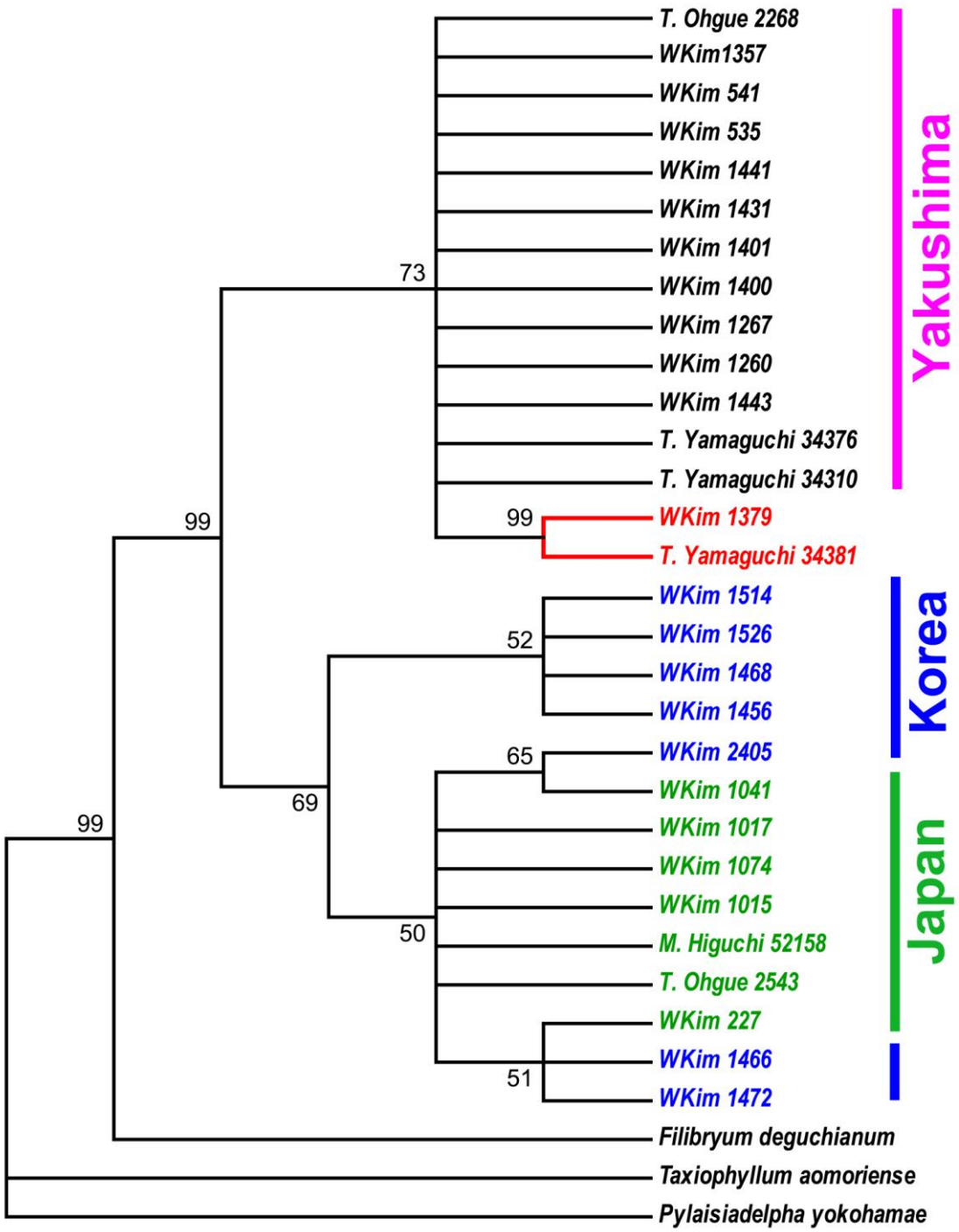

Figure 9. The Neighbor-Joining method analysis tree by using combine sequences (ITS and *psa*A-*rps*4). The optimal tree with the sum of branch length = 0.10649804. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 3816 positions in the final dataset.

2. 5 Mitochondrial gene *nad*5

Obtained 757 nucleotieds (exon 1-73, intron 74-757) of which was 3 variable sites, 2 parsimony-informative sites. A parsimony informative site (595) shows no relationship related to geographical distribution (Table 9). However, another site (85) is only founded in the Yakushima population. Then, thses genetic variation are not shown to interspecific variation. Thsese substitution sites are all transition mutation sites.

Table 9. Variable sites and parsimony informative sites from *nad*5 partial.

No.	Sepceis	Voncher No.	Population	Subpopulation	85	595	685
1	*Filibryum ogatae*	HG521586	Japan	Ibaraki	T	C	G
2	*F. ogatae*	Ohgue2268	Yakushima	Yakusugirando	•	T	•
3	*F. ogatae*	Ohgue2543	Japan	Nara	•	T	•
4	*F. ogatae*	WKim1015	Japan	Kochi	•	•	•
5	*F. ogatae*	WKim1017	Japan	Kochi	•	•	•
6	*F. ogatae*	WKim1041	Japan	Okayama	•	•	•
7	*F. ogatae*	WKim1074	Japan	Oita	•	•	•
8	*F. ogatae*	WKim1260	Yakushima	Maedake	C	•	•
9	*F. ogatae*	WKim1267	Yakushima	Maedake	•	T	•
10	*F. ogatae*	WKim1357	Yakushima	Aikodake	•	T	•
11	*F. ogatae*	WKim1400	Yakushima	Jomonsugi	C	•	•
12	*F. ogatae*	WKim1401	Yakushima	Jomonsugi	C	•	•
13	*F. ogatae*	WKim1410	Yakushima	Jomonsugi	C	•	•
14	*F. ogatae*	WKim1431	Yakushima	Yakusugirando	•	•	•
15	*F. ogatae*	WKim1441	Yakushima	Yakusugirando	•	•	•
16	*F. ogatae*	WKim1443	Yakushima	Jomonsugi	•	•	•
17	*F. ogatae*	WKim1456	Korea	Gyeonggi	•	•	•
18	*F. ogatae*	WKim1466	Korea	Gyeonggi	•	T	•
19	*F. ogatae*	WKim1468	Korea	Gyeonggi	•	•	•
20	*F. ogatae*	WKim1472	Korea	Gyeonggi	•	T	•
21	*F. ogatae*	WKim1514	Korea	Gangwon	•	•	•
22	*F. ogatae*	WKim1526	Korea	Gangwon	•	•	•
23	*F. ogatae*	WKim227	Japan	Hiroshima	•	T	•
24	*F. ogatae*	WKim2405	Korea	Jeju	•	•	•
25	*F. ogatae*	WKim535	Yakushima	Onnagawa	C	•	•
26	*F. ogatae*	WKim541	Yakushima	Onnagawa	•	•	A
27	*F. ogatae*	YG34310	Yakushima	Aikodake	•	T	•
28	*F. ogatae*	YG34376	Yakushima	Aikodake	C	•	•
29	*F. yakoushimae*	YG34381	Yakushima	Yakushima	•	T	•
30	*F. yakoushimae*	WKim1379	Yakushima	Yakushima	•	T	•

Table 10. Interspecific sites on the *ycf*3 intron1 region in *Filibryum*.

Species	Pop.	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849
F. ogatae	Yakushima	A	A	A	G	A	A	A	A	T	-	-	-	-	-	-
	Japan	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-
	Korea	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-
F. yakoushimae	Yakushima	C	T	T	T	.	.	.	.	.	-	-	-	-	-	-
F. deguchianum	Yakushima	.	.	.	.	.	.	.	.	.	T	T	T	T	C	G

Discussion

1. Potential DNA barcode region for pleurocarpous mosses

It is known for pleurocarpous mosses to have low molecular diversity (Shaw *et al.*, 2002; Merget and Wolf, 2010) and short branch length of the phylogenetic tree through the rapid adaptation at the early evolution (Buck *et al.*, 2000). Therefore, pleurocarpous mosses have difficulty to analysis the molecular phylogeny. This study verified the interspecific genetic variation between *F. ogatae* and *F. yakoushimae*. This genetic variation was shown on the chloroplast DNA *ycf*3 gene. It is knonw that *ycf*3 gene produces the essential protein Ycf3 to accumulate the photosystem I (PSI), and is conserved in cyanobacteria, alage, and plant (Helle *et al.*, 2001). As the present study, *Filibryum* has *ycf*3 gene, which is total 1,930 bp including 3 exons and 2 introns (Exon1-147 bp; Intron 1-737 bp; Exon 2-228 bp; Intron 2-692 bp; Exon 3-126 bp). Such as two introns in the *ycf*3 reading frame, it is also known to *Anthoceros* and *Physcomitrella* in bryophytes (Knoop et al., 2004). Then, this study also confirmed 3,233 sequences from *psaA* partial gene to *rps*4 gene. This sequence contains three DNA barcod regions, which are one coding gene (*rps*4) and two non-coding spacers (*psaA*-*ycf*3, *trnS*-*rps*4) (Nishiyama *et al*., 2004; Forrest *et al.*, 2011; Stech & Quandt, 2010). In the case of *Filibryum*, *psaA*-*ycf*3 spacer is 262 bp, and there is an informative site (300 site, Table 10), whereas anthor spacer, *trnS*-*rps*4, there is no interspecific

variation between *F. ogatae* and *F. yakoushimae*. On the other hads, the distinct genetic variation between both species were shown on *ycf*3 gene, especially at the Intron 1 region. This regions has not been paied attention to anlaysis of phylogeny. In the other hands, there were not interspecific variation on ITS sequences. This is known to very useful for analyzing the intraspecific as well as the interspecific genetic variation (Pisa *et al*., 2013). Especially, ITS2 sequence has been used to resolve moss phylogenies at the genus or species level (Olsson *et al*., 2009; Vanderpoorten *et al*., 2006). The ITS2 is a spacer region between two conserved core, genes, 5.8 and 28 S ribosomal DNA. This promotes intra-genomic homogeneity of the repeat units, although high-throughput sequencing showed the occurrence of frequent variations within plant species (Song *et al*,, 2012), and tolerates a highe mutation rate, which results in a more variable DNA sequence. As the result of this study, it was not verified to interspecific variations between *F. ogatae* and *F. yakoushimae* on ITS sequence. There was only an intraspeciefic variation of *F. ogatae*, it was related to the geographical distribution. However, *ycf*3 gene displayed an intraspecific variation as well as an interspecific variation. Unfortunatelly, there are not many studies on *ycf*3 gene in order to analyze the molecular phylogeny. Just, it considers a potential DNA barcode as *psa*A-*ycf*3 spacer. Therefore, this study suggests that the *ycf*3 gene is valuable to analyze the phylgeony of bryophytes. And, it is necessary to examine for an application in more various mosses as the new DNA barcode region.

2. Geographical genectic variation and cryptic speciation on Yakushima Island

It is commonly accepted that rates of morphological and molecular evolution are highly correlated (Barraclough and Savolainen, 2001; Soltis *et al.*, 2002). However, some studies argues when of molecular and morphological variation are uncoupled. It is asserted that some lineages accumulate morphological transformations at a much faster rate than others suggestes that many differences in complex morphological traits (Aigoin *et al.*, 2009; Brakefield, 2006; Devos and Vanderpoorten, 2009) do not result from accumulated muations in multiple genes, but are rather based on one or a few point mutations, or even to changes in the mechanisms of gene regulation (Brakefield, 2006). Also, Hedenäs & Eldenäs (2008) argued that a single or a few genes may be responsible for dramatic morphological modifications in some mosses. On the other hands, mosses exhibiting distinct morphological difference yet share identical non-coding sequences with the common species (Stech & Frahm 1999; Werner et al. 2007; Olsson *et al*. 2009; Sotiaux *et al*. 2009). Then, the cryptic species is considered to hidden species by the traditional classification. As the present study, there are geographical genetic variations in *Filibryum ogatae* (Table 7-8). Firtly, one is on ITS 2 region, this variable site separates two populations, which are Japan and Yakushima population. And, Korea population shares each variation with both populations (Table 7, Fig. 7). It is known that the ITS2 promotes intra-genomic

homogeneity of the repeat units, although high-throughput sequencing showed the occurrence of frequent variations within plant species (Song *et al*., 2012), and tolerates a high mutation rate, which results in a more variable DNA sequence. Secondly, the others are on *psaA*-*ycf*3 spacer, *ycf*3 Intron 1 and *rps*4. These regions distinctly separate Yakushima population from the others (Table 8, Fig. 8). Therefore, this present study verified the intraspecific genetic variation in *F. ogatae*. Additionally, this intraspecific genetic variation is related to the geographical distribution. Although Yakushima population of *F. ogatae* was distinguished from genentic variations, it did not show any morphological differentiation. Therefore, it is possible to consider that Yakushima population of *F. ogatae* is potential cryptic species undergoing the anagenetic divergence through the distinct genetic variation on the variable genes. Kimura *et al*. (2014) reported that *Cryptomeria japonica* (vascular plant) has undergone the anagenetic divergence on Yakushima. This advanced study analyzied the genetic structure of Japanese populations of *C. japonica* by using microsatellite. As the result, Yakushima population of *C. japonica* is distinguished from other Japanese populations. So that, Kimura *et al*. (2014) infers that such as the genetic difference results from geographical isolation. In addition, Kimura *et al*. (2014) considered that Kikai caldera eruption influenced on the geographical isolation of Yakushima Island. Machida and Arai (1992) reported that the Kikai Caldera near the Yakushima Island erupted 7,300 years ago in the Holecene. This eruption is known to influence to the

Akahoya eruption, which is the largest eruption in the Holocene, and it caused catastrophic damage to the forests in Kyushu district and Yakushima Island (Kimura *et al.*, 1996). So that, Yakushima Island could probably be isolated from other gene pools since the LGM. This isolation was drived to the genetic dirft or the bottneck effect into the Yakushima Island. This study considered that such as the affection from the geographical isolation might also influence to the byrophytes in Yakushima Island. So that, it was presented throughout the genetic difference of Yakushima populations in *F. ogatae*. Furthermore, this study also inferred that *F. yakoushima*, endemic species of Yakuahima Island, derived from *F. ogatae* after Kikai caldera eruption. Because, the genetic distinction of *F. yakoushimae* was more similar to the Yakushima group than the Japan - Korea group of *F. ogatae*. Therefore, it is possible to infere that extremely environmental change such as a volcanic eruption was the reason for that morphological difference is unclear despite clear genetic variations, these presented Yakushima Islnad enedemic species, *F. yakoushimae* or Yakushima population of *F. ogatae*. As the advanced studies, it was asserted that extreme environmental conditions might impose stabilizing selection on morphology, reducing or eliminating morphological change that can accompany speciation (Bickford *et al*., 2007; Schröngge *et al*., 2002). In constrary, Nevo (2001) argued that evolving under severe environmental extremes can also limit changes in morphology, because there are a limited number of ways in which an organism can adapt to harsh conditions.

Therefore, this study inferred that environmental change in Yakushima Island following the kikai caldera eruption derived genetic variations by changing its gene pool. On the other hands, the environmental change might limit morphological change. Consequently, this might influence characters of Yakushima's *Filibrum*, which are that the moprhologcial difference between *F. yakoushimae* and *F. ogatae* is unclear, and Yakushima population of *F. ogatae* has the possibility as a cryptic species.

Conclusion

As the result of analyzing the genetic variation, it is considered that *Filibryum* distributes in Japan Archipelago and Korean peninsula from the LGM until now. And, it is able to infer that the Yakushima population of *F. ogatae* is undergoing the cryptic speciation. This cryptic speciation is derived from the geographical events, such as the Kikai caldera eruption (Kimura *et al.*, 2014) during the Holocene. Additionally, chloroplast gene *ycf*3 Intron 1 is possible to apply to the interspecific maker region for *Filibryum*. Furthermore, it is necessary to investigate the possibility of the new DNA barcode region in order to apply to analyzing the phylogeny or the genetic variation of pleurocarpous mosses. And, analyzing the super DNA barcode (Li *et al.*, 2015), chloroplast complete genome is also necessary to more clearly clarify the circumscription between *F. ogatae* and *F. yakoushimae*.

Chapter 4. Phylogeny of *Glossadelphus sesnsu Brotheri*

Abstact

It was pointed out that the diagnostic character was unclear in *Glossadelphus* M.Flesich. after originally described. Therefore, Buck (1987) retreated this genus as a synonym of *Phylldon* Shimp., and some species were treated to other genera, such as *Taxiphyllum* M.Fleisch., *Ectropothecium* Mitt., and *Bryocrumia* L.E.Anderson. Althoguh the bryologist agreed to Buck's concept, there was no study about the phylogenetic relationship of *Phyllodon*. So, this study verified the taxonomical position of *Phyllodon* in Hypnales, and what genera was related to *Phyllodon*. This study was carried out using chloroplast DNA *rbc*L and *rps*4 and nuclear DNA ITS. As the result, *Phyllodon* is closely related to *Symphyodon*, *Chaetomitrium* and *Chaetomitriopsis*. And, *Bryocrumia* is also closely realted to *Phyllodon* and Symphyodontaceae. Additionally, an unknown moss was discovered from Taiwan. This moss is similar to *B. vivicolor*. Furthermore, this moss was also closely related to *B. vivicolor* on the phylogeny tree. Therefore, the unknown moss was considered as a new species of *Bryocrumia* and newly named *Bryocrumia taiwaniana*. Consequenctly, this study suggests that *Phyllodon* and *Bryocrumia* are newly placed in Symphyodontaceae. In addition, it is necessary to re-examin the phylogeny and taxonomy of Symphyodontaceae.

Introdution

As having seen in the study, *Glossadelphus* M. Fleisch. proposed by Flescher (1923) was treated to a synonym of *Phyllodon* by Buck (1987). Moreover, Buck (1987) recognized that some species were not members of *Phyllodon* (Schimp.) W.R. Buck. Therefore, he treated some species to other genera, such as *Bryocrumia* L.E. Anderson, *Ectropothecium* Mitt. and *Taxiphyllum* M. Fleisch.. Then, *Phyllodon* has been placed in the family Hypnaceae by Buck (1987). In addition, he considered that *G. vivicolor* (Broth. & Dixon) Broth., and *Bryocrumia andersonii* (E.B. Bartram) L.E. Anderosn were same species. So, that he treated *B. andersonii* as a synonym of *G. vivicolor*. Moreover, he agreed to the concept of *Bryocrumia* by Anderoson (1980). Consequently, *G. vivicolor* was treated to *Bryorumia* (Buck, 1987), and this genus is a monotypic. Then, there was not phylogenetic study about *Phyllodon* and its related genera after Buck's study (1987). Therefore, this study investigated the taxonomic position and phylogenetic relationship between *Phyllodon* and its related genera. Then, an unknown moss was collected during the field survey in Tawin. This was similar to *B. vivicolor* (Broth. & Dixon) W.R. Buck, however it was distinguished from the lateral leaf form. So, it is suggested that this moss is a new member of *Bryocrumia* L.E. Anderosn. Therefore, this study described a new species in *Bryocrumia*, named to *B. taiwaniana* W. Kim, T. Yamagu., K.-Y. Yao & J.-D. Yang and also investigated its phylogenetic position.

Material

Many sequences using in the phylogeny analysis are collected from GeneBank (http://www.ncbi.nlm.nih.gov/genbank, Appendix 2). In addition, partial sequences are newly obtained from fresh samples or specimens (Table 11, Appendix 3).

Table 11. List of voucher specimens.

Species	Voucher No.	Coll. Site
Bryocrumia vivicolor	*Akiyama, Irie, Printarakul & Kanzai 1289*	Thailand; Chiang Mai, Doi Inthanon National Park, alt. 2,250 m.
B. taiwaniana	*W.Kim 1606*	Taiwan; Nantou County, around the Nature Education Area at Mt. Xitou, alt. 1,286 m, around the rainbow bridge trail
Chaetomitrium leptoma	*AY23428*	Malaysia, Sabah (Boreneo Isl.); Kinabalu National Park, Liwagu trail, on shrub branches, alt. 1,690m
Entodontopsis sp.	*Sun et al. C1078*	Cambodia; Mondulkiri Prov. Mondulkiri Protected Forest, coll. date. 19 Dec. 2011
Homalia pennatula	*Sun et al. C1005*	Cambodia; Mondulkiri Prov. Mondulkiri Protected Forest, coll. date. 17 Dec. 2011
Phyllodon lingulatus	*W. Kim 522*	Japan; Yakushima Island, Onoaida trail, Yakushima-cho, Kumage-gun, Kagoshima Prov.
P. glossoides	*Sun et al. C2069*	Cambodia; Mondulkiri Prov. Mondulkiri Protected Forest, coll. date. 18 Dec. 2010
Symphyodon sp.	*H. Akiyama 22870*	Japan: Miyazaki Pref., Tsuno-cho, at the southern foot of Osuzu Mts., en route from camping site to Yatogi Fall, ca 500 m alt.
Symphyodon scbrisetus	*AY23977*	Vietnam: Lam Dong Co., Da Lat, Bidoup Nui Ba National Park, in the vicinity of Giang Ly Station, alt. 1,470 m

Method

The molecular method including such as DNA extraction and PCR, etc. is following the prior.

Phylogenetic analyses

Phylogenetic relationships were inferred by using Neihbor-Joining Method (NJ) , Maximum Parsimony (MP) and Byesian inference (BI) method.

1) Neihbor-Joining Analysis

Total 231 OTU sequence of chloroplast *rbc*L were used for analyzing the NJ method, in order to investing the position of *Phyllodon* and *Bryocrumia* in Hypnales. Sequences were allinged by using MAFFT (www.ebi.ac.uk/Tools/msa/mafft/), then it was checked manually by using MEGA ver. 7.0 (Kumar *et al*. 2916). NJ analyses and 1,000 bootstrap replicates were performed in PAUP* ver. 4.0a150 (Swofford, 2002). The evolutionary distances were computed using the Kimura 2-parameter meothd (Kimura 1980) and are in the units of the number of of base substitution per site. Codon positons included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. All positions gaps and missing data were eliminated.

2) Maximum Parsimony and Bayesian Analysis

Maximum Parsimony (MP) and Bayseian (BI) analysis were performed by using *rps*4 sequences. And, taxa were selected through the NJ

method analysis. MP analyses were conducted with heuristic searches, maximum branch length equaled zero during the branch swqpping procedure. The heuristic search procedure consisted of 1,000 replicates each with step wise rondom taxon addition to construct the strting tree followed by tree bisection and reconnection (TBR) branch swapping. Bootstrap analyses (Felsenstein 1985) were conducted with 1,000 replicates of the MP analyses. For the Bayesian analysis, choice of the sequence evolution model was performed using the program MrModeltest 2.3 (Nylander 2004). MrModeltest retrieved GTR + G as the optimal model of evolution. A total of 1,000,000 generations were run, sampling every 500^{th} generation using settings: Nst = 6, rates = gamma, statefreqpr = dirichlet (1,1,1,1). A 50% majority rule consensus tree was constructed using the “sumt” command of MrBayes. The tree was edited using TreeView version 1.6.6 (Page 1996).

Taxonomic treatment

Anderson (1980) originally suggested this genus for an American species, *B. andersonii* (E.B. Bartram) L.E. Anderson. This species was originally reported as a member of *Glossadelphus* M. Fleisch. by Bartram (1951). However, Crum (1965) considered that this species was a member of *Taxiphyllum* M. Fleisch., and especially resemble to *T. scalpellifolium* (C.M.) Broth. Later, Anderson (1980) argued that this species was distinguished by morphological characters, such as broad leaf tips, elliptic or oblong-ovate leaf shape and short leaf cells from *Taxiphyllum* or related genus *Isopterygium* Mitt. Therefore, he suggested the monotypic genus *Bryocrumia*. Later, Buck (1987) judged that *G. vivicolor* (Broth. & Dixon) Broth. was a same species to *B. andersonii*. Therefore, *B. andersonii* was treated to a synonym of *B. vivicolor* by him. The distribution of *B. vivicolor* is known to South India, China and Thailand, North America, Uganda, Zaire, Kenya, Tanzania (Crum & Anderson 1981; Buck 1987; Redfern *et al*. 1996; O'Shea & Buck 2001; Kis 2002; Printarakul *et al*. 2013; Ma *et al*. 2016). During bryophyte field survey at Xitou in the middle of Taiwan, a curious moss was colleceted. This curious moss is resembled to *B. vivicolor*. However, this curious moss significantly differes from *B. vivicolor* in the lateral leaf feature. The stem lateral leaf feature of *B. vivicolor* is rounded or slightly truncated at the apex, however a Taiwan curious moss is an obtuse at the apex. Therefore, this study describes this curious moss as a new species in the genus *Bryocrumia*.

Key to the species of *Bryocrumia*

1. Stem lateral leaves elliptical or oblong form and obtuse at the apex .....
..... 1. *B. taiwaniana*

1. Stem lateral leaves sub-orbicular, lingulate or sub-spathulate form and rounded or slingtly truncated at the apex ..... 2. *B. vivicolor*

1. *Bryocrumia taiwaniana* W. Kim ,T. Yamag, K.-Y. Yao & J.-D. Yang. *sp. nov.*

Type: Taiwan: Nantou County, around the Nature Education Area at Mt. Xitou, alt. 1,286 m, around the rainbow bridge trail, *W.Kim* 1606 (*holotype* in KB; *isotypes* in HIRO, TAIE)

(Figures. 11-13)

Plants robust, growing in tufts, creeping, not glossy, green to dark green, old parts brown or whitish yellow. **Stems** sympodially and irregularly branched, branches often attenuate; in cross-section with small and thick-walled outer cortical cells, and large, loosely areolated and thick-walled inner cortical cells. **Leaves** dimorphic, imbricate dorsal stem leaves elliptical or oblong, round or obtuse of the apex, slightly concave, margin serrate above 1/3 and serrulate near to the apex; costae double, short and indistinct, ca. 0.60 x 0.27 mm, median laminal cells vermiculate, prorate, 18-27 μm long; apical laminal cells irregularly form, smaller and shorter than median cells. **Lateral stem leaves** oblong-ovate, concav, obtuse, costae double, indistinct; median laminal cells linear-vermiculate, somewhat prorate at the end, 23-39 μm long; margins entire below, serrate at the spex; alar regions flat, not differentiate, basal corner cells small quadrate. **Branch leaves** ovate, slightly contract the basal, obtuse and serrate at the apex, 0.75 x 0.24-0.27 mm width, median cells vermiculate, 23-43 μm length. **Pseudoparaphylla** like a leaf. **Setae** 1.5-2 cm,

reddish, smooth**. Pericathial leaves** ecostate, 0.9-1.5 mm long, inner leaves longer than outer leaves, lanceolate with broad base, and gradually narrowed above 1/2 length**. Capsuls** small, *ca*. 0.7 mm long without operculum, inclined to horizontal. **Exostome teeth** ca. 320-325 μm long, ca. 70 μm wide at base, curved inwardly when dry, yellowish-brown or amber-colored, densely cross-striolate. **Calyptrae** not seen.

Habitat: Growing on moist rocks

Distribution: Endemic to Taiwan

This species is similar to *B. vivicolor*. It is known that *B. vivicolor* has dimorphic, broadly ovate or oblong-ovate leaf form and leaf arrange imbricated, and conspicuous shining. And, it is newly verified that *B. vivicolor* has distinctly orbiculate dorsal leaves, somewhat spatulate lat eral leaf form and small leaves through re-examining its type specimens. However, this species has no spatulate leaves and shining. Especially, this has ovate lateral leaves, which are obtuse at the apex. Although this is resembled to *Phyllodon similans*, this differs in having no papilose on the leaf median cells. Also, this is distinguished from *P. glossoides*, which has bifid cells at the leaf apex. However, this species has no bifid cells at the apex.

2. *Bryocrumia vivicolor* (Broth. & Dixon) W. R. Buck (Figure 14)

Basionym: *Taxitelium vivicolor* Broth. & Dixon, Rec. Bot. Surv. India 6 (3): 86-87, pl. 1, f. 4. 1914. Type: India . Mahableshwar, Western Ghats; alt. circa 4 000 ft.; Shembaganur, Madura, *M.Foreau* (isotype PC!)

Glossadelphus andersonii Batram., Bryologist 54: 81, f. 1-6. 1951. Type: North America. South Carolina, *Leewis E. Anderson No. 9237* (holotype DUKE) ≡ *Taxiphyllum andersonii* (Bartram.) Crum, Bryologist 68: 220. 1965; ≡ *Bryocrumia andersonii* (Bartram.) L.E.Anderson; Phytologia 45: 66. 1980.

Glssadelphus serpyllifolius P.de la Varde, Ark. Bot. ser. 2, 3: 193. f.33. 1955. Type: Uganda. Rwenzori: Mubuku valley, at a small stream in montane rain forest, 2 100 m, 19 Mar 1948, *Hedberg* 329c p.p. (PC!)

Sepcim. exam.: India, Mahableshwar, Western Ghats, January 1909, *Sedgwick 23* (isotype: PC 0083253, PC 0097020), Shembaganur, Madura, 1911, *Foreau 10* (isotype: PC 0083252); Thailand, Chiang Mai, Doi Inthanon National Park, near Plot E09, *Akiyama, Irie, Printarakul & Kanzai 1289* (HYO).

Habitat: Growing on the moist rock near to the forest stream or around a waterfall.

Distribution: India (Maharashtra, Tamil Nadu), Sri Lanka (Nuwara Eliya), China (Yunnan), Thailand (, North America (Carolina), Africa (Uganda, Kenya, Zaire, Tanzania)

This species is resembled to the genus *Homalia* species, especially very similar to *H. pennatula* (Mitt. ex Dixon) S. He & Enroth at the cells of the leaf apex, the secondary stem leaf arrangement and the alar region cells. However, *H. pennatula* differs in the truncate leaf apex and culti-spathulate leaf shape.

Figure 10. Habitat of *Bryocrumia taiwaniana* W.Kim, T.Yamag.,K.-Y.Yao & J.-D.Yang. (A - C) Habitat, (D) Patch of *B. taiwaniana*.

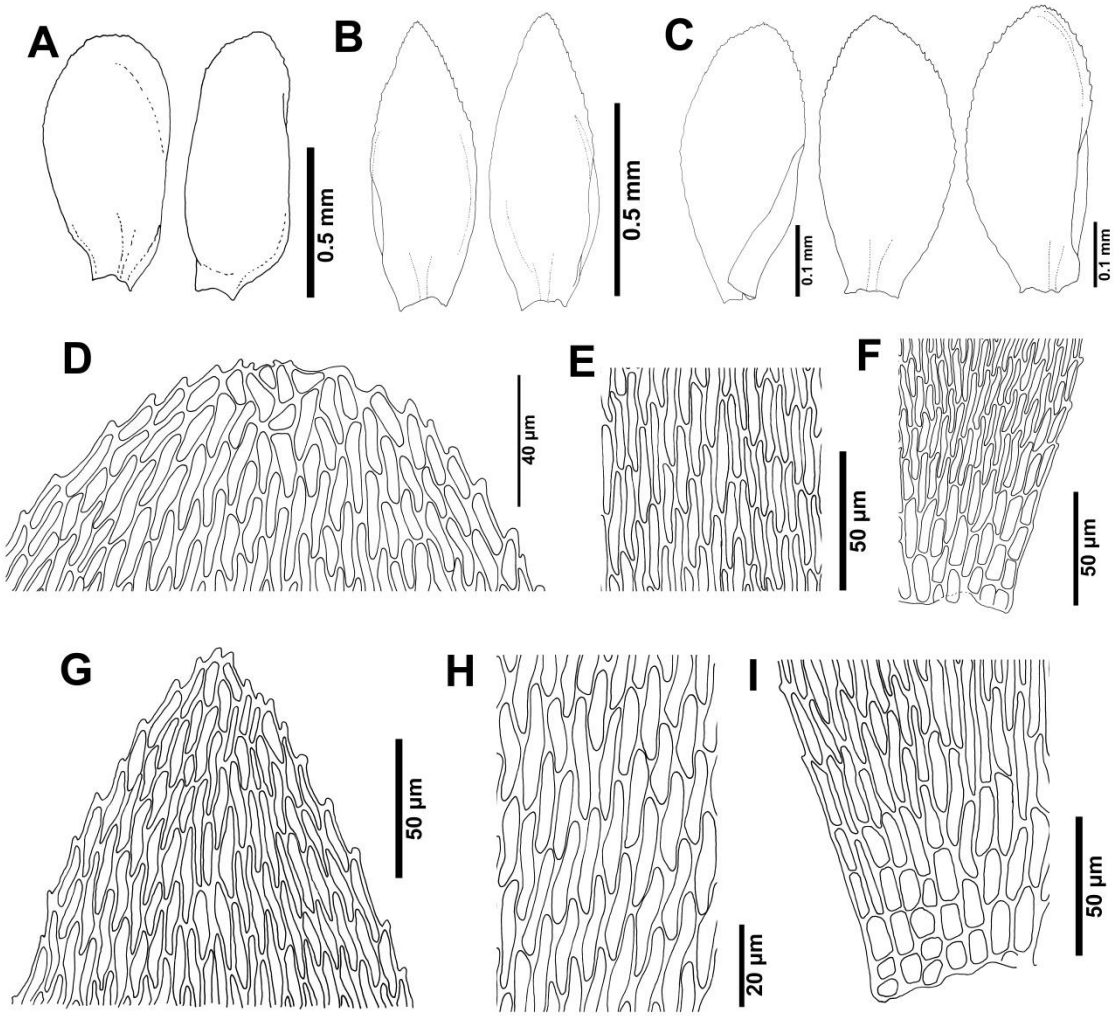

Figure 11. *Bryocrumia taiwaniana* W.Kim, T.Yamag.,K.-Y.Yao & J.-D.Yang. (A) Stem dorsal leaves. (B) Stem lateral leaves. (C) Branch leaves. (D) Apex of stem dorsal leaf. (E) Median cells of stem dorsal leaf. (F) Alar region of dorsal leaf. (G) Apex of stem lateral leaf. (H) Median cells of stem lateral leaf. (I) Alar region of stem lateral leaf. (All from the holotype, *W. Kim* 1606, KB).

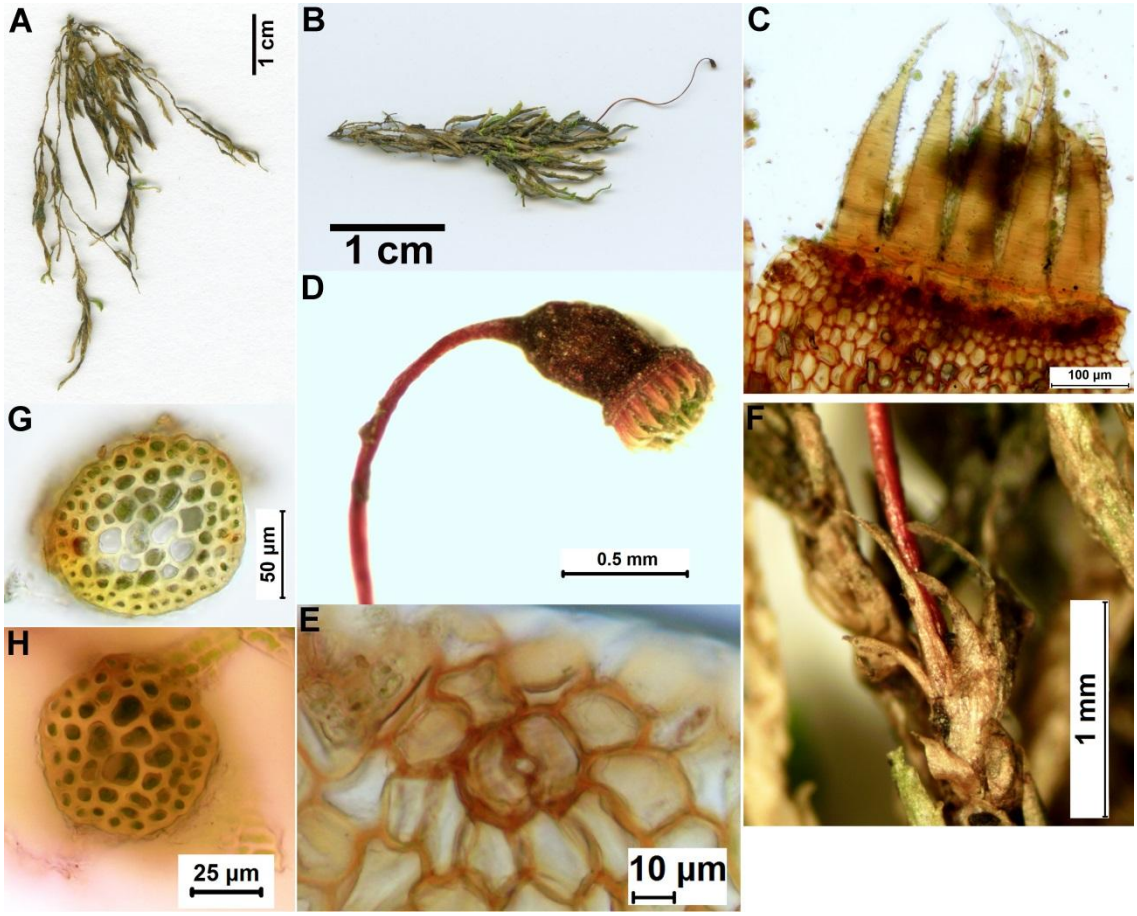

Figure 12. *Bryocrumia taiwaniana* W.Kim, T.Yamag.,K.-Y.Yao & J.-D.Yang. (A & B) Habit. (C) Peristome. (D) Capsule. (E) Stoma cells of capsule. (F) Perichatial leaves, (G) Portion of cross-section of stem. (H) Portion of cross-section of branch. (All from the holotype, *W. Kim* 1606, KB).

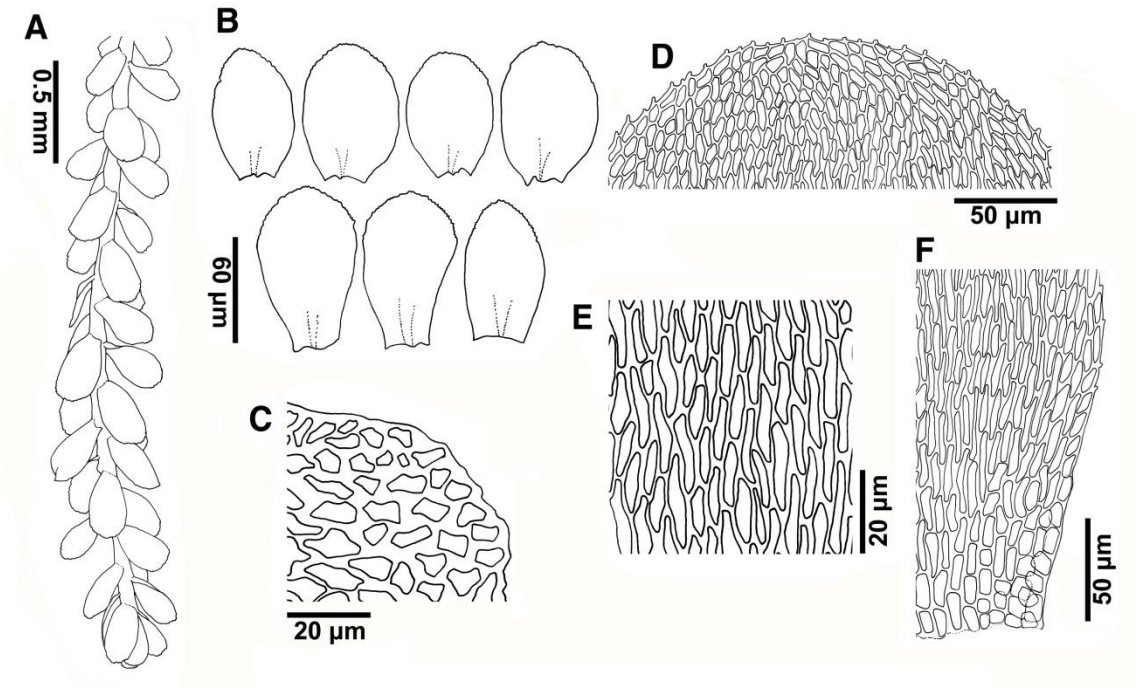

Figure 13. *Bryocrumia vivicolor* (Broth. & Dixon) W.R. Buck. (A) Secondary stem. (B) Secondary stem leaves. (C) Secondary stem cross-section. (D) Cells of the secondary stem leaf apex. (E) Median cells of the secondary stem leaf. (F) Alar region of the secondary stem leaf. (All from isotype, PC).

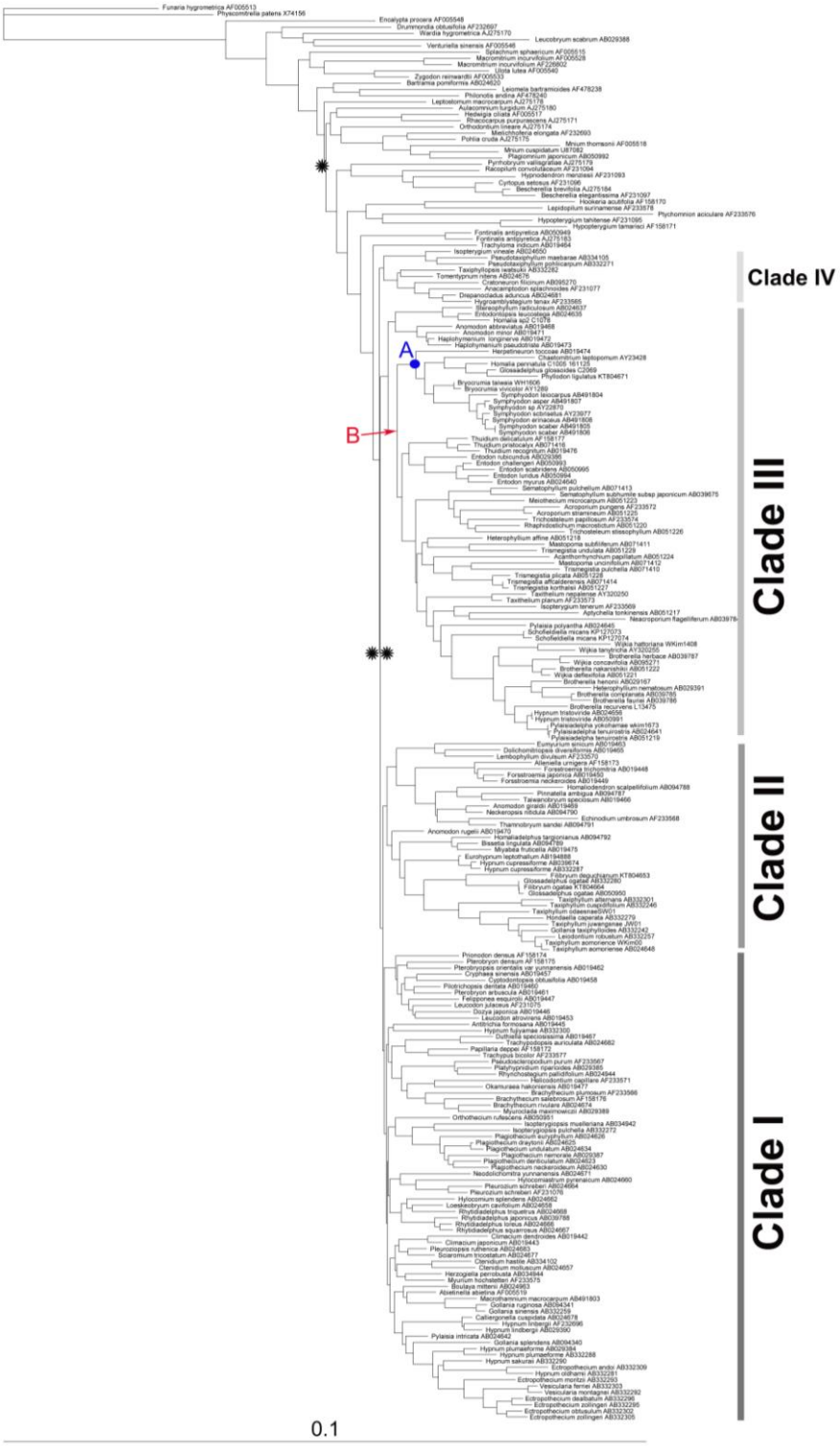

Figure 14. Backbone of Hypnales. Profile Neighbor-Joining method with Kimura 2-parameter distance. Bootstrap values (% of 1000 replicates) of 50% and above are depicted.

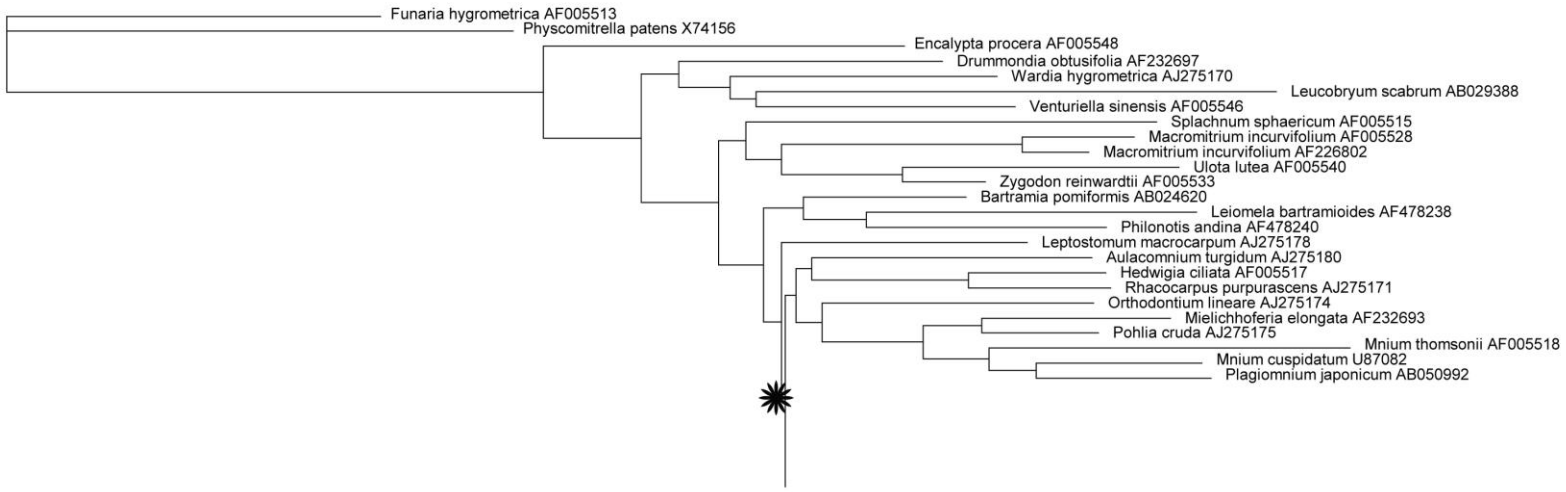

Figure 15. NJ phylogram deducated from *rbc*L, out group.

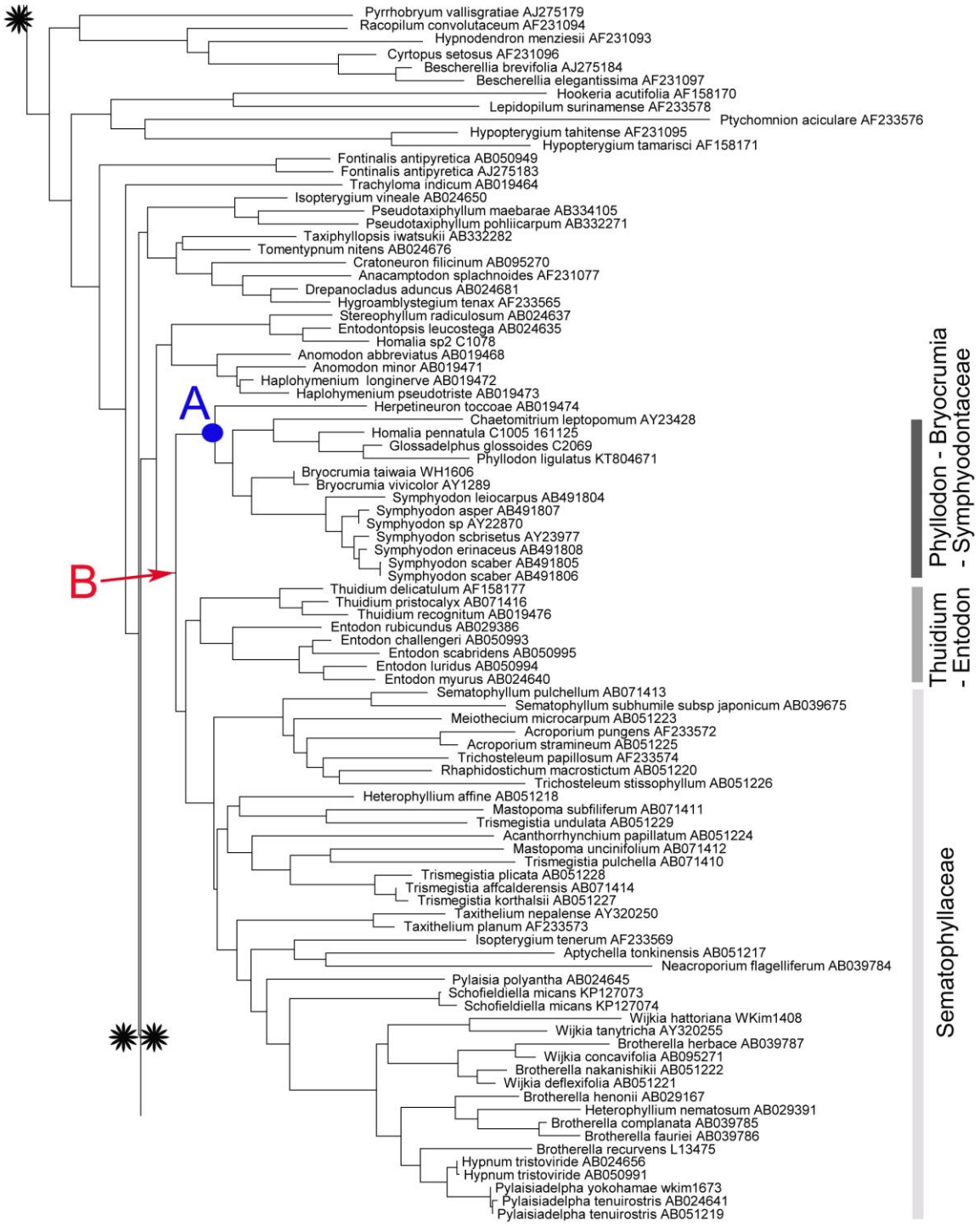

Figure 16. NJ phylogram with details on the hypnalian families Symphyodontaceae, Sematophyllaceae, and Hypnaceae.

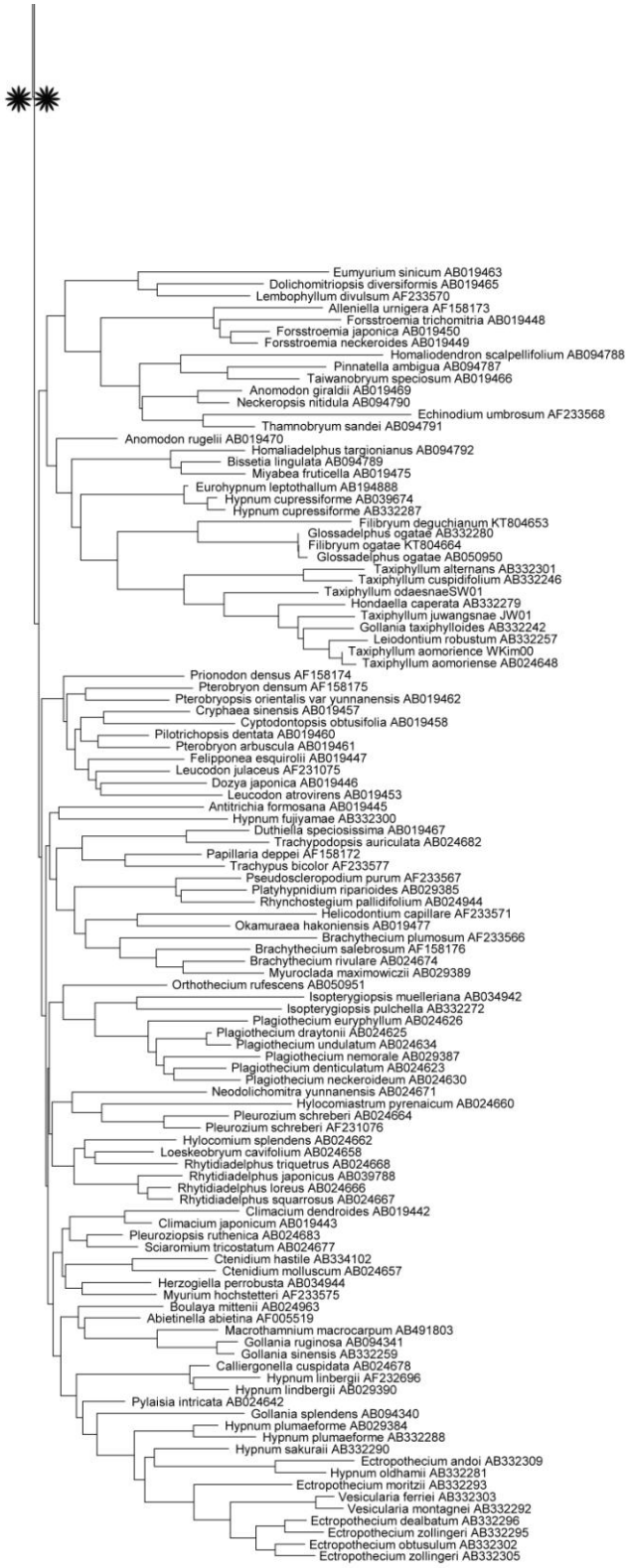

Figure 17. NJ phylogram with details on the hypnalian families Neckeraceae, Hypnaceae, Leucodontaceae, Hylocomiaceae, and Plagiotheciacea.

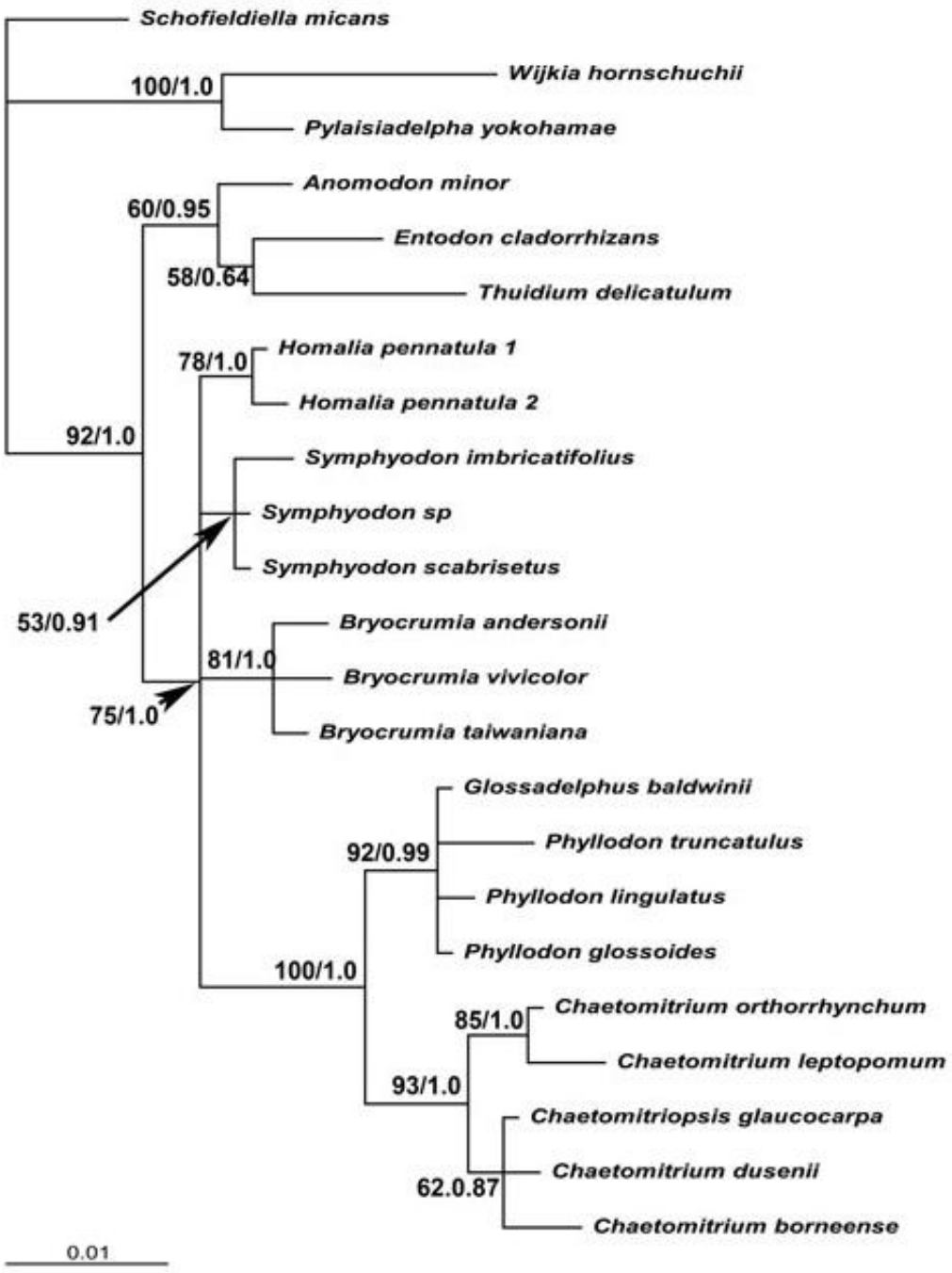

Figure 18. Bayesian 50% Majority-rule consensus tree obtained *rps*4, showing the relationsp among *Phyllodon*, *Brocrumia* and Symphyodontaceae. Numbers above the branches are MP bootstrap values/ Bayesian posterior probabiities.

Result and Discussion

1. Taxonomical position of *Phyllodon* and its related genera

As the result by using *rbc*L sequences, Hypnales was consisted by four big Clads (Fig. 15). Clade I consisted of Hypnaceae, Plagiotheciaceae, Brachytheciaceae and Leucodontaceae, and Clade II consisted of *Taxiphyllum*, *Filibryum*, Anomodontaceae, Leptodontaceae, Neckeraceae and Lembophyllaceae. Then, Clade III consisted of Sematophyllaceae, Entodontaceae, Thuidiaceae and Symphyodontacea. Moreover, *Phyllodon* and *Bryocrumia* was placed in Clade III. Then, Clade Ⅳ consisted of *Isopteryium*, *Taxiphyllopsis*, *Pseudotaxiphyllum*, and *Tomentypnum* and *Hygroamblystegium*. Fristly, *Ectropothecium zollingeri* (≡ *G. zollongeri*), which was a member of the traditional *Glossadelphus* sect. *Ananstigma*, was close to *Vesicularia* (Fig. 18). Secondly, the position of *Phyllodon* and *Bryocrumia* were close to Symphyodontaceae. *Phyllodon* was close to *Chaetomitrium*, and *Bryocrumia* was close to *Symphyodon* (Fig. 17). This result was same to the advanced study's result by using five DNA regions (Pokorny *et al*., 2012). Buck (1987) recognized that *Phyllodon* and *Bryocrumia* were members of the the family Hypnaceae. However, this study showed that both genera, *Phyllodon* and *Bryocrumia* were more closely related to the family Symphyodontaceae than the family Hypnaceae.

2. Phylogenetic relationship between *Phyllodon* and related genera

Verifying accurately relationship among taxa included in the Clade III (Fig. 17) by using *rps*4 sequences. As the result, *Phyllodon* was monophyletic, and close to both genera, *Chaetomitriopsis* and *Chaetomitrium*, and this relationship was strongly supported by bootstrap value (100%, Fig. 19). And, *Bryocrumia* was also monophyletic. However, clade of *Bryocrumia* included an individual of *P. glossoides*, which was collected in Malaysia. So that, it was considered that the individual was uncorrectly identified, because they were not well known and theirs morphological features were somewhat similar. Therefore, it is necessary to re-examine morphological characteristic of the Malaysian individual. On the other hads, an individual of *P. glossoides* collected in Cambodia was placed in the clade of *Phyllodon*. Additionally, morphological features of the Cambodian specimen corresponded to the description and the characteristics of the type specimens of *P. glossoides*. Therefore, this study only recognized that the Cambodian individual was a species, *P. glossoides*. Then, *B. taiwainia* was the closeset to *B. vivicolor*, and there were genetic variations, which were 5 variations sites and one informative site on *rps*4 sequences. Therefore, this study suggests that it is necessary to accurately exaime the phylogenetic relationship and genetic variations between Asian population and American population of *Bryocrumia* by using variable genetic regions. Moreover, it is also necessary to examine the taxonomical position of *Homalia pennatula*. Although it was known that this was similar to *B. vivicolor* (Bartram, 1951), this

was a member of the family Neckeraceae. As the result, *B. vivicolor* and *H. pennatula* shared morphological characteristics, which were subdistichous leaves and the alar region and the apxe of leaf cells. Moreover, this was closer to Symphyodontaceae than Neackeraceae (Fig. 18). Then, it is known that *Chaeotomitrium* Dozy & Molk. and *Chaetomitriopsis* M. Fleisch. are tropical genera, and usually distributed in Southeast Asian regions, which are New Guinea, the Philippines, Indonesia, south China and Malesian peninsula (Akiyama and Suleiman, 2001; Redfearn *et al*., 1996). Additionally, *Chaetomitriopsis* is considered as a monotypic genus. And, it is known that *Chaetomitrium* has papilose or spiny seta, and *Symphyodon* and *Chaetomitriopsis* has smooth seta. In addition, Symphyodontaceae is considered to having spinose capsules. Although, *Phyllodon* and *Bryocrumia* have no spinose capsules, they share characteristics, which are smooth seta, flat alar region and prorate laminall cells, with *Chaetomitrium* and *Chaetomitriopsis*. Therefore, it is necessary to re-examin the diagnostic features of Symphyodontaceae and the relationship between morphorlogical characters and molecular phylogenetic relationship among *Phyllodon*, *Bryocrumia*, and genera of the family Symphyodontaceae. Because, it was verified that *Phyllodon* and *Bryocrumia* were the closeset to genera of Symphyodontaceae in this study.

Conclusion

In first, a Taiwan crurious moss is close to *B. vivicolor*, however distinguished from morphological characters. Therefore, it is considered to a new species of *Bryocrumia*, newl named to *B. taiwaniana*. Secondly, *Homalia pennatula* is very similar to *B. vivicolor*, and this species is shown to have the phylogenetic relationship among *Bryocrumia*, *Phyllodon* and Symphyodontaceae. Therefore, it is necessary to re-examin the taxonomical position of *H. pennatula*. Finally, *Phyllodon* and *Bryocrumia* are related to the family Symphyodontaceae follow the result of the molecular phylogenetic analysis by using chloroplast DNA *rbc*L and *rps*4. Therefore, this study suggests that *Phyllodon* and *Bryocrumia* are placed in the family Symphyodontaceae, and it is necessary to re-examine the phylogenetic relationship among genera of Symphyodontaceae including other genera, such as *Trachythecium* M. Fleisch., *Unclejackia* Ignatov, T. Kop. & D. Norris.

General Discussion

Firstly, *Glossadelphus* was parialy re-examined and treated as a synonym of *Phyllodon* by Buck (1987). So, this study re-exaimed East Asian *Glossadelphus*, which was excepted from Buck's study at that time, and verified the phylogenetic relationships among them. As the result, *G. ogatae*, which was only distributed in Japan and Korea, has no diagnostic characters of Phyllodon, and was not shown the relationship between *G. ogatae* and Phyllodon. Moreover, an unknown moss was discovered from Kyushu, Japan. This unknown moss was similar to *G. ogatae*, and closely related to *G. ogatae*. Therefore, this study considered that it was necessary to describe a new geneus for *G. ogatae* and an unknown species. Consequently, this study suggested a new gneus, *Filibryum*, and placed *F. ogatae*, *F. yakoushima*, and a new species, *F. deguchianum*. Furtheremore, this study anlayzed the genetic variation of *F. ogatae*, which was related to geographical distribution. As the result by using chloroplast DNA *rps*4-*pas*A region and nuclear DNA ITS, it was shown the significantly genetic variation in the Yakushima Population. However, individuals of the Yakushima population were not shown to any morphological variation. Therefore, this study considered that Yakushima population of *F. ogatae* is undergoing the cryptic specieation. And, this study inferred that the distinctive genetic variation of the Yakushima population was caused from the geogaraphical isolation or the change of gene pool, which were influenced by

the volcanic eruption or other geographical events. Secondly, *Phyllodon* was placed in the family Hypnaceae by Buck (1987). And, he treated *G. vivicolor* as to *Bryocrumia*. So that, *Bryocrumia* was a monotypic genus in the family Hypnaceae. However, the recent advanced study showed that *Phyllodon* was related to Symphyodontaceae (Porkony *et al*., 2012). Therefore, this study verified the phylogenetic relationship among taxa of the traditional *Glossadelphus* by using chloroplast DNA *rbc*L and *rps*4. As the result, *Phyllodon* was the closeset to *Chaetomitirium* and *Chaetomitriopsis*. And, *Bryocrumia* was also closely related to *Phyllodon* and Symphyodontaceae. Cosequentlly, this study suggests that *Phyllodon* and *Bryocrumia* are new members of Symphyodontacea. Additionally, it is considered to re-examine the taxonomical position of *Homalia pennantula*. Because this species is resemble to *B. vivicolor* and has close phylogeneitc relationship with *B. vivicolor* and *Symphyodon spp*. Therefore, it is necessary to re-exmine the phylogenetic study and morphological characteristic of Symphyodontaceae. Finally, this study suggests a new *Bryocrumia* species. This new species was discovered from Taiwan, and has the phylogenetic relationship with *B. vivicolor*. However, this new *Bryocrumia* spcecies differed from the lateral leaf form. So, this new species is newly named to *B. taiwaniana*.

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Appendix 1. List of DNA vouchers used in the phylogeny analysis of *Filibryum*. Taxon sampling and DDBJ/EMBL/GenBank accession numbers. Accession information is species name, accession number, locality, and specimen number (herbarium) for chloroplast DNA *rbc*L.

Bissetia lingulata (Mitt.) Broth., AB094789, Japan, *H. Akiyama 14195* (HYO); *Brotherella complanata* Reim. & Sak., AB039785, Japan, *HT-2295* (HIRO); *Callicladium haldanianum* (Grev.) H.A.Crum, AB332268, Japan, *M. Higuchi 42196* (TNS); *Ctenidium molluscum* (Hedw.) Mitt., AB334103, Japan, *N. Nishimura 1l139* (HIRO); *Ctenidium hastile* (Mitt.) Lindb., AB332299, Japan, *N. Nishimura 12109* (TNS); *Ectropothecium obtusulum* (Cardot) Z.Iwats., AB332302, Japan, *N. Nishimura 12055* (TNS); *E. zollingeri* (Müll. Hal.) A.Jaeger., AB332305, *Y. Tateishi 20556* (TNS); *Entodon challengeri* (Paris) Cardot, AB050993, Japan, *H. Tsubota 2727* (HIRO); *Eurohypnum leptothallum* (Müll. Hal.) Ando, AB194888, Japan, *H. Tsubota 4386* (HIRO); *Fontinalis antipyretica* Hedw., AB050949, Japan, *H. Tsubota 3423* (HIRO); *Filibryum ogatae* (Broth. & Yas.) W.Kim & T.Yamag (as *Glossadelphus ogatae* Broth. & Yasuda in database), AB332280, Japan, *M. Higuchi 47056* (TNS); *Gollania japonica* (Cardot) Ando & Higuchi; AB332248, Taiwan, *M. Higuchi 42036* (TNS); *Gollania sp*.; AB332247, Taiwan, *M. Higuchi 43851* (TNS); *Gollania varians* (Mitt.) Broth.; AB332249, Japan, *M. Higuchi 44796* (TNS); *Heterophyllium affine* (Hook.) M.Fleisch., AB051218, Japan, *T. Arikawa 1351* (TNS); *Homomallium japonico- adnatum* (Broth.) Broth., AB332243, Japan, *M. Higuchi 47039* (TNS); *Hondaella caperata* (Mitt.) B.C.Tan & Z.Iwats., AB332279, Japan, *M. Higuchi 47036* (TNS); *Hypnum cupressiforme* Hedw., AB194889, Spain, *H. Tsubota 4384* (HIRO); *Hypnum cupressiforme* var. *filiforme* Brid., AB332261, Russia, *T. Yoshida s.n. 23 Sep. 2005* (TNS); *Hypnum plumaeforme* Wilson, AB029384, Japan, *H. Tsubota 395* (HIRO); AB332288, Japan, *N. Nishimura 12029* (TNS); *Leptocladiella delicatula* (Broth.) J.R.Rohrer, GQ254039, China, *M. Z. Wang 12795-a*; *L. psilua* (Mitt.) M.Fleisch., AB491802, Myanmar, *Murata et al. 22380*

(HYO); *Macrothamnium macrocarpum* (Reinw. & Hornsch.) M.Fleisch., AB491803, Thailand, *H. Akiyama Th-65* (HYO); *Miyabea fruticella* (Mitt.) Broth.; AB019475; *Neckera crispa* Hedw., DQ463111, UK, *N. Bell 1296* (BM); *Phyllodon lingulatus* (Cardot) W.R.Buck, KT804671, Japan, *W. Kim 522* (KB; *Pleurozium schreberi* (Willd. ex Brid.) Mitt., AF231076, USA, *E. L. Conklin 23 Oct 1975*; *Ptilium crista-castrensis* (Hedw.) De Not., AB332269, Japan, *M. Higuchi 4106* (TNS); *Pylaisiadelpha tenuirostris* (Bruch & Schimp. ex Sull.) W.R.Buck, AB024641, Japan, *M. Higuchi 32486* (TNS); *Rhytidiadelphus japonicus* (Reimers) T.J.Kop., AB039788, Japan, *HD-33244* (HIRO); *Rhytidium rugosum* (Ehrh. ex Hedw.) Kindb., AB332273, Japan, *M. Higuchi 43103* (TNS); *Schofieldiella micans* (Mitt.) W.R.Buck, KP127073, Japan, *W. Kim 1018* (KB, HIRO); KP127074, Japan, *W. Kim 1032* (KB, HIRO); *Sematophyllum pulchellum* (Cardot) Broth., AB071413, Japan, *H. Tsubota 3736* (HIRO); *Stereodontopsis pseudrevoluta* (Reimers) Ando, AB332245, Japan, *K. Kawai 2810* (TNS); *Stereophyllum radiculosulum* (Müll. Hal.) A.Jaeger, AB024637, USA, *M. Higuchi 32537* (TNS); *Symphyodon asper* (Mitt.) A.Jaeger, AB491807, Thailand, *H. Akiyama 21543* (HYO); *Symphyodon erinaceus* (Mitt.) A.Jaeger, AB491808, Thailand, *H. Akiyama 21562* (HYO); *Taxiphyllopsis iwatsukii* Higuchi & Deguchi; AB332282, Japan, *H. Kiguchi s.n.* (TNS); *Taxiphyllum aomoriense* (Besch.) Z.Iwats., AB024648, Japan, *T. Arikawa 556* (TNS); *T. cuspidifolium* (Cardot) Z.Iwats., AB332246, Japan, Okayama, *M. Chishiki 4900* (TNS); *T. taxiphylloides* (Ando & Higuchi) M.Higuchi (≡*Gollania taxiphylloides*), AB332242, Japan, *M. Higuchi 47030* (TNS); *Taxithelium planum* (Brid.) Mitt., AF233573, *De Luna 54*; *Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee, AY532392, *C. Cox 147* (RNG); *Vesicularia ferriei* (Cardot & Thér.) Broth., AB332303, Japan, *N. Nishimura 12045* (TNS); *Vesicularia montagnei* (Schimp.) Broth., AB332292, Taiwan, *N. Nishimura 12064* (TNS); *Wijkia concavifolia* (Cardot) H.A.Crum, AB095271, Japan, *H. Deguchi 36051* (HIRO); *Wijkia tanytricha* (Mont.) H.A.Crum, AY320255, *Y. Chang cy0112*.

Appendix 2. List of DNA vouchers used in the phylogeney analysis of *Phyllodon* and related genera. Taxon sampling and DDBJ/EMBL/GenBank accession numbers. Accession information is species name, accession number, locality, and specimen number (herbarium) for chloroplast DNA *rbc*L.

Funaria hygrometrica Hedw., AF005513, *Priddle 1408* (ALTA); *Physcomitrella patens* (Hedw.) Bruch & Schimp., X74156; *Encalypta procera* Bruch, AF005548, *Vitt 37966* (ALTA); Drummondia obtusifolia Müll. Hal., AF232697; *Wardia hygrometrica* Harv. & Hook., AJ275170, *Hedderson 12820* (RNG); *Leucobryum scabrum* Sande Lac., AB029388, *Japan, H. Tsubota 2093* (HIRO); *Venturiella sinensis* (Venturi) Müll. Hal., AF005546; *Splachnum sphaericum* Hedw., AF005515, *Goward 95-1470* (UBC); *Macromitrium incurvifolium* (Hook. & Grev.) Schwägr., AF005528, *Sreimann 49345* (ALTA); Macromitrium incurvifolium (Hook. & Grev.) Schwägr., AF226802, *New Caledonia, Wall 855* ; *Ulota lutea* (Hook. F. & Wilson) Mitt., AF005540, *Fife 8042* (ALTA); *Zygodon reinwardtii* (Hornsch.) A. Braun, AF005533, *Goffinet 636* (ALTA); *Bartramia pomiformis* Hedw., AB024620, *Japan, Arikawa 372* (TNS); *Leiomela bartramioides* (Hook.) Paris, AF478238, *Magombo 5880* (MO); *Philonotis andina* (Mitt.) A. Jaeger, AF478240, *Magombo 5729* (MO); *Leptostomum macrocarpum* (Hedw.) Bach. Pyl., AJ275178, *Visch. s.n. Jan 1973, Rotomanu, Aust.* (DUKE); *Aulacomnium turgidum* (Wahlenb.) Schwägr., AJ275180, *Godizik et al. Exiccata 38* (BM); *Hedwigia ciliate* (Hedw.) P. Beauv., AF005517, *Goffinet 3324* (ALTA); *Rhacocarpus purpurascens* (Brid.) Paris, AJ275171, *Hedderson 11750* (RNG); *Orthodontium lineare* Schwägr., AJ275174, *Hedderson s.n.* (RNG); *Mielichhoferia elongata* (Hoppe & Hornsch.) Ness & Hornsch., AF232693; *Pohlia cruda* (Hedw.) Lindb., AJ275175, *Hedderson 10468* (RNG); *Mnium thomsonii* Schimp., AF005518, *Vitt 35884* (ALTA); *Mnium cuspidatum* Hedw., U87082, ?; *Plagiomnium japonicum* (Lindb.) T.J. Kop., AB050992, *Japan, H. Tsubota 3830* (HIRO); *Pyrrhobryum vallis-gratiae* (Hampe

ex Müll. Hal.) Manuel, AJ275179, *Hedderson 11755* (RNG); *Racopilum convolutaceum* (Müll. Hal.) Reichardt, AF231094, *New Zealand, Wellington, Haurangi Range, Glenny 4941* (Welt); *Hypnodendron menziesii* (Hook.) Paris, AF231093, *New Caledonia, Withey 739* (DUKE) ; Cyrtopus setosus (Hedw.) Hook. f., AF231096, *New Zealand, Glenny 4827* (Welt); *Bescherellia brevifolia* Hampe, AJ275184, *Streimann 38462* (RNG); *Bescherellia elegantissima* Duby, AF231097, *New Caledonia, Withey 732* (DUKE); *Hookeria acutifolia* Hook. & Grev., AF158170, *Mishler 22* ; *Lepidopilum surinamense* Müll. Hal., AF233578, *De Luna 51*; *Ptychomnion aciculare* (Brid.) Mitt., AF233576, *De Luna 53*; *Hypopterygium tahitense* Ångström, AF231095, *New Zealand, Withey 570* (DUKE); *Hypopterygium tamarisci* (Hedw.) Müll. Hal., AF158171, *De Luna 7*; *Fontinalis antipyretca* Hedw., AB050949, *Japan, H. Tsubota 3423* (HIRO); Fontinalis antipyretica Hedw., AJ275183, *Hedderson 11849* (RNG); *Trachyloma indicum* Mitt., AB019464, *H. Akiyama s.n.* (HYO); *Isopterygium vineale* E.B. Bartram, AB024650, USA, Hawaii, *Arikawa 944* (TNS); *Pseudotaxiphyllum maebarae* (Sakurai) Z. Iwats., AB334105, Japan, *H. Tsubota 4956* (HIRO); *Pseudotaxiphyllum pohliaecarpum* (Sull. & Lesq.) Z. Iwats., AB332271, Japan, *M. Higuchi f. no. 75* (TNS); Taxiphyllopsis iwatsukii Higuchi & Deguchi, AB332282, Japan, Okayama, 30 Aug. 2006, *H. Kiguchi s.n.* (TNS); *Tomentypnum nitens* (Hedw.) Loeske, AB024676, Czech Republic, *CCALA M-110. Keil 1950-784*; *Cratoneuron filicinum* (Hedw.) Spruce, AB095270, *H. Tsubota 4773* (TNS); *Anacamptodon splachnoides* (Froel. ex Brid.) Brid., AF231077, USA, Portland Arch, IN, *ML Sargent's culture collection*; *Drepanocladus aduncus* (Hedw.) Warnst., AB024681, Czech Republic, *CCALA M-42. Keil 1947-565*; *Hygroamblystegium tenax* (Hedw.) Jenn., AF233565, *De Luna 49*; *Stereophyllum radiculosum* (Müll. Hal.) A. Jaeger, AB024637, USA, *M. Higuchi 32537* (TNS); *Entodontopsis leucostega* (Brid.) W.R. Buck & Ireland, AB024635, USA, *M. Higuchi 32507* (TNS);

Homalia sp. C1078; *Anomodon abbreviatus* Mitt., AB019468, Akiyama?; *Anomodon minor* (Hedw.) Lindb., AB019471; *Haplohymenium longinerve* (Broth.) Broth., AB019472, Akiyama? ; Haplohymenium pseudotriste (Müll. Hal.) Broth., AB019473, Akiyama? ; *Herpetineuron toccoae* (Sull. & Lesq.) Cardot, AB019474; *Chaetomotrium leptopoma* (Schwägr.) Bosch & Sande Lac., AY23428; *Phyllodon glossoides* (Bosch & Sande Lac.) P. Câmara, C2069; *Phyllodon lingulatus* (Cardot) W.R. Buck, KT804671, Japan, *WKim 522* (KB, HIRO); *Bryocrumia taiwaniana* W.Kim & T.Yamag., Taiwan, *WKim 1606*; *Bryocrumia vivicolor* (Broth. & Dixon) W.R. Buck, AY1289; *Symphyodon leiocarpus* H. Akiyama & H. Tsubota, AB491804, Thailand, *Akiyama Th-127* (HYO); *Symphyodon asper* (Mitt.) A. Jaeger, AB491807, Thaland, *Akiyama 21543* (HYO); *Symphyodon sp.* AY22870; *Symphyodon scbrisetus ??* AY23977; *Symphyodon erinaceus* (Mitt.) A. Jaeger, AB491808, Thailand, *Akiyama 21562* (HYO); *Symphyodon scaber* (Tixier) S. He & Snider, AB491805, Myanmar, *Murata et al. 23237* (HYO); *Symphodon scaber* (Tixier) S. He & Snider, AB491806, Thailand, *Akiyama 21559* (HYO); *Thuidium delicatulum* (Hedw.) Schimp., AF158177, *Mishler 12*; *Thuidium pristocalyx* (Müll. Hal.) A. Jaeger, AB071416, Japan, *H. Tsubota 3862* (HIRO); *Thuidium recognitum* (Hedw.) Lindb., AB019476, Japan, Akiyama s.n. (HYO); *Entodon rubicundus* (Mitt.) A. Jaeger, AB029386, Japan, *H. Tsubot 2313* (HIRO); Entodon challengeri AB050993; Entodon scbridens AB050995; Entodon luridus AB050994; *Entodon myurus* (Hook.) Hampe, AB024640, China, *Matsui 6864* (TNS); Sem*atophyllum pulchellum* (Cardot) Broth., AB071413, Japan, *H. Tsubota 3736* (HIRO); *Sematophyllum subhumile* subsp. *japonicum* (Broth.) Seki, AB039675, Japan, *H. Tsubota 2558* (HIRO); *Meiothecium microcarpum* (Harv.) Mitt., AB051223, Singapore, *B.C. Tan s.n.* (HIRO); *Acroporium pungens* (Hedw.) Broth., AF233572, Mexico, De Luna 46; *Acroporium stramineum* (Reinw. & Hornsch.) M. Fleisch., AB051225,

Malaysia, *H. Akiyama Sarawak-75* (HYO); *Trichosteleum papillosum* (Hornsch.) A. Jaeger, AF233574, Mexico, *De Luna 59*; *Rhaphidostichum macrostictum* (Broth. & Paris) Broth., AB051220, Japan, *H. Deguchi 35017* (HIRO); *Thrichosteleum stissophyllum* (Hampe & Müll. Hal.) A. Jaeger, AB051226, Malaysia, *H. Akiyama Sarawak-112* (HYO); *Heterophyllium affine* (Hook.) M. Fleisch., AB051218, Japan, *T. Arikawa 1351* (TNS); *Mastopoma subfiliferum* Horik. & Ando, AB071411, Thailand, *H. Akiyama Th-2* (HIRO); Trismegistia undulata Broth. & Yasuda, AB051229, Taiwan, *H. Akiyama Taiwan-67* (HYO); *Acanthorrhynchium papillatum* (Harv.) M. Fleisch., AB051224, Malaysia, *H. Akiyama Sarawak-43* (HYO); *Mastopoma uncinifolium* (Broth.) Broth., AB071410, Malaysia, *H. Akiyama Maliau-501* (HYO); *Trismegistia plicata* H. Akiy., AB051228, Malaysia, *H. Akiyma Sarawak-68* (HYO); *Trismegistia aff. caldrensis* (Sull.) Broth., AB071414, Malaysia, *H. Tsubota 4387* (HIRO); *Trismegistia korthalsii* (Dozy & Molk.) Broth., AB051227, Malaysia, *H. Akiyama Sabah-5* (HYO); *Taxithelium nepalense* (Schwägr.) Broth., AY320250, Singapore, *cy0159* ; *Taxithelium planum* Besch., AF233573, Mexico, *De Luna 54*; *Isopterygium tenerum* (Sw.) Mitt., AF233569, Mexico, *De Luna 50*; *Aptychella tonkinensis* (Broth. & Paris) Broth., AB051217, Japan, *H. Deguchi 34995* (HIRO); Neacroporium flagelliferum (Sakurai) Z. Iwats. & Nog., AB039784, Japan, *H. Deguchi 33075* (HIRO); Pylaisia polyantha AB024645, Czech Republic, *CCALA M-95. Keil 1949-752*; *Schofieldiella micans* (Mitt.) W.R. Buck, 1 KP127073, Japan, *W.Kim 822* (HIRO, KB), *Schofieldiella micans* (Mitt.) W.R. Buck 2 KP127074, Japan,*W.Kim 837* (HIRO, KB); *Wijkia hornschuchii* (Dozy & Molk.) H.A. Crum, Japan, *W. Kim 1408* (KB); *Wijkia tanytricha* (Mont.) H.A. Crum, AY320255, Singapore, *cy0112*; *Brotherella herbace* AB039787, Japan, *H. Deguchi 33243* (HIRO); *Wijkia deflexifolia* (Mitt. ex Renauld & Cardot) H.A. Crum, AB051221, Japan, *H. Deguchi 35041* (HIRO); *Brotherella henonii* (Duby) M. Fleisch., AB029167;

Heterophyllium nematosum AB029391; Brotehrella complanata AB039785; Brotherella fauriei AB039786; Brotherella recurvens L13475; *Hypnum tirstoviride* (Broth.) Paris, AB024656, Japan, *T. Arikawa 644* (TNS); *Hypnum tristoviride* (Broth.) Paris, AB050991, Japan, *H. Tsubota 2915* (HIRO); Pylai*siadelpha yokohamae* (Broth.) W.R. Buck, Korea, *W. Kim 1673* (KB); *Pylaisiadelpha tenuirostris* (Bruch & Schimp. ex Sull.) W.R. Buck, AB024641, Japan, *M. Higuchi 32486* (TNS); *Pylaisiadelpha tenuirostris* (Bruch & Schimp. ex Sull.) W.R. Buck, 2 AB051219, Japan, *H. Tsubota 3069* (HIRO); Eumyurium sinicum AB019463, Japan, *H. Akiyama s.n.* (HYO); *Dolichomitriopsis diversiformis* (Mitt.) Nog., AB019465, Japan, *H. Akiyama s.n.* (HYO); *Lembophyllum divulsum* (Hook. f. & Wilson) Lindb. ex Paris, AF233570, Mexico, *De Luna 58*; *Alleniella urnigera* (Müll. Hal.) S. Olsson, Enroth & D. Quandt, AF158173, Mexico, *De Luna 8*; *Forsstroemia trichomitria* (Hedw.) Lindb., AB019448, *H. Akiyma s.n.* (HYO); *Forsstroemia japonica* (Besch.) Paris, AB019450, *H. Akiyama s.n.* (HYO); *Forsstroemia neckeroides* Broth., AB019449, *H. Akiyma s.n.* (HYO); *Homaliodendron scalpellifolium* (Mitt.) M. Fleisch., AB094788, *H. Akiyama 14234* (HYO); *Pinnatella ambigua* (Bosch & Sande Lac.) M. Fleisch., AB094787, Taiwan, *H. Akiyma Taiwan-177* (HYO); *Taiwanobryum speciosum* Nog., AB019466, *H. Akiyma s.n.* (HYO); *Anomodon giraldii* Müll. Hal., AB019469, *H. Akiyama s.n.* (HYO); *Neckeropsis nitidula* (Mitt.) M. Fleisch., AB094790, *H. Akiyama 14210* (HYO); *Echinodium umbrosum* (Mitt.) A. Jaeger, AF233568, Mexico, *De Luna 56*; *Thamnobryum sandei* (Besch.) Z. Iwats., AB094792, *H. Akiyama 14359* (HYO); *Bissetia lingulata* (Mitt.) Broth., AB094789, *H. Akiyama 14195* (HYO); *Miyabea fruticella* (Mitt.) Broth., AB019475, *H. Akiyama s.n.* (HYO); *Eurohypnum leptothallum* (Müll. Hal.) Ando, AB194888, Japan, *H. Tsubota 4386* (HIRO); *Hypnum cupressiforme* Hedw., AB039674, Japan, *H. Tsubota 2793* (HIRO); *Hypnum cupressiforme* Hedw., AB332287, Japan, *N. Nishimura 12008* (TNS); *Filibryum*

deguchianum W. Kim & T. Yamag., KT804653, Japan, *W. Kim* (HIRO, KB); *Filibryum ogatae* (Broth. & Yasuda) W. Kim & T. Yamag., AB332280, Japan, ; *Filibryum ogatae* (Broth. & Yasuda) W. Kim & T. Yamag., 2 KT804664, Japan, *W. Kim 00* (HIRO, KB) ; *Filibryum ogatae* (Broth. & Yasuda) W. Kim & T. Yamag., 3 AB050950, Japan, ; *Taxiphyllum alternans* (Cardot) Z. Iwats., AB332301, Japan, *M. Chishiki 4900* (TNS); *Taxiphyllum cuspidifolium* (Cardot) Z. Iwats., AB332246, Japan, *M. Higuchi f. no. 108* (TNS); *Taxiphyllum sp1*., Korea, *W. Kim sw01* (KB); *Hondaella caperata* (Mitt.) B.C. Tan & Z. Iwats., AB332279, Japan, *M. Higuchi f. no. 150* (TNS); *Taxiphyllum sp2*., Korea, *W. Kim* JW01 (KB); *Taxiphyllum taxiphylloides* (Ando & Higuchi) Higuchi, AB332257, China, *M. Higuchi 29673* (TNS); *Leiodontium robustum* Broth., AB332257, China, *M. Higuchi 29673* (TNS); *Taxiphyllum aomoriense* (Besch.) Z. Iwats., Korea, *W. Kim00* (KB); *Taxiphyllum aomoriense* (Besch.) Z. Iwats., 2 AB024648, Japan, *T. Arikawa 556* (TNS); *Prionodon densus* (Sw. ex Hedw.) Müll. Hal., AF158174, Mexico, *De Luna 21*; *Pterobryon densum* Hornsch., AF158175, Mexico, *De Luna 22*; *Pterobryopsis orientalis* susbsp. *yunnanensis* (Broth.) Nog., AB019462, *H. Akiyama s.n.* (HYO); *Cryphaea sinensis* E.B. Bartram, AB019457, *H. Akiyama s.n.* (HYO); *Cyptodontopsis obtusifolia* (Nog.) Nog., AB019458, *H. Akiyama s.n.* (HYO); *Pilotrichopsis dentata* (Mitt.) Besch., AB019460, *H. Akiyama s.n.* (HYO); *Pterobryon arbuscula* Mitt., AB019461, *H. Akiyama s.n.* (HYO); *Felipponea esquirolii* (Thér.) H. Akiy., AB019447, *H. Akiyama s.n.* (HYO); *Leucodon julaceus* (Hedw.) Sull., AF231075, Canada, *ML Sargent's culture collection*; *Dozya japonica* Sande Lac., AB019446, *H. Akiyama s.n.* (HYO); *Leucodon atrovirens* Nog., AB019453, *H. Akiyama s.n.* (HYO); *Antitrichia formosana* Nog., AB019445, *H. Akiyama s.n.* (HYO); *Hypnum fujiyamae* (Broth.) Paris, AB332300, Japan, *K. Kawai 3541* (TNS); *Duthiella speciosissima* Broth. ex Cardot, AB019467, *H. Akiyama s.n.* (HYO); *Trachypodopsis auriculata* (Mitt.) M. Fleisch.,

AB024682, USA, Hawaii, *T. Arikawa 929* (TNS); *Papillaria deppei* (Hornsch. ex Müll. Hal.) A. Jaeger, AF158172, Mexico, *De Luna 6*; *Trachypus bicolor* Reinw. & Hornsch., AF233577, Mexico, *De Luna 55*; *Pseudoscleropodium purum* (Hedw.) M. Fleisch., AF233567, Mexico, *De Luna 57*; *Platyhypnidium riparioides* (Hedw.) Dixon, AB029385, Japan, *H. Tsubota 2210* (HIRO); *Rhynchostegium pallidifolium* (Mitt.) A. Jaeger, AB024944, Japan, *H. Tsubota 393* (HIRO); *Helicodontium capillare* (Hedw.) A. Jaeger, AF233571, Mexico, *De Luna 48*; Okamuraea hakoniensis (Mitt.) Broth., AB019477, *H. Akiyama s.n.* (HYO); *Brachythecium plumosum* (Hedw.) Schimp., AF233566, Mexico, *De Luna 47*; *Brachythecium salebrosum* (Hoffm. ex F. Weber & D. Mohr) Schimp., AF158176, Mexico, *Mishler 32*; *Brachythecium rivulare* Schimp., AB024674, Japan, *T. Arikawa 645* (TNS); *Myuroclada maximowiczii* (G. G. Borshch.) Steere & W.B. Schofield, AB029389, Japan, *H. Tsubota 2221* (HIRO); *Orthothecium rufescens* (Dicks. ex Brid.) Schimp., AB050951, Japan, *H. Tsubota 3712* (HIRO); *Isopterygiopsis muelleriana* (Schimp.) Z. Iwats., AB034942, Japan, *T. Arikawa 1100* (TNS); *Isopterygiopsis pulchella* (Hedw.) Z. Iwats., AB332272, Japan, *M. Higuchi 41549* (TNS); *Plagiothecium euryphyllum* (Cardot & Thér.) Z. Iwats., AB024626, Japan, *T. Arikawa 360* (TNS); *Plagiothecium draytonii* (Sull.) E.B. Bartram, AB024625, USA, Hawaii, *T. Arikawa 739* (TNS); *Plagiothecium undulatum* (Hedw.) Schimp., AB024634, Czech Repblic, *CCALA M-66. Keil 1949-646*; *Plagiothecium nemorale* (Mitt.) A. Jaeger, AB029387, Japan, *H. Tsubota 2142* (HIRO); *Plagiothecium denticulatum* (Hedw.) Schimp., AB024623, Slovakia, *CCALA M-27. Keil 1946-522*; *Plagiothecium neckeroideum* Schimp., AB024630, Taiwan, *Itouga 1113* (Tottori); *Neodolichomitra yunnaensis* (Besch.) T.J. Kop., AB024671, Japan, *Y. Tateishi 8700* (TNS); *Hylocomiastrum pyrenaicum* (Spruce) M. Fleisch. ex Broth., AB024660, Japan, *T. Arikawa 560* (TNS); *Pleurozium schreberi* (Brid.) Mitt., AB024664; *Pleurozium schreberi* (Brid.) Mitt.,

AF231076; *Hylocomium splendens* (Hedw.) Schimp., AB024662, Japan, *T. Arikawa 585* (TNS); *Loeskeobryum cavifolium* (Sande Lac.) M. Fleisch. ex Broth., AB024658, Japan, *T. Arikawa 1039* (TNS); *Rhytidiadelphus loreus* (Hedw.) Warnst., AB024666, *Sato G1* (TNS); *Rhytidiadelphus squarrosus* (Hedw.) Warnst., AB024667, *Sato G2* (TNS); *Climacium dendroides* (Hedw.) F. Weber & D. Mohr, AB019442, *H. Akiyama s.n.* (HYO); *Climacium japonicum* Lindb., AB019443, *H. Akiyama s.n.* (HYO); *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton, AB024683, Japan, *T. Arikawa 459* (TNS); *Sciaromium tricostatum* (Sull.) Mitt., AB024677, USA, Hawaii, *T. Arikawa 800* (TNS); *Ctenidium hastile* (Mitt.) Lindb., AB334102, Japan, *N. Nishimura 11145* (OKAY, TNS, HIRO); *Ctenidium molluscum* (Hedw.) Mitt., AB024657, Czech Republic, *CCALA M-115 Keil 795*; *Herzogiella perrobusta* (Broth.) Z. Iwats., AB034944, Japan, *A. Tanaka 1380* (TNS); *Myurium hochstetteri* (Schimp.) Kindb., AF233575, Mexico, *De Luna 52*; *Boulaya mittenii* (Broth.) Cardot, AB024963, Japan, *H. Tsubota 2141* (HIRO); *Abietinella abietina* (Hedw.) M. Fleisch., AF005519, *Goffinet 4106* (ALTA); *Macrothamnium macrocarpum* (Reinw. & Hornsch.) M. Fleisch., AB491803, Thailand, *H. Akiyama Th-65* (HYO); *Gollania ruginosa* (Mitt.) Broth., AB094341, Japan, *T. Arikawa 2855* (TNS); *Gollania sinensis* Broth. & Paris, AB332259, China, *M. Higuchi 29776* (TNS); *Calliergonell cuspidata* (Hedw.) Loeske, AB024678, Czech Republic, *CCALA M-43. Keilova 1947-567*; *Hypnum lindbergii* Mitt., AF232696, *Goffinet s.n.*; *Hypnum lindbergii* Mitt., 2 AB029390, Japan, *H. Tsubota 2257* (HIRO); *Pylaisia intricata* Kindb., AB024642, Japan, *T. Arikawa 648* (TNS); *Gollania splendens* (Broth. ex Ihsiba) Nog., AB094340, Japan, *T. Furuki & M. Higuchi 39333* (TNS); *Hypnum plumaeforme* Wilson, AB029384, Japan, *H. Tsubota 395* (TNS); *Hypnum plumaeforme* Wilson, AB332288, Japan, *N. Nishimura 12029* (TNS); *Hypnum sakuraii* (Sakurai) Ando, AB332290, Japan, *N. Nishimura 12030* (TNS); *Ectropothecium andoi* N. Nishim., AB332309,

Japan, *Y. Tateishi 22286* (TNS); *Hypnum oldhamii* AB332281; *Ectropothecium moritzii* A. Jaeger, AB332293; *Vesicularia ferriei* (Cardot & Thér.) Broth., AB332303, Japan, *N. Nishimura 12045* (TNS); *Vesicularia montagnei* (Schimp.) Broth., AB332292, Taiwan, *N. Nishimura 12064* (TNS); *Ectropothecium dealbatum* (Reinw. & Hornsch.) A. Jaeger, AB332296, Taiwan, *N. Nishimura 12085* (TNS); *Ectropothecium zollingeri* (Müll. Hal.) A. Jaeger, AB332295, Taiwan, *N. Nishimura 12108* (TNS); *Ectropothecium obtusulum* (Cardot) Z. Iwats., AB332302, Japan, *N. Nishimura 12055* (TNS); *Ectropothecium zollingeri* (Müll. Hal.) A. Jaeger, AB332305, Japan, *Y. Tateishi 20556* (TNS).