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Re-assessment of the familial position of *Pseudohypnella* (Symphyodontaceae, Bryophyta), with a new record of *P. verrucosa* in Vietnam

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Abstract

A unique moss species, *Pseudohypnella verrucosa* with two kinds of papillae on laminal cells and double costae reaching to midleaf is newly reported from Vietnam. Its familial position in the Symphyodontaceae is newly proposed based on molecular analysis using ITS1&2 and *trn*L-F and supported by its morphology.

Key words: Hypnaceae, ITS1&2, moss, Sematophyllaceae, range extension, *trn*L-F

Introduction

The monotypic genus *Pseudohypnella* (Brotherus, 1908: 1093) Fleischer (1923: 1368) was first described based on *Hypnum verrucosum* Dozy & Molkenboer (1844: 309) that was placed under *Taxithelium* subg. *Pseudohypnella* Brotherus (1908: 1093). The type specimens were collected in Java by C.L. Blume (see Touw 2007). Since that time, the genus has long been classified under various families, for example, Hookeriaceae (Brotherus 1925, Touw 1992), Hypnaceae (Goffinet *et al.* 2009, Cairns *et al.* 2019, Lee & Norhazrina 2019), Pilotrichaceae (Buck *et al.* 2005, Câmara 2011), Sematophyllaceae (Fleischer 1923, Bartram 1944, Akiyama 1993, 2009) until the present study. Its sole species, *Pseudohypnella verrucosa* (Dozy & Molkenboer 1844: 14) Fleischer (1923: 1368) was reported across Southeast Asia, including Pahang in Peninsular Malaysia (Lee & Norhazrina 2019), Mindanao in Philippines (Bartram 1944, Linis 2006, Linis & Schwarz 2023), Java (Fleischer 1923, Gradstein *et al.* 2010), Banka (Fleischer 1923), Flores (Touw 1992), and Seram (Akiyama 1993, 2009) in Indonesia, Morobe District in Papua New Guinea (Akiyama 2009) and extends to Sri Lanka to the East (Fleischer 1923, O'Shea 2002) and north-east Queensland in Australia to the West (Ramsay & Cairns 2004, Ramsay 2012, Cairns *et al.* 2019).

In a survey conducted in 2023 as part of the floristic study of Bidoup Nui Ba National Park in southern Vietnam, which has been ongoing since 2018 with the cooperation of Vietnamese and Japanese researchers (see Middleton *et al.* 2019, Tagane *et al.* 2020, Ngoc *et al.* 2021), the first author collected an unfamiliar moss growing on a tree trunk. After examination under microscope, we determined that it is *Pseudohypnella verrucosa* on the basis of its unique morphological features such as obtuse leaves with well-developed double costae reaching to midleaf and presence of two types of laminal cell ornamentation on papillosity, i.e., type 1: single, tall, terminally stellate projection at the upper end of each lamina, and type 2: short, slightly curved, slender, horn-like seriate papillae on laminal lumina (sometimes on intercellular walls). The first type of papillae is also evident on the upper leaf margins. Fleischer (1923) indicated that the second type of papillae is found on both surfaces of leaves. However, based on our observations, both types of ornamentation are confined to the dorsal surface of leaves while the ventral surface is smooth (FIG. 1 A–M). Dozy & Molkenboer (1861–1870) gave a detailed illustration of *Pseudohypnella verrucosa* under the illegitimate name *Hypnum dozyanum* Müll.Hal. (Tab. CCXLV), and they also pointed out that it has raised papillae at the leaf margins and dorsal side of laminal cells.

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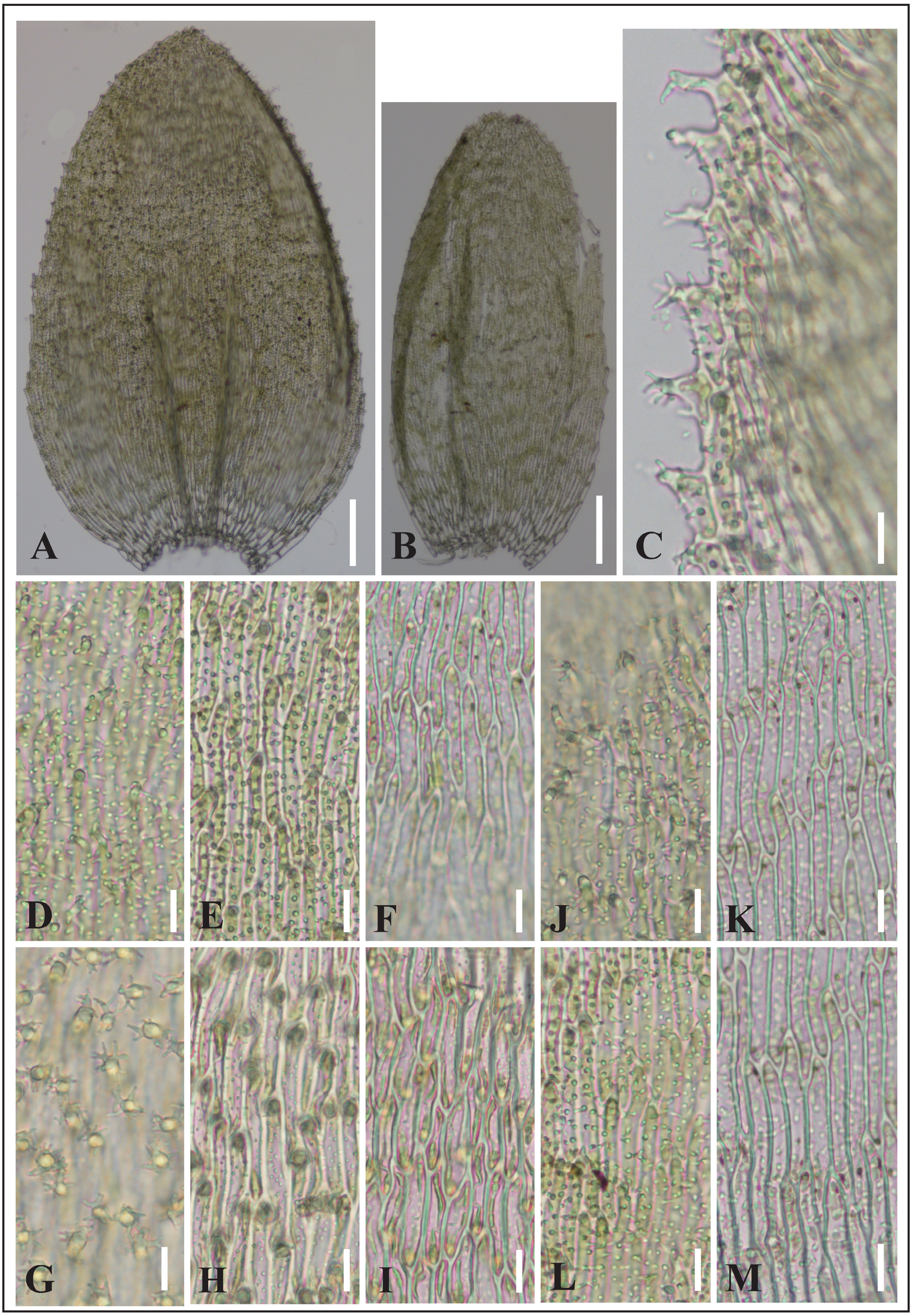

FIGURE 1. Leaves and laminal cells of *Pseudohypnella verrucosa* (A–F, J–M: Vietnam, *Myotoishi V-99*. G–I: Indonesia, Seram, *Akiyama C-15094*). A: Stem leaf. B: Branch leaf. C: Uppermost margin with prominent projection at upper lamina with stellate-papillose apices. D, E, G & H: Dorsal surfaces of median laminal cells [shifting the focus from higher proration (D & G) to lower seriate papillae (E & H)]. F & I: Ventral surface of median laminal cells. J: Dorsal surface of upper laminal cells. K: Ventral surface of upper laminal cells. L: Dorsal surface of lower laminal cells. M: Ventral surface of lower laminal cells. Note that the shadow of papillae at opposite side is visible because of the thin cell walls in F, I, K & M. Scales bars: A & B = 100 µm. Others = 10 µm.

The present study represents for the first time that this unique moss species occurs in Vietnam and also a significant extension to its northernmost distribution range. Additionally, we conducted molecular phylogenetic analyses with a few selected taxa to reveal the correct familial position of *Pseudohypnella verrucosa*.

Materials and methods

To assess the molecular position of *Pseudohypnella verrucosa*, we newly obtained DNA sequence data from the sample collected from Vietnam. Two loci were selected for the phylogenetic analyses: *trn*L$_{\mathrm{UAA}}$-*trn*F$_{\mathrm{GAA}}$ region including the *trnL* intron and the *trn*L-*trn*F intergenic spacer (*trn*L-F), and nuclear internal transcribed spacer (ITS) including 5.8S rRNA. The protocol for DNA extraction, PCR amplification, and DNA sequencing followed Inoue & Aung (2023). The primer pairs for PCR were trnT71F and trnF1F for *trn*L-F (Tsubota *et al.* 2015); and 18S1659B and 26S166BR for ITS (Oguri *et al.* 2003). The primers for cycle sequencing were a [*trn*T(UGU)F-a], c [*trn*L(UAA)5exonF-c], *trn*F8F, and d [*trn*L(UAA)3exonR-d] for *trn*L-F (Taberlet *et al.*, 1991; Tsubota *et al.* 2015); 18S1764B, 5srRNA, 26S102BR, and 5srRNAR for ITS (Oguri *et al.* 2003). Sequences obtained in the present study have been submitted to the DNA Data Bank of Japan (DDBJ), a member of International Nucleotide Sequence Database Collaboration (INSDC).

Based on BLAST searches and the results by Kim *et al.* (2020) and Akiyama *et al.* (2021), we selected 16 accessions from Symphyodontaceae as ingroup and three accessions from Neckeraceae and Taxiphyllaceae as outgroup (TABLE 1). Sequences of the two loci were aligned separately using MAFFT ver. 7.511 (Katoh & Standley 2013) with some manual adjustment on the sequence editor MEGA ver. 7.0.26 (Kumar *et al.* 2016). Gaps were treated as missing data. The concatenated matrix of the two loci was used for maximum likelihood tree inference with IQ-TREE ver. 2.2.2.7 (Minh *et al.* 2020). The optimal nucleotide substitution model and partitioning scheme were selected based on the Bayesian information criterion (BIC) using ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in IQ-TREE. Branch support was estimated using the value of SH-like approximate likelihood ratio test (SH-aLRT; Guindon *et al.* 2010), fast local bootstrap probability (LBP; Adachi & Hasegawa 1996), ultrafast bootstrapping (UFBoot; Hoang *et al.* 2018) with 10,000 replicates, respectively, and approximate Bayes test (aBayes; Anisimova *et al.* 2011).

TABLE 1. Alphabetical list of taxa for the phylogenetic analyses with voucher information and accessions. Newly obtained accessions are shown in bold. -: No data in GenBank.

Taxon	Voucher	Accession	
		trnL-F	**ITS**
Ingroup			
Chaetomitriopsis glaucocarpos (Reinw. ex Schwägr.) M.Fleisch.	China. *Wang, M. Z. 4046A* Dec 19 1986 (MO)	HQ443858	HQ443721
Chaetomitrium bornense Mitt.	Brunei. *Tan 95-1116* (NY)	AY306714	HQ613413
Chaetomitrium dusenii Müll.Hal. ex Broth.	Equatorial Guinea. *Heras 499/94* (NY)	AY306715	HQ613414
Chaetomitrium horridulum Bosch & Sande Lac.	Vietnam. *Akiyama 23956* (HYO)	LC620860	LC620246
Chaetomitrium orthorrhynchum (Dozy & Molk.) Bosch & Sande Lac.	Malaysia. *Akiyama 23432* (HYO)	LC620861	LC620247
Dimorphocladon bornensis Dixon	Brunei. *Tan 95-1060* (NY)	AY306732	HQ443732
Glossadelphus glossoides (Bosch & Sande Lac.) M.Fleisch.	Malaysia. *Hedenäs, B57848* (S)	AM990368	FM161106
Phyllodon lingulatus (Cardot) W.R.Buck	Japan. *Wonhee 522* (KB, HIRO)	LC574036	KT804691
Phyllodon truncatulus (Müll.Hal.) W.R.Buck	French Guiana. *Buck 33300* (NY)	HQ443880	HQ443764
Pseudohypnella verrucosa (Dozy & Molk.) M.Fleisch.	Australia. NE100911	-	MN688910
Pseudohypnella verrucosa (Dozy & Molk.) M.Fleisch.	Vietnam. *Myotoishi V-99* (HYO)	**LC797998**	**LC797999**
Rheoshevockia fontana Ignatov, W.Z.Ma & D.G.Long	China. *Shevock 46419* (MHA ex UC)	MK257806	MK164023

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TABLE 1. (Continued)

Taxon	Voucher	Accession	
		trnL-F	**ITS**
Symphyodon chrysobasilaris (Broth.) W.Kim & H.Akiyama	U.S.A. Hawaii, *Shevock et al. 46302* (CAS)	LC620857	LC620244
Symphyodon erinaceus (Mitt.) A.Jaeger	Thailand. *Akiyama & Printarakul 1460* (HYO)	LC620801	LC620189
Symphyodon imbricatifolius (Mitt.) S.P.Churchill	Colombia. *Churchill & Betancur 18734* (MO)	LC620809	LC620197
Outgroup			
Filibryum ogatae (Broth. & Yasuda) W.Kim & T.Yamag.	Japan. *Akiyama 23015* (HYO)	LC620862	LC620248
Homaliodendron flabellatum (Sm.) M.Fleisch.	Locality unknown. H-3071675 (H)	FM210290	FM161132
Taxiphyllum taxirameum (Mitt.) M.Fleisch.	Japan. *Akiyama 24505* (HYO)	LC620863	LC620249

Results & Discussion

The data matrix consisted of a total length of 1197 bp, of which, 277 sites (23%) were variable and 155 sites (56% of the variable sites) parsimony-informative. The best-fit models per partition are HKY+F+G4 for *trn*L-F, and HKY+F+R2 for ITS.

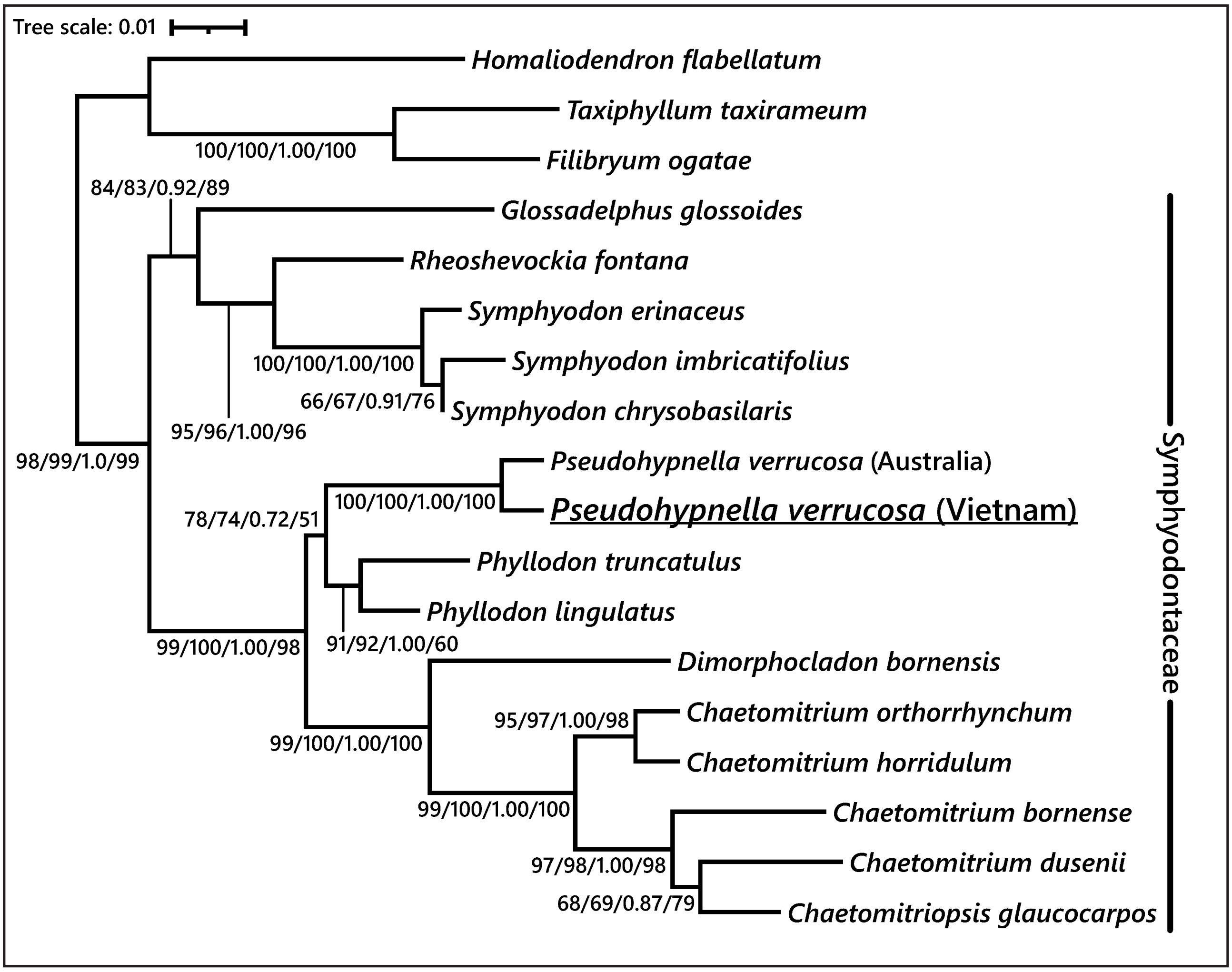

FIGURE 2. The maximum likelihood tree based on concatenated *trn*L-F and ITS sequences, depicted by IQ-TREE. Supporting values more than 50% are shown on each branch in order of SH-aLRT (%), LBP (%), aBayes, and UFBoot (%).

The sample from Vietnam is resolved and included in a monophyletic Symphyodontaceae. Both accessions of *Pseudohypnella verrucosa* from Vietnam and Australia formed a clade sister to the clade of *Phyllodon lingulatum* and *P. truncatulus*. (FIG. 2). The sister relationship of Vietnamese and Australian *P. verrucosa* was well-supported in the preliminary phylogenetic inference, even with a missing *trn*L-F sequence of the Australian material. Distinctive combination of morphological features in *Pseudohypnella verrucosa*, such as round to broadly obtuse leaf apices, moderately developed double costae, linear-rhomboidal laminal cells with prominent proration, and weakly developed alar regions with quadrate to rectangular cells (Akiyama *et al.* 2021) are in sufficient agreement to its molecular phylogenetic placement in the Symphyodontaceae.

Pseudohypnella verrucosa (Dozy & Molk.) M.Fleisch., Musci Buitenzorg 4: 1368, f. 222 (1923)

Basionym: *Hypnum verrucosum* Dozy & Molk., Musc. Frond. Archip. Ind.: 14 (1844). Type: Java, *Blume s.n.* (holotype L [L0623786]) (*fide* Touw 2007).

Synonyms: *Hypnum dozyanum* Müll.Hal., Syn. Musc. Frond. 2: 276 (1851), nom. illeg. superfl.; *Ectropothecium dozyanum* A.Jaeger, Ber. Thätigk. St Gallischen Naturwiss. Ges. 1877–1878: 267 (1880) [replacement name]; *Hypnella dozyana* (A.Jaeger) Renauld & Cardot, Rev. Bryol. 23: 104 (1896); *Hookeria dozyana* (A.Jaeger) Paris, Index Bryol. Suppl. 1: 185 (1900); *Taxithelium dozyanum* (Müll.Hal.) Broth., Nat. Pflanzenfam. I(3): 1093, f. 774: F–H (1908), nom. illeg.

Specimens examined: VIETNAM. Bidoup Nui Ba National Park, Dinh lar Reing, 12.1514° N, 108.5363° S, 1460 m elev., on tree trunk, 26 February 2023, *Myotoishi V-99* (FU, HYO, TNS). INDONESIA. Seram Island, Tehoru, Gunung Watane, 1650 m elev., *Akiyama C-15013* (HYO); Piliana—Gunung Watane, 1600 m elev., *Akiyama C-15001* (HYO); Piliana—Hau Harunoe, 1300 m elev., *Akiyama C-15094* (HYO); ibid., Hau Harunoe—Ena Puti, 2018 m elev., *Akiyama C-15128b* (HYO).

Distribution: Australia, Indonesia, Malaysia, Papua New Guinea, Philippines, Sri Lanka, Vietnam.

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