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RESEARCH PAPER

Note on taxonomic position and distribution of *Hygrobiella nishimurae* N. Kitag. (Hygrobiellaceae, Marchantiophyta)

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ABSTRACT

The taxonomic position of *Hygrobiella nishimurae*, an enigmatic East Asian rarity, was identified using integrative approach, including DNA analysis, conducted for the first time for the species. This taxon was described under *Hygrobiella*, then placed into *Metahygrobiella* and finally to *Cephalozia*, however belongs to the former genus – the point of view that is not widely accepted now. In the course of our works this taxon (before regarded as Japanese endemic) was revealed for the first time in Korean Peninsula. The paper provides review of the genus taxonomy, distribution, descriptions and figures based on available material.

Keywords: *Hygrobiella*, East Asia, Hygrobiellaceae, taxonomy, Hepaticae

РЕЗЮМЕ

Бакалин В.А., Вильнет А.А. О таксономическом положении и распространении *Hygrobiella nishimurae* N. Kitag. (Hygrobiellaceae, Marchantiophyta). Таксономическое положение *Hygrobiella nishimurae* – малоизвестного восточноазиатского вида, определено с помощью интегративного подхода, включая молекулярно-генетические исследования, проведенные для этого вида впервые. Таксон, который был описан как *Hygrobiella*, затем перемещен в *Metahygrobiella* и, наконец, в *Cephalozia*, принадлежит к первому роду, что в настоящее время не является широко признанным. В ходе работы вид, ранее рассматривавшийся как эндем Японии, был выявлен впервые на Корейском полуострове. Работа содержит замечания по таксономической структуре рода, а также описание и иллюстрации на основании изучения доступного материала.

Ключевые слова: *Hygrobiella*, Восточная Азия, Hygrobiellaceae, таксономия, Hepaticae

Hygrobiella nishimurae N. Kitag. was described based on the complex of features uncharacteristic in the genus, including strongly distichously leaved plants, conduplicate and keeled leaves and the absence of underleaves. These features were as drastically different from that of *H. laxifolia* – the only taxon known in the genus at that time – as this new species was at the same time placed to the newly described subgenus *Hygrobiella* subg. *Pterocephalozia* N. Kitag. (Kitagawa 1982). This strongly different look resulted the position of this genus was doubted soon after the description. As soon as two years later it was transferred to *Metahygrobiella* on the basis of absence of underleaves (Grolle 1984), despite no *Metahygrobiella* had similarly distichously leaved plants and at least the most of them are soft textured and resembling *Cephalozia*. The placement of *Hygrobiella nishimurae* to *Metahygrobiella* was not followed by Yamada & Iwatsuki (2006).

Despite aforementioned contradiction, *Hygrobiella nishimurae* was housed in *Metahygrobiella* for 30 years, until Váňa et al. (2013) have transferred it to *Cephalozia*, as *C. nishimurae* (N. Kitag.) Váňa. The latter transfer (Váňa et al. 2013) was the result of formalistic approach to provide new names for the 9 taxa before regarded to *Metahygrobiella* on the basis of two taxa (*M. albula* (Steph.) Grolle and *M. macgregorii* (Steph.) R.M. Schust.) which were found by Feldberg et al. (2013) as members of *Cephalozia* s. str. No more '*Metahygrobiella*' taxa were studied genetically by Feldberg et al. (2013). Therefore, the first transfer *Hygrobiella nishimurae* to *Metahygrobiella* was accomplished by the second transfer to *Cephalozia*, regardless the striking contrast in appearance of *Hygrobiella nishimurae* with other *Cephalozia*. The latter point of view was adopted in the World checklist of liverworts and hornworts (Söderström et al. 2016).

Recently V. Bakalin was able to study type materials of the species as well as some other materials in Japanese herbaria and to collect this peculiar species both in Japanese Shikoku and southernmost flank of the Republic of Korea. To check the position of this species and to discuss the distribution of the taxon were the primary goal of the present paper that was conducted using integrative approach.

MATERIAL AND METHODS

Herbarium materials

The material discussed in the paper was collected in the course of field researches in Japan and the Republic of Korea in summer of 2015. After collecting the material was transferred to VBGI and morphologically studied. Other herbarium specimens cited below were studied in 2014 when the first author visited KYO and HIRO. The only specimen we were able to extract DNA was the Japanese specimen (J-11-42-15). The Korean specimen was not sequenced due

to very scarce amount of the material. The total list of specimens examined is placed below:

Specimens examined: JAPAN. Hiroshima Pref., Ohouki Mt., 790 m alt., wet rocks in the valley, Nakashima M., 3672, 15.IX.1981 (KYO, s.n.); ibid., Hiramidani, 620 m alt., stone near stream in deciduous forest, Nakashima M., 3850 & 3849(a), 26.X.1981 (HIRO, s.n.); ibid., 640 m alt., wet rocks near stream, Nakashima M., 3748, 17.X.1981 (HIRO); Kochi Pref., Nagaoka-gun, Ou, Shiofuri Falls (33°48'09"N 133°41'15"E), 500 m alt., Bakalin V.A., J-11-42-15, 25.III.2015 (VBGI); Oita Pref., Kamitue-mura, Sasano, 600 m alt., on wet rock, riverside, Nishimura N., 8459, 29.VII.1981 (holotype of the taxon, KYO; isotype HIRO); Wakayama Pref., Tanagadani , 300 m alt., moist rock, Kodama T., 46730, 26.XII.1973 (KYO). REPUBLIC OF KOREA. Jeju Island, Donnaeko Valley (33°17'56"N 126°35'08"E), 200–350 m alt., Bakalin V.A., Kor-30-58-15, 14.V.2015 (VBGI).

Taxon sampling

To test phylogenetic affinity of Japanese specimen (J-11-42-15) morphologically identified as *Hygrobiella nishimurae* we implemented a BLAST search (https://blast.ncbi.nlm.nih.gov) for the firstly sequenced DNA loci. The similarity of tested specimen with the genus *Higrobiella* achieved 87–91 % in ITS2, 96 % in *trn*L-F and 96–98 % in *trn*G-intron, the less level of similarity was suggested with other representatives of suborder Jungermanniineae and did not reveal relation to the genus *Cephalozia*. Thus, to support this find we produced a new dataset included three multiply sampled species of the genus *Hygrobiella* (Bakalin & Vilnet 2014) and representatives of suborder *Jungermanniineae* according with phylogeny demonstrated in Shaw et al. (2015) and World checklist of liverworts and hornworts (Söderström et al. 2016). In total, 27 nucleotide sequences of ITS1-5.8 *r*DNA, 50 of *trn*L-F and 45 of *trn*G-intron cpDNA presented 59 specimens from 38 species belonging to 33 genera and 18 families of suborder Jungermanniineae, were combined in the dataset. The nucleotide sequences data for 76 accessions were taken from previous studies (Yatsentyuk et al. 2004, Vilnet et al. 2010, 2012, Bakalin & Vilnet, 2012, 2014, Bakalin et al. 2015, Mamontov et al. 2018, unpublished data) and 31 accessions were downloaded from GenBank. For *Mesoptychia sahlbergii* (Lindb.) A. Evans DNA data were combined from two specimens. The genus *Southbya* Spruce was represented by sequence data from *Southbya gollanii* Steph. and *Southbya nigrella* (De Not.) Henriq. For eight specimens three ITS1-5.8, four *trn*L-F and seven *trn*G-intron nucleotide sequences were newly generated. All analyzed specimens are listed in Table 1, including GenBank accession numbers and voucher details.

DNA isolation, PCR amplification and DNA sequencing

DNA was extracted from dried liverwort tissue using the NucleoSpin Plant Kit (Macherey-Nagel, Germany). The amplification and sequencing were performed using primers suggested by White et al. (1990) for ITS1-5.8S rDNA, Taberlet et al. (1991) for *trn*L-F, Shaw et al. (2005) for *trn*G-intron.

PCR were carried out in 20 μl volumes according to the following procedure: 3 min at 94°C, 30 cycles (30 s 94°C, 40 s 56°C (ITS1-5.8S rDNA, *trn*L-F) or 64°C (*trn*G-intron), 60 s 72°C) and 2 min of extension time at 72°C. Amplified fragments were visualized on 1% agarose TAE gels by EthBr staining, purified using the GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, USA), and then used as a template in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analysis

Three datasets (ITS1-5.8S rDNA, *trn*L-F and *trn*G-intron) were automatically aligned in BioEdit 7.0.1 (Hall 1999) with ClustalW option and then manually corrected. The preliminary phylogenetic analyses revealed a lack of incongruence between all datasets and then a combined alignment was produced. All positions of the final alignment were included in the phylogenetic analysis, absent data was coded as missing.

The combined ITS1-5.8S+*trn*L-F+*trn*G-intron alignment was analyzed only by the maximum likelihood method (ML) with PhyML v. 3.0 (Guindon et al. 2010) due to absence of nucleotide sequence data for one or two loci at a number of specimens. The TN+I+G model was selected as the best-fit evolutionary model of nucleotide substitutions for alignment using the ModelGenerator software (Keane et al. 2004). In ML analysis this model was used and the rate heterogeneity among sites was modeled using a gamma distribution with four rate categories. Bootstrap support (BS) for individual nodes was assessed using a resampling procedure with 300 replicates. According to the stopping frequency criterion (FC) for bootstrapping procedure (Pattengale et al. 2010) for our dataset 300 replicates were enough for reaching BS convergence with Pearson average $\varrho 100 = 0.994064$ as estimated by RAxML v. 7.2.6 (Stamatakis 2006). The calculation of infrageneric and infraspecific distances (p-distances) for the genus *Hygrobiella* was provided with Mega 5.1 (Tamura et al. 2011), obtained results are shown in Table 2.

RESULTS

The newly generated 14 nucleotide sequences were deposited in GenBank. The combined ITS1-5.8S + *trn*L-F + *trn*G-intron alignment for 57 specimens consists of 1871 character sites, among them 551 sites belong to ITS1-5.8S, 588 sites – to *trn*L-F and 732 sites – to *trn*G-intron. The number of invariable sites in ITS1-5.8S, *trn*L-F and *trn*G-intron are 326 (59.17 %), 247 (42 %) and 221 (30.19 %), variable positions are 209 (37.93 %), 327 (55.61 %) and 480 (65.57 %), parsimony informative positions are 117 (21.23 %), 229 (38.94 %) and 327 (44.67 %). In the combined alignment there are 794 (42.43 %) invariable sites, 1016 (54.30 %) variable and 673 (18.49 %) parsimony informative positions.

The ML calculation resulted in a tree with Log likelihood of -16135.234549, the obtained tree topology is shown on Fig. 1 with indication of bootstrap support values (BS). The phylogenetic affinity within suborder Jungermanniineae is

Table 1. The list of taxa, specimen vouchers and GenBank accession numbers. The newly obtained sequences are in bold, the sequences downloaded from GenBank are in italic.

Taxon	Specimen voucher	GenBank accession number		
		ITS1-5.8 nrDNA	trnL-F cpDNA	trnG-intron cpDNA
Acrobolbus ochrophyllus (Hook. f. & Taylor) R.M. Schust.	Chile, Engel, 26633 (F)	no data	*KJ802071*	*KJ802044*
Anthelia julacea (L.) Dumort.	Scotland, Long, 31292 (E)	no data	*KJ802043*	*KJ802070*
Arnellia fennica (Gottsche) Lindb.	Finland, Shaw, 1250 (DUKE)	no data	*KJ802096*	*KJ802058*
Balantiopsis cancellata (Nees) Steph.	Chile, Hyvonen, 5946	no data	*AY463545*	no data
Blepharidophyllum vertebrale (Taylor ex Gottsche, Lindenb. & Nees) Ångstr. ex C. Massal.	New Zealand, Engel & von Konrat (F)	no data	*KJ802086*	no data
Calypogeia muelleriana (Schiffn.) Müll. Frib.	Russia: Perm Prov., Konstantinova, K 367-1-04 (KPABG)	JX629935	JX630062	no data
Delavayella serrata Steph.	Chuna: Yunnan, Long, 34809 (E)	no data	*KJ802066*	*KJ802037*
Endogemma caespiticia (Lindenb.) Konstant., Vilnet & A.V. Troitsky	Austria, Sova (DUKE)	no data	*KF943088*	*KF942925*
Eremonotus myriocarpus (Carrington) Pearson	Russia: Karachaevo-Cherkesia Rep., Konstantinova, K 446-6-05 (KPABG)	EU791839	EU791716	no data
Gymnomitrion commutatum (Limpr.) Schiffn.	Russia: Kamchatka Territory, Bakalin, K-51-7-03, 105602 (KPABG)	MF521469	JF421596	no data
Geocalyx graveolens (Schrad.) Nees	China: Yunnan, Long, 34828 (E)	no data	*KJ802067*	*KJ802038*
Gongylanthus renifolius (Mitt.) Steph.	South Africa, de Roo s.n. (BOL)	no data	no data	*AM397717*
Gyrothyra underwoodiana M. Howe	USA: Oregon, Shaw, F886 (DUKE)	no data	*KJ802103*	*KJ802063*
Harpanthus flotovianus (Nees) Nees	Norway, Long & Schill, 31355 (E)	no data	*KJ802065*	*KJ802039*
Hygrobiella intermedia Bakalin et Vilnet	Russia: Central Kamchatka, Bakalin, 90-8-01-VB, 103960 (KPABG)	no data	KF008581	KF008638
	Russia: Sakhalin Prov., Kuril I. Chain, Iturup Isl., Bakalin, K-70-3-05 (VLA), duplicate 115584 (KPABG), 1	KF008621	no data	no data
	Russia: Sakhalin Prov., Kuril I. Chain, Iturup Isl., Bakalin, K-71-8-05 (VLA), duplicate 115583 (KPABG), 2	no data	KF008583	KF008640
	Russia: Sakhalin Prov., Kuril I. Chain, Shikotan Isl., Bakalin, K-48-13-07 (VLA), duplicate 115589 (KPABG)	KF008620	KF008582	KF008639
Hygrobiella laxifolia (Hook.) Spruce	Canada, Urmi, 8852 (dupla in VGBI), 1	**MH580595**	**MH580590**	**MH580598**
	Canada, Urmi, 8861 (dupla in VGBI), 2	no data	**MH580591**	**MH580599**
	Russia: Commander Islands, Bering Isl., Bakalin, K-6-6-02-VB, 103449 (KPABG)	KF008616	KF008571	KF008636
	Russia: Khanty-Mansi Autonomous Area, Lapshina, 13-256 t. 11 (dupla in KPABG), 1	no data	no data	**MH580601**
	Russia: Khanty-Mansi Autonomous Area, Lapshina, 13-262 t.11 (dupla in KPABG), 2	no data	no data	**MH580600**
	Russia: Khanty-Mansi Autonomous Area, Lapshina, 13-267 t.11 (dupla in KPABG), 3	no data	no data	**MH580602**
	Russia: Murmansk Prov., Konstantinova, 115/1 (KPABG), 1	KF008613	KF008568	KF008632
	Russia: Murmansk Prov., Konstantinova, 5171 (KPABG), 2	KF008612	KF008567	KF008631
	Russia: Murmansk Prov., Konstantinova, 15-1-98, 6984 (KPABG), 3	JX629933	JX630052	KF008627
	Russia: Murmansk Prov., Konstantinova, 29-1-94 (KPABG), 4	KF008611	AF519187	KF008626
	Russia: Murmansk Prov., Konstantinova, 152-7-87, 6103 (KPABG), 5	KF008614	KF008569	KF008633
	Russia: Murmansk Prov., Konstantinova, K 110-1-11, 20180 (KPABG), 6	KF008610	KF008565	KF008625
	USA, Konstantinova, A108/1-95 (KPABG)	KF008619	KF008572	KF008635
Hygrobiella nishimurae N. Kitag.	Japan, Bakalin, J-11-42-15 (KPABG, VGBI)	**MH580594**	**MH580592**	**MH580603**
Hygrobiella squamosa Bakalin et Vilnet	Russia: Commander Islands, Medny Isl., Bakalin, K-67-2-04, 106771 (KPABG)	KF008618	KF008578	KF008647
	Russia: Central Kamchatka, Bakalin, KK 50-24a-02-VB, 104117 (KPABG)	no data	KF008575	KF008641
	Russia: Krasnoyarsk Territory, Taimyr, Fedosov, 11-232 (KPABG)	no data	no data	**MH580597**
	Russia: Sakhalin Prov., Kuril I. Chain, Iturup Isl., Bakalin, K-12-6-07 (VLA), duplicate 115588 (KPABG), 1	no data	KF008584	KF008648
	Russia: Sakhalin Prov., Kuril I. Chain, Iturup Isl., Bakalin, K-12-8a-07 (VLA), duplicate 115587 (KPABG), 2	no data	KF008585	KF008649
	Russia: Sakhalin Prov., Kuril I. Chain, Paramushir Isl., Bakalin, K-106-7-04, 107584 (KPABG)	no data	KF008580	KF008645
Isotachis lyallii Mitt.	New Zealand, Engel, 21825 (F)	no data	*AY608130*	*AY608178*

Table 1. Continued.

Taxon	Specimen voucher	GenBank accession number		
		ITS1-5.8 nrDNA	trnL-F cpDNA	trnG-intron cpDNA
Jungermannia atrovirens Dumort.	Russia: Caucasus, Karachaevo-Cherkesia Rep., Ignatov, Ignatova MHA 4.8.02 (KPABG)	GQ220782	GQ220766	no data
Lethocolea glossophylla (Spruce) Grolle	Ecuador, Davis, 259 (DUKE)	no data	*KJ802080*	*KJ802051*
Liochlaena lanceolata Nees	Russia: Vladimir Prov., Kokoshnikova, 112323 (KPABG)	KF516120	KF516228	KF516172
Marsupella funckii (F. Weber & D. Mohr) Dumort.	Russia: Karachaevo-Cherkessia Rep., Konstantinova, K516-1-05, 109804 (KPABG)	EU791820	EU719700	no data
Mesoptychia chinensis Bakalin, Vilnet et Xiong	China: Guizhou Prov., Bakalin, China-51-11-13 (KPABG)	no data	KM501488	KM501472
Mesoptychia sahlbergii (Lindb.) A. Evans	Russia: Chukotka, Afonina, 10.VIII.1979 (KPABG)	EU791840	AF519189	no data
	Russia: East Siberia, Ignatov, s.n. (BOL)	no data	no data	*AM397757*
Metacalypogeia cordifolia (Steph.) Inoue	Russia: Primorsky Kray, Bakalin, P-66-18a-06 (KPABG)	JX629934	JF421597	no data
Metasolenostoma orientale Bakalin & Vilnet	Russia: Kamchatka, Bakalin, HPE 42, 29.08.2001 (KPABG)	GQ220773	AY327777	JQ828698
Nardia compressa (Hook.) Gray	Canada, Konstantinova, A97/1-95 (KPABG)	EU791837	AF519188	no data
Notoscyphus lutescens (Lehm. & Lindenb.) Mitt.	Australia, Pocs, 01098/d (DUKE)	no data	*KJ802099*	*KJ802060*
Plectocolea obovata (Nees) Mitt.	Russia: Kemerovo Prov., Konstantinova, 72-2-00, 102004 (KPABG)	GQ220769	GQ220753	JQ828678
Poeltia campylata Grolle	China: Sichuan, Bakalin, China-48-2-17, 37210 (VGBI)	**MH580596**	**MH580593**	no data
Prasanthus suecicus (Gottsche) Lindb.	Norway: Svalbard, Konstantinova, K 121-5-06 (KPABG)	EU791825	EU791704	no data
Saccogynidium australe (Mitt.) Grolle	New Zealand, Engel, von Konrat & Braggins, 24166 (F)	no data	*KF942960*	*KF942832*
Solenostoma confertissimum (Nees) Schljakov	Russia: Karachayevo-Cherkessian Rep., K 459-8a-05, 109680(KPABG)	GQ220774	GQ220758	JQ828781
Southbya gollanii Steph.	Nepal, Long, 30537 (E)	no data	no data	*AM397739*
Southbya nigrella (De Not.) Henriq.	Italy, Buryova, 621 (DUKE)	no data	*KJ802083*	no data
Stephaniella paraphyllina J.B. Jack	Venezuela, Soederstroem, 2004/030b (BOL)	no data	no data	*AM397698*
Trichotemnoma corrugatum (Steph.) R.M. Schust.	Glenny, 8426	no data	*AY463591*	no data

Table 2. The value of infrageneric and infraspecific *p*-distances for the genus *Hygrobiella,* n/c – non calculated value due to single specimen only, "–"non calculated value due to unsequenced DNA locus.

Taxon	Infraspecific p-distances, (ITS1-5.8/*trn*L-F /*trn*G-intron) %	Infrageneric p-distances, (ITS1-5.8/trnL-F/trnG-intron) %				
		H. laxifolia	*H. squamosa*	*H. intermedia*	*H. nishimurae*	*Anthelia julacea*
Hygrobiella laxifolia	0.8/0/0.1					
H. squamosa	n/c/0.1/0.1	5.0/2.1/3.0				
H. intermedia	0/0/0.2	4.5/2.6/2.7	5.2/2.1/3.0			
H. nishimurae	n/c/n/c/n/c	7.5/3.0/2.3	6.6/3.4/2.3	9.0/3.6/2.2		
Anthelia julacea	–/n/c/n/c	–/7.1/9.0	–/7.2/8.5	–/6.5/8.8	–/8.1/8.1	

common with those obtained by Shaw et al. (2015). The newly sequenced Japanese specimen of *Hygrobiella nishimurae* formed the basal branch in the clade of family Hygrobiellaceae, its relation to three other *Hygrobiella* species obtained 96 % bootstrap support. The level of ITS1-5.8S sequence divergence of *Hygrobiella nishimurae* from the rest of *Hygrobiella* species counts 6.6–7.5 % that almost in 1.5 higher then divergence between *H. laxifolia*, *H. squamosa* and *H. intermedia* (4.5-5.2 %, Table 2). The value of *trn*L-F p-distances between *Hygrobiella nishimurae* and *Hygrobiella* spp. are 3.0–3.6 % opposite to 2.1–2.6 % between other *Hygrobiella* species. The *trn*G-intron data suggested lower level of divergence of *Hygrobiella nishimurae* (2.2–2.3 %) whereas three *Hygrobiella* species are more diverged from each other (2.7–3.0 %).

Implemented phylogenetic estimation robustly proved initial treatment of *Hygrobiella nishimurae* as a member of the genus *Hygrobiella* and the level of nrDNA sequence divergence with a number of morphological features support its separation in the subgenus *Pterocephalozia*.

DISCUSSION

As it was found above, the robust morphological difference is accompanied with strong distance in the term of genetics. *Hygrobiella nishimurae* belongs to Hybrobiellaceae and is quite distanced from other bulk of the taxa of the genus therefore the subgenus *Pterocephalozia* should be recognized as it was proposed by Kitagawa (1982). The differences of *H. nishimurae* from other taxa are in strongly distichous

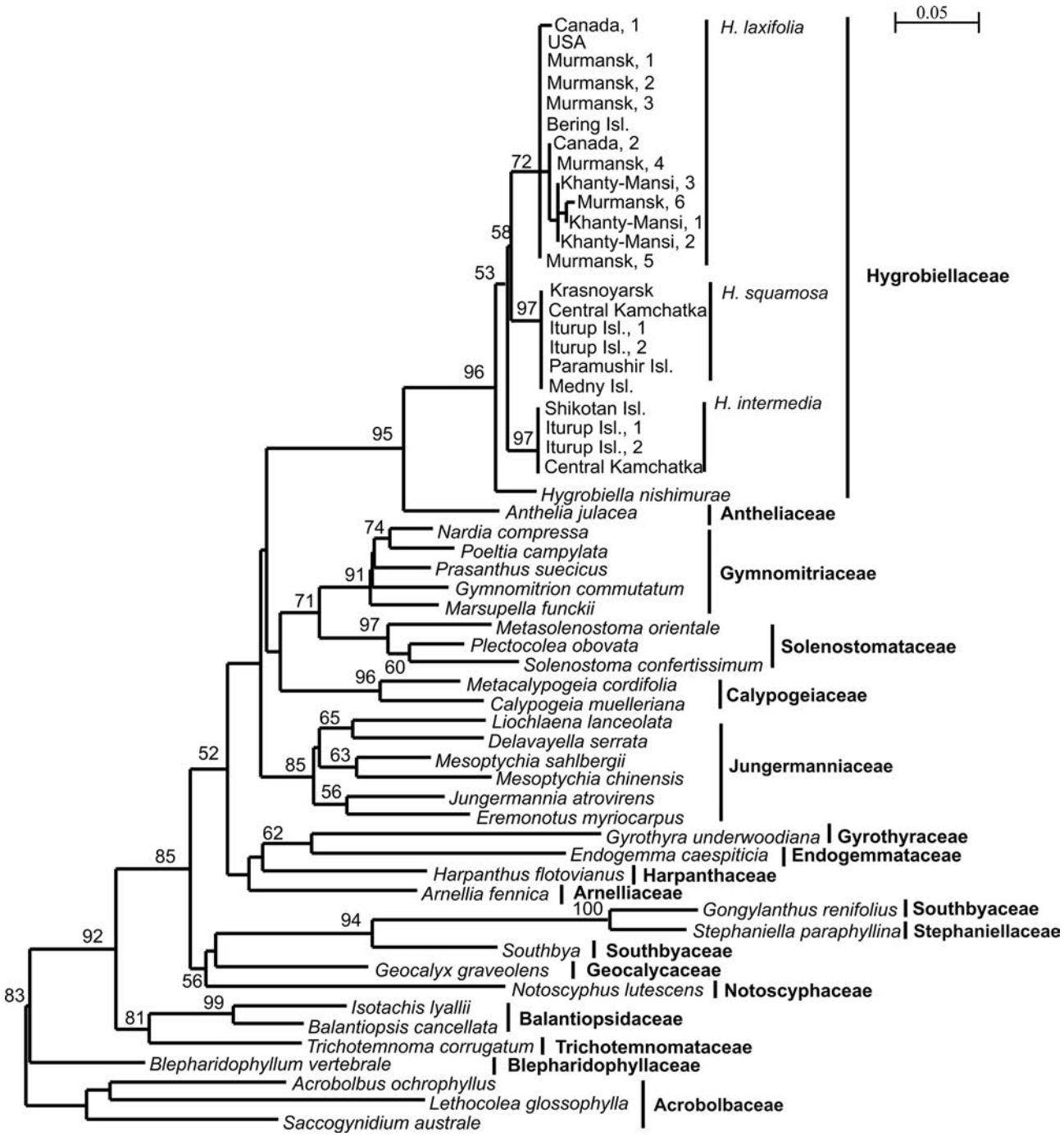

Figure 1 Maximum likelihood phylogenetic tree for the suborder Jungermanniineae based on combined dataset ITS1-5.8S *nr*DNA+*trn*L–F+*trn*G-intron.Bootstrap support values are indicated

leaf arrangement and absence of underleaves. However, it worth to be noted although leaves are conduplicate in the majority of cases the keel wing may not be developed. The plants in paratype from Wakayama Pref. are even hardly winged. The plant size is the subject of the great variation, and sometimes differs in 2 or even 3 times that may be obvious from stem cross sections obtained from Japanese and Korean material (Fig. 2: 7–9). The color of plants is also the subject for variation. Indeed, the type specimen of the species is greenish brown, as indicated in original description; however, green coloration of plants is somewhat common in shady areas. Moreover some confusion in the coloration may arise from the color changes in the herbarium. The plants we collected in Shikoku were bright green in the wild conditions (Fig. 3: 1), but after complete dying become brownish pigmented. Since the material revealed in the course of this study enlarge data on the species variability below we provide the morphological description of the species. Since we did not find the generative structures in specimens collected by Bakalin, the information on them is adopted and shortened from Kitagawa (1982) and put into square brackets.

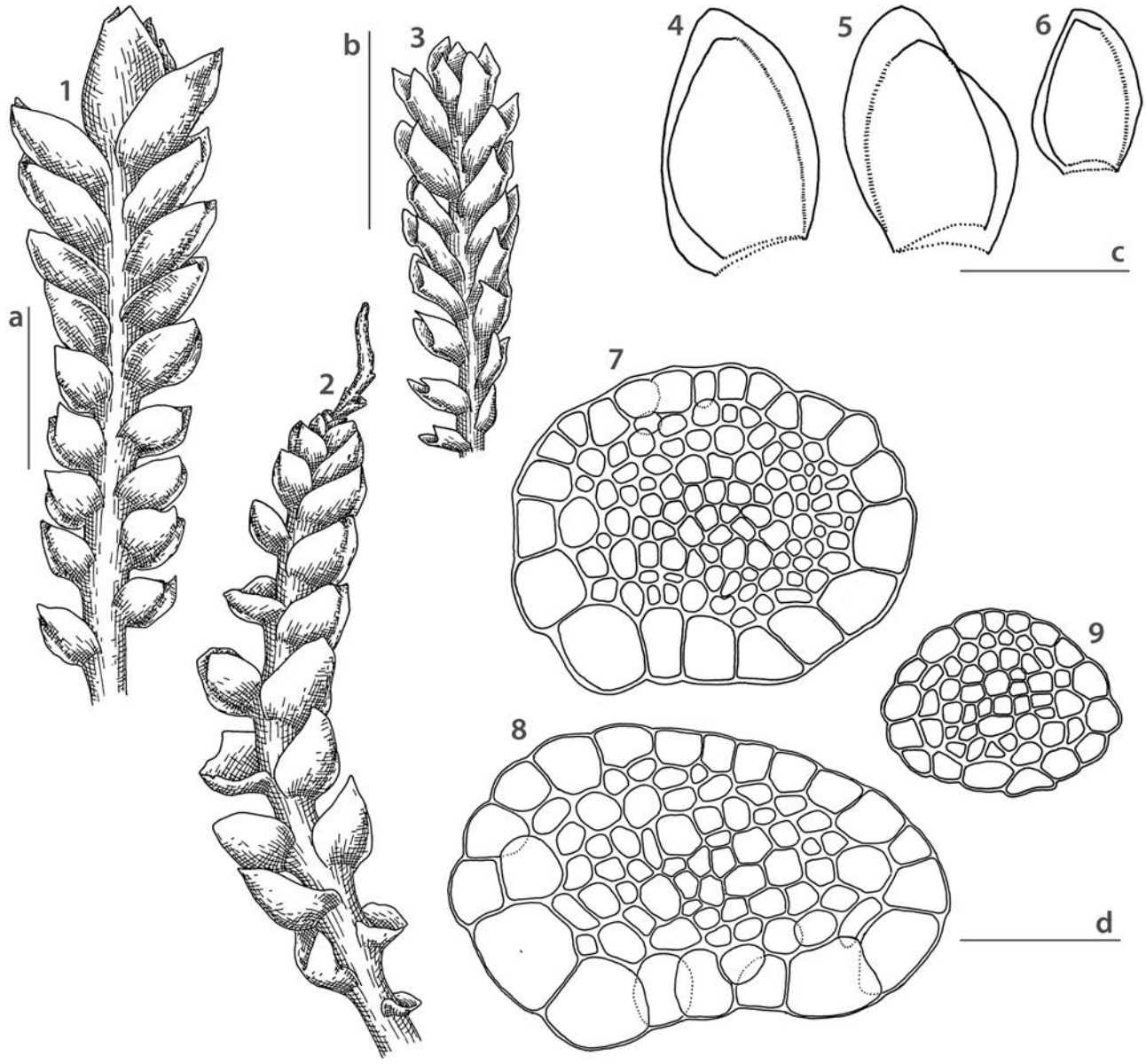

Figure 2 *Hygrobiella nishimurae* N. Kitag.: 1, 3 – plant habit, dorsal view; 2 – plant habit, ventral view; 4–6 – leaves; 7–9 – stem cross sections. Scales: a – 1 mm, for 1, 2; b – 1 mm, for 3; c – 500 μm, for 4-6; d – 100 μm, for 7-9. 1, 2, 4-7 – from Bakalin J-11-42-15 (VBGI); 3, 9 – from Bakalin Kor-30-58-15 (VBGI); 8 – from Kodama 46730 (KYO)

Hygrobiella nishimurae N. Kitag., Misc. Bryol. Lichenol. 9(4):69, 1982.

Plants bright green to brownish green when alive, become brownish to greenish brown in herbarium, strongly distichously leaved, with leaves concave to conduplicate with keel sometimes developed (regardless leaves strongly conduplicate or not), 0.5–1.2 mm wide and 5–15 mm long, forming loose pure patches or as admixture to other hepatics and mosses; apical part of shoots sometimes become depauperate with scale-like leaves. Rhizoids sparse, mostly in the area of branch origin, brownish, erect spreading, separated or in loose fascicles. Stem brownish, from rhizomatous and freely branched base, above branching sparse, ventral, produces normally leaved branches or ventral leafless stolons; cross section somewhat to distinctly transversely elliptic, 110–250 × 200–300 μm, with hyaloderm cells in one layer, with somewhat thickened walls, cells very large, 40–60(–70) μm in diameter, inward suddenly become smaller, inner layer 7–10 cells high, thick-walled, with vestigial to small trigones, 15–25 μm in diameter. Leaves dichotomously arranged, canaliculate to conduplicate, with commonly present keel (both in conduplicate and concave leaves), when flattened in the slide always remain conduplicate, when trying unfold commonly lacerate, from scale like and very small to 700 × 500 μm (folded size, therefore unfolded should reach 700 × 1000 μm), divided by narrow sinus descending to 1/5–1/3 of leaf length into two obtuse to acute lobes. Underleaves absent. Cells are similar across the leaf, in the leaf middle with slightly thickened walls, 20–38 × 17–30 μm, trigones virtually absent, cuticle smooth. [Dioicous. Leaves become large to gynoecia. Gynoecia terminal on leading branches, without subfloral innovations; perianth deeply triplicate, 2–3-stratose in lower half and unistratose

above, to 1.2 mm long and 0.6 mm wide; bracts folded, but not or hardly winged, margins entire or with a few teeth; bracteole present 1(–2) per gynoecium, unequally bilobed with smaller lobe commonly reduced to the tooth and then bracteole looks lanceolate. Androecia virtually intercalary, but branch never realize after androecia and die, bracts monandrous, antheridia spherical, 100–120 μm in diameter on short biseriate stalk. (Kitagawa 1982)] (Figures 2, 3).

The newly obtained data permit to discuss the distribution of the species, also basing on the result of our revision of available material. Holotype of the species is from Kyushu (Oita Pref.). Two more paratypes cited by Kitagawa (1982) are from Honshu: Hiroshima Pref. and Wakayama Pref. (all were studied in KYO and HIRO). Besides HIRO houses three more specimens of the species; all of them are from nearly the same place (see specimens examined in Material and Methods). VB collected *Hygrobiella nishimurae* in two localities. One of them is in Shikoku (Kochi Pref.) and is the new record of the taxon for that island. Another collection is from Jeju Island in the Republic of Korea. Therefore all known records of this species (both published before and revealed by us) are in the narrow band between 33 and 35°N stretching from Japanese Honshu, Shikoku and Kyushu to Jeju Island in Korea. Within that latitudinal limit the species is also limited altitudinally, all known collections are between 300 and 800 m a.s.l.

The species distribution is restricted to warm temperate moist forests with many evergreen broadleaved trees and the area type of the taxon may be called as Japan-Korea warm temperate. The species may decrease the population due to absence or rarity of sporophyte production. We were not able to find androecia or perianths in our recent collections. The holotype and the specimen from Wakayama Pref. although bears perianths and androecia, but have no traces of spore production. The latter may explain noticeable rarity of the taxon. The population observed in Jeju Island is very small, represented by dwarf sterile plants and may disappear within a few years. The probability of occurrence of the taxon in other areas of Japan and Korean Peninsula seems to be high. However we are not inclined to expect its distribution in adjacent areas like China and the southern flank of the Russian Far East. We estimate this taxon as strict Korean-Japanese endemic species that require conservation actions.

By ecological requirements this taxon is acidophilic that is similar to another taxa of *Hygrobiella*, although in the moisture requirements it looks more drought tolerant in comparison with other taxa of the genus and seems survive in the course of dry periods. Two recent collections are from moist rocks that however sometimes (probably rarely) become dry. The collections available in the herbaria have unclear references to moist to wet rocks without indication whether these rocks could be dry or not. We estimate ecologically this species may be called as acidophilic hygrophyte, but not hydrophyte as e.g. *H. laxifolia*.

Figure 3 *Hygrobiella nishimurae* N. Kitag.: 1 – plant cushion; 2 – plant habit, dorsal view. Scales a – 1 mm, for 1; b – 500 μm, for 2. 1 – from Bakalin J-11-42-15, 2 - from Bakalin Kor-30-58-15 (all in VBGI).

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