

*RHEOSHEVOCKIA* (SYMPHYODONTACEAE, BRYOPHYTA),  
A NEW RHEOPHYTIC MOSS GENUS FROM YUNNAN, CHINA

*RHEOSHEVOCKIA* (SYMPHYODONTACEAE, BRYOPHYTA),  
НОВЫЙ РЕОФИТНЫЙ МОХ ИЗ ПРОВИНЦИИ ЮННАНЬ, КИТАЙ

WEN ZHANG MA<sup>1</sup>, DAVID G. LONG<sup>2</sup>, OXANA I. KUZNETSOVA<sup>3</sup>,  
ELENA A. IGNATOVA<sup>4</sup> & MICHAEL S. IGNATOV<sup>3,4</sup>

ВЕН ЖАНГ МА<sup>1</sup>, ДЭВИД Д. ЛОНГ<sup>2</sup>, ОКСАНА И. КУЗНЕЦОВА<sup>3</sup>,  
ЕЛЕНА А. ИГНАТОВА<sup>4</sup>, МИХАИЛ С. ИГНАТОВ<sup>3,4</sup>

Abstract

A rheophytic moss from Yunnan superficially similar to *Hygrohypnum* was found to belong to the Symphyodontaceae, and is resolved in a position sister to the genus *Symphyodon* in phylogenies by all four studied DNA markers: nuclear ITS, chloroplast *trnL-F* and *rps4* and mitochondrial *nad5*. It is described as a new genus and species, *Rheosheockia fontana* Ignatov, W.Z. Ma & D.G. Long, as it is strikingly different from species of the mainly epiphytic genus *Symphyodon*. This is not a unique case where molecular phylogenetic studies reveal a close though unexpected relationship between epiphytic and rheophytic mosses.

Резюме

Водный мох из Юннаня, растущий в быстро текущих ручьях и внешне сходный с *Hygrohypnum*, по результатам молекулярно-филогенетического анализа оказался в сестринском положении к роду *Symphyodon* из семейства Symphyodontaceae, которое включает преимущественно тропические эпифиты. Вместе с тем, о таком его систематическом положении свидетельствуют независимо все четыре изученных участка ДНК: ядерный ITS, хлоропластные *trnL-F* и *rps4* и митохондриальный *nad5*. Этот мох описан как новый род и вид, *Rheosheockia fontana* Ignatov, W.Z. Ma & D.G. Long, поскольку среди видов *Symphyodon* сходных видов нет. Отмечается, что это не первый случай, когда молекулярно-филогенетические исследования выявляют близкое родство эпифитов и реофитов.

KEYWORDS: new taxa, *Rheosheockia fontana*, *Glossadelphus*, *Phyllocladon*, molecular phylogeny, ITS, *trnL-F*, *rps4*, *nad5*

INTRODUCTION

The Chinese moss flora with nearly 2000 species reported makes that country probably one the richest for bryophytes in the world (Redfean *et al.*, 1996; the eight volume *Moss Flora of China*, 2002–2014), and Yunnan Province is the most species rich province within the country (Li, 2002, 2005). Although actively studied for about a century since the pioneering collections of Handel-Mazzetti (Brotherus, 1924; 1929), numerous additions to the flora have appeared since the classical works of P.-C. Chen (1978) and his student X.-J. Li (2002, 2005), and in various treatments of the *Moss Flora of China*. These additions continue steadily in recent studies in the course of broader international collaboration and biodiversity inven-

tory activities (Bednarek-Ochyra & Ochyra 2010; Bell *et al.*, 2017; Blom *et al.*, 2011; Enroth *et al.*, 2010, 2018; Higuchi & Long, 2002; Ma *et al.*, 2014, 2016a; Ma & Shevock 2015a,b, 2016; Shevock *et al.*, 2018; Yi *et al.*, 2015).

This paper describes one more enigmatic moss from Yunnan, collected in streams within the upper forest belt in the Gaoligong Shan region of westernmost Yunnan by James R. Shevock and the first author. This new moss is superficially similar to *Hygrohypnum* (especially *H. fontinaloides*) in both morphology and ecology, but does not fit any known species of this genus, likewise any other moss known from China. Therefore we undertook a DNA study to further elucidate its relationship to other pleurocarpous mosses, and as in many cases molecular insights

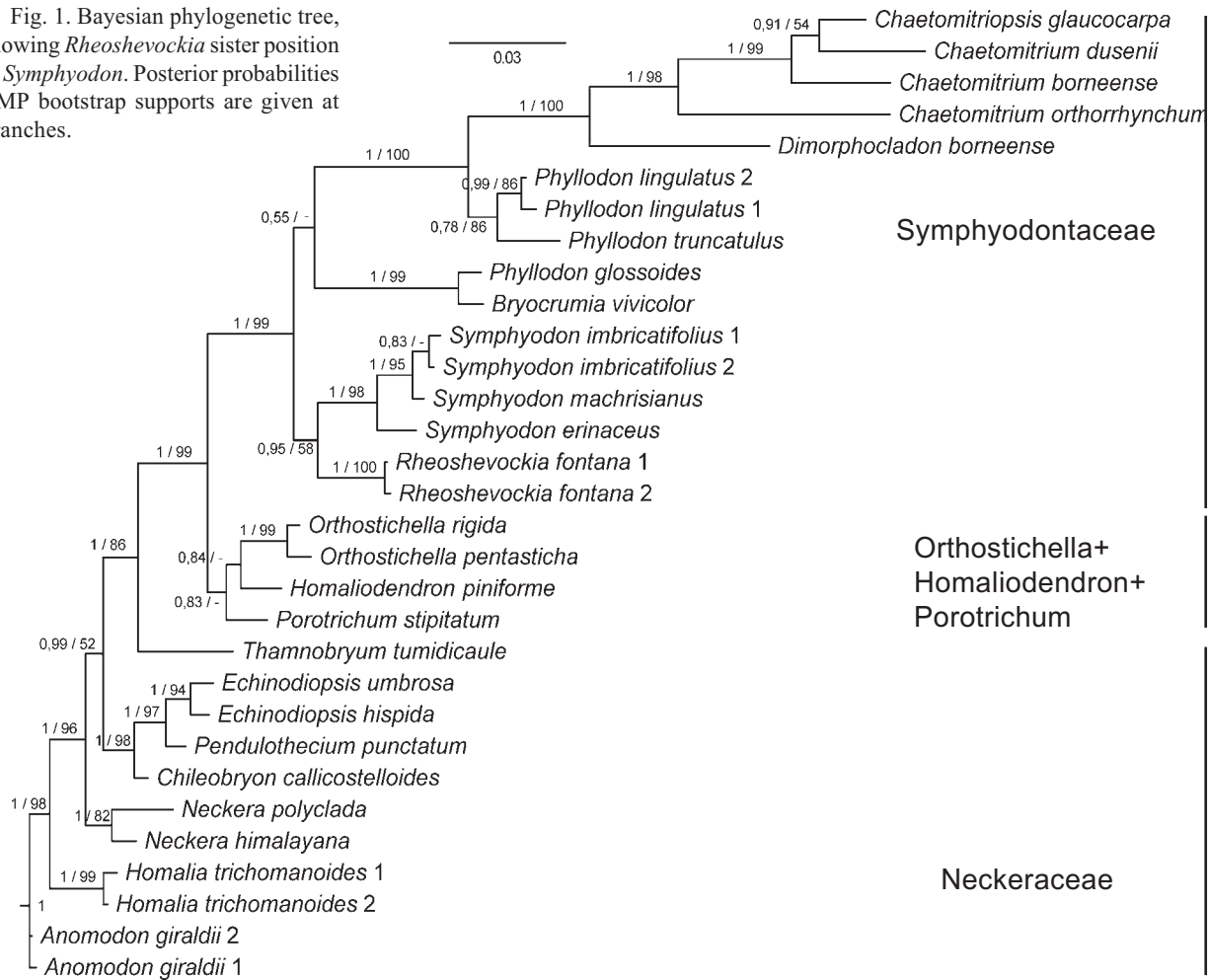
<sup>1</sup> – Herbarium, CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

<sup>2</sup> – Royal Botanic Garden, 20A Inverleith Row, Edinburgh EH3 5LR, UK

<sup>3</sup> – Tsitsin Main Botanical Garden, Russian Academy of Sciences, Botanicheskaya Str., 4, Moscow 127276 Russia – Россия 127276 Москва, Ботаническая 4, ГБС РАН.

<sup>4</sup> – Lomonosov Moscow State University, Biological Faculty, Geobotany Dept., Leninskie Gory Str. 1-12, Moscow 119234 Russia – Россия, 119234, Москва, Ленинские Горы, д. 1 стр. 12, Московский государственный университет, биологический факультет, кафедра геоботаники. E-mail: misha\_ignatov@list.ru

Fig. 1. Bayesian phylogenetic tree, showing *Rheosheockia* sister position to *Symphyodon*. Posterior probabilities / MP bootstrap supports are given at branches.



help to resolve morphological puzzles which probably would be impossible to resolve otherwise (e.g. Ignatov *et al.*, 2014; Shevock *et al.*, 2018).

MATERIAL AND METHODS

Molecular studies of the Yunnan specimen started with nuclear ITS, as the most variable and useful gene region in pleurocarpous mosses. As the preliminary BLAST tests unexpectedly revealed that the Yunnan moss appears to be most similar to *Symphyodon*, an epiphytic tropical moss genus (He & Sniders, 2000), we then expanded the markers, including also chloroplast *trnL-F* and *rps4* and mitochondrial *nad5*, and added more *Symphyodon* species verified from herbarium collections. Additional taxa for the present analysis were selected from GenBank using two criteria: 1) maximal similarity with the Yunnan moss in their sequences; and 2) represented by maximal, ideally four gene regions from the same specimen, although the latter condition was not always fulfilled (Table 1), as data were limited.

Total genomic DNA was extracted from dry plants using the Nucleospin Plant Extraction Kit (Macherey-Nagel, Germany). Laboratory protocol was essentially the same as in previous moss studies, described in detail by, e.g., Gardiner *et al.* (2005) and Fedosov *et al.* (2016).

Sequences were aligned by Clustal and modified manually using BioEdit 7.0 (Hall, 1999). Bayesian analysis of the ITS dataset was conducted in MrBayes (Huelsenbeck & Ronquist, 2001) using the GTR+G model. It was run for 10 000 000 generations with sampling every 1000 generations. The first 25% of sampled trees were discarded for the burn-in. Supplementary maximum parsimony analysis was performed in Nona (Goloboff, 1994) in Winclada shell (Nixon, 1999), with bootstrap calculation for 2000 replications (N searches 100, starting trees per rep 100, max trees 100, do max).

Moss floras of China (Wu & Crosby, 1999–2011), East Himalayas (Gangulee, 1969–1980), Java (Fleischer, 1906–1908, 1923), Japan (Noguchi *et al.*, 1994), and a revision of the genus *Glossadelphus* (Tixier, 1988), which partly now belongs to *Phyllodon* in current classifications, and revision of *Symphyodon* (He & Snider, 2000), were all consulted.

RESULTS

The obtained tree is shown in Fig. 1. In general it represents a grade of taxa traditionally classified within or near Neckeraceae and the group introduced by Olsson *et al.* (2009) as *Orthostichella* + *Porotrichum* + *Porothamnium* –clade. The terminal part of the tree includes



Fig. 2. *Rheosheockia fontana* (from isotype, MHA). Shoots, showing fasciculate branching (natural size).

two subclades: one is poorly supported but includes two smaller clades: (1) a maximally supported clade with most *Phyllodon* species, *Dimorphocladon*, *Chaetomitrium* and *Chaetomitriopsis*, and (2) another one, highly supported (PP=1, BS=99) with *Bryocrumia* and *Phyllodon glossoides*. The second terminal subclade includes a maximally supported smaller clade with two specimens of the Yunnan moss and another smaller clade with three species of *Symphyodon* (PP=1, BS=98).

As the genus *Dixonia* has some morphological and ecological similarity with our Yunnan moss, it was a subject for additional comparison. The most variable DNA region, the nuclear ITS of *D. thamnoides* (FM161097) was inserted in our main alignment, and the simple maximum parsimony analysis (not shown), found *D. thamnoides* in strict consensus tree with *Orthostichella* + *Poro-trichum* + *Porothamnium* species, i.e. far from Symphyodontaceae.

#### DISCUSSION

Goffinet *et al.* (2009) defined the family Symphyodontaceae M. Fleisch. as containing six genera: *Chaetomitriopsis* M. Fleisch., *Chaetomitrium* Dozy & Molk., *Dimorphocladon* Dixon, *Symphyodon* Mont., *Trachythecium* M. Fleisch., and *Unclejackia* Ignatov, T. Kop. & D. Norris. The last one, according to recent molecular results, belongs to the Brachytheciaceae (Ignatov & Huttunen, 2002; Huttunen & Ignatov, 2004). According to unpublished data obtained by the last author, *Trachythecium* belongs to the Pylaisiaceae. Cox *et al.* (2010) confirmed four genera in Symphyodontaceae, *Chaetomitriopsis*, *Chaetomitrium*, *Dimorphocladon* and *Symphyodon*, which form a clade along with two genera classified in Hypnaceae, *Phyllodon* and *Bryocrumia*. The genera *Dimorphocladon* and *Bryocrumia* were not shown in the tree in Cox *et al.* (2010)' publication, but data are available in GenBank, and were therefore included in the present analysis, confirming that they are closely related to *Phyllodon* and *Symphyodon* (Fig. 1).

*Symphyodon* was revised worldwide by He & Snider (2000), who noted that the diagnostic characters of the

genus are mostly sporophytic, and therefore in the case of sterile plants, they are difficult to differentiate from *Glossadelphus*. The genus *Glossadelphus* was recently addressed in phylogenetic studies, which resulted in its splitting into two genera, *Filibryum* (Kim & Yamaguchi, 2017), a group related to Taxiphyllaceae (Ignatov *et al.*, 2012), and *Phyllodon* (earlier treated as *Glossadelphus*, which appears to be a later synonym of *Phyllodon*, cf. Buck, 1987). Most species of *Symphyodon* are epiphytic (while this new Yunnan moss is aquatic) and both *Symphyodon* and most species of *Phyllodon* have prorate cells and serrate to serrulate leaves (He & Snider, 2000; Tixier, 1988), whereas the new Yunnan moss has smooth laminal cells and an entire leaf margin.

The same distinctions in leaf laminal cells occur between the new Yunnan moss and *Bryocrumia*, an Arcto-Tertiary species disjunct between East Asia and eastern North America that has stem central strand, prorate laminal cells and serrulate leaf margins, and leaves that are also considerably smaller (0.7–0.8 mm vs. 0.75–1.1 mm long) in *Rheosheockia*.

*Dimorphocladon*, *Chaetomitrium* and *Chaetomitriopsis* have even more projecting cell ends in both gametophyte and sporophyte (Akiyama, 2011; Suleiman & Akiyama, 2014), and therefore, distant from the new Yunnan moss not only genetically, but also morphologically.

Given the above distinctions from other members of Symphyodontaceae, we see no better solution than to describe the Yunnan rheophytic moss as a genus new for science.

#### TAXONOMY

***Rheosheockia fontana*** Ignatov, W.Z. Ma & D.G. Long, gen. and species nova. Figs. 2–3.

Type: China, Yunnan Province, Gaoligongshan Region, Teng-chong County, Western slope of the Gaoligongshan, 3.1 km above Danzha Forestry Farm Headquarters from Zhaobitan Bridge along the Zhaobitan River, 25°34'01.6" N, 98°11'48.7" E, 25 Apr 2015, elev. 2685 m. Mixed hardwood forest. Cascading stream over



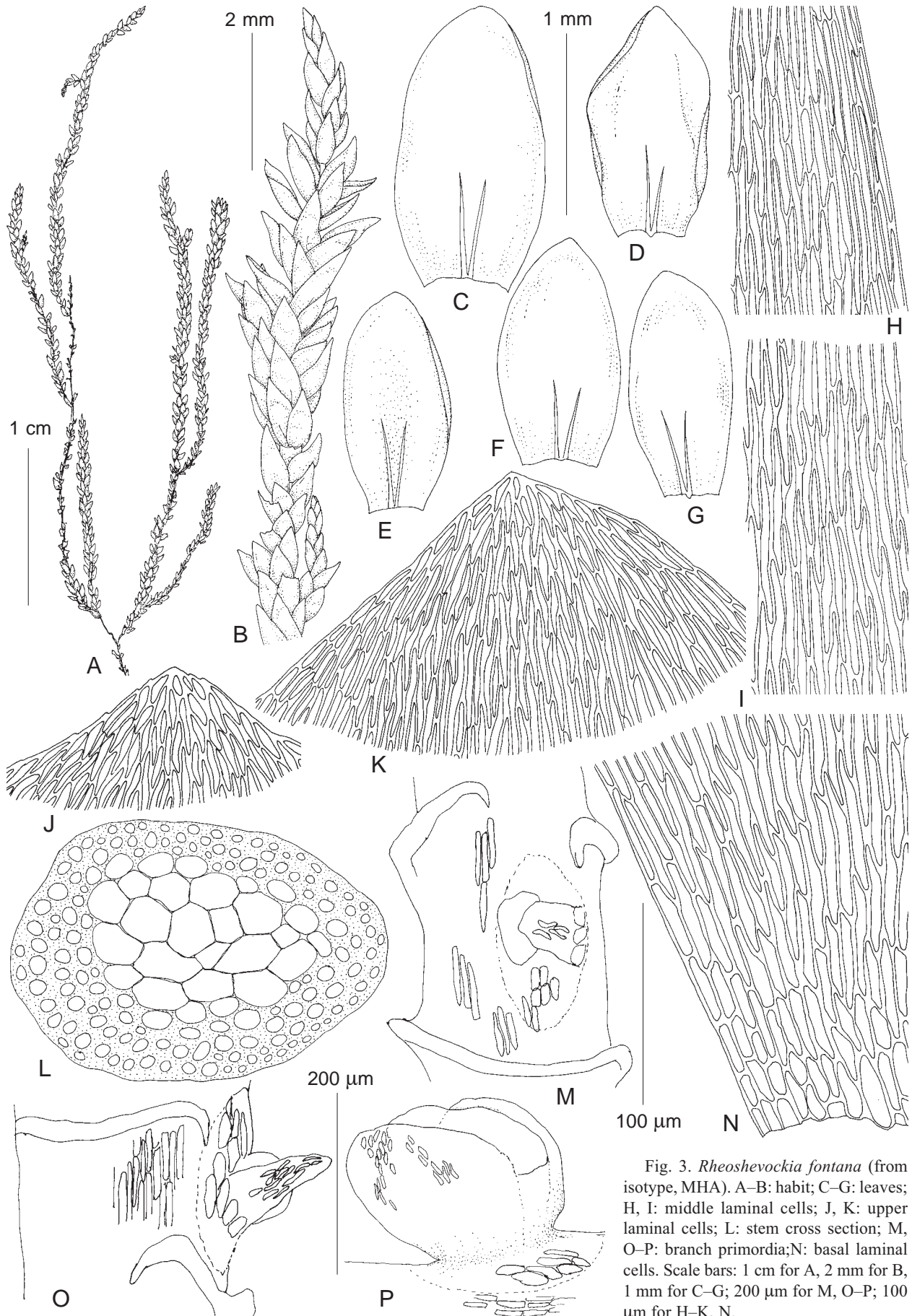


Fig. 3. *Rheoshevockia fontana* (from isotype, MHA). A–B: habit; C–G: leaves; H, I: middle laminal cells; J, K: upper laminal cells; L: stem cross section; M, O–P: branch primordia; N: basal laminal cells. Scale bars: 1 cm for A, 2 mm for B, 1 mm for C–G; 200 μm for M, O–P; 100 μm for H–K, N.



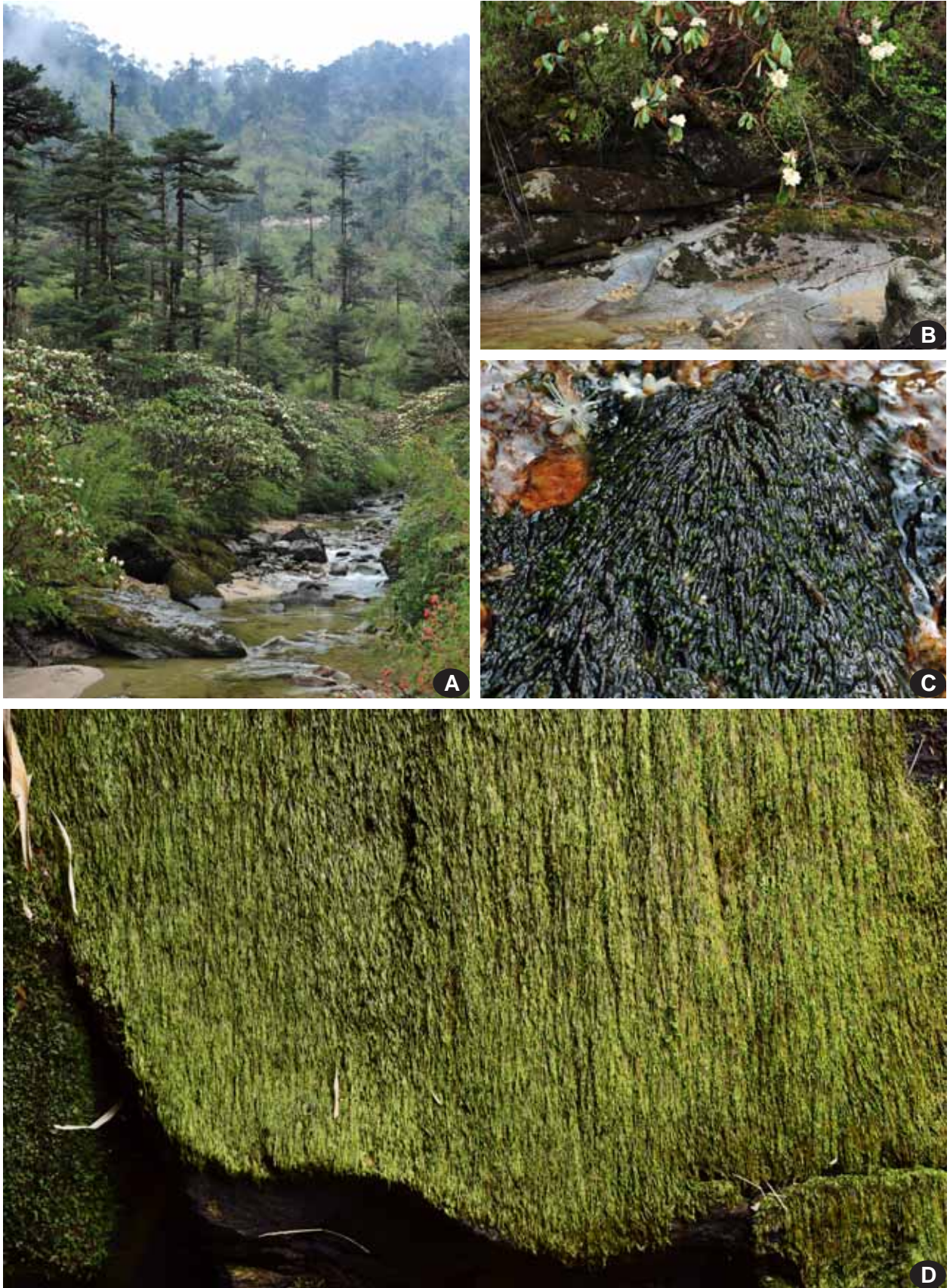


Fig. 4. A: *Rheosheevokia fontana* habitat along streams in an open conifer and hardwood forest with *Rhododendron* understorey; B: plants in dense pendulous mats closely affixed to the rock substrate; C: specimen W.Z. Ma # 15-6319; D: specimen W.Z. Ma # 17-8912 (photos by W.Z. Ma).



granitic bedrock along roadside. On vertical wet rocks wall in shade. *Shevock, W.Z. Ma & Y.L. Yao 46419* (Holotype: KUN; Isotypes: CAS, E, H, KRAM, MHA, MO, NY, PE, SZG, TAIE, TNS).

**Etymology:** the name is deriving from *rheo* – stream (Greek), indicating its characteristic growth in running water, and from James Robert Shevock (1950–), a bryologist and the collector of the type material of this plant.

**Diagnosis.** Differs from all other Symphyodontaceae in its entire leaves and submerged growth in rapidly running water.

**Description.** Plants medium-sized to moderately robust, in rather dense mats, deep green to brownish-green, glossy. Stem floating in running water to prostrate across emergent boulders and rockslabs, thin, firm, 4–10 cm long, 0.15–0.20 mm in diameter, without hyalodermis and central strand, with sclerodermis consisting of 3–4 layers of small cells with thick, brown walls, and 2–3 layers of large, thin-walled medullary cells; terete densely foliate, with few to numerous branches 5–20 mm long to densely copiously fasciculate branching, with numerous reiterating shoots. Branch initials occurring in the central of zone of pellucid cells, contrasting with otherwise dark red-brown cortical cells; the outermost branch leaves in lateral position (4 and 11 o'clock), ovate-triangular. Stem and branch leaves not differentiated; loosely appressed when dry, erectopatent when moist, 0.75–1.1 mm long, 0.5–0.45(–0.5) mm wide, oblong-ovate to occasionally obovate, moderately concave, broadly acute to subobtuse; gradually tapered to base, not decurrent; margins plane to shallowly incurved, serrulate distally, entire or slightly uneven along most of leaf; costa double, ± thick, reaching to 1/4–1/3 the leaf length; median leaf cells linear, 50–90 µm long, ca. 4 µm wide (luminae 3 µm wide), non-porose, with rounded ends; cells at leaf apex shorter; cells in basal 2–4 rows shorter and wider, thick-walled and porose; marginal and alar cells not differentiated. Gametangia and sporophytes unknown.

**Distribution.** Currently endemic to China, although likely to also be discovered in other mountainous regions and countries adjacent to the Yunnan border between 2400–3000 m, such as Myanmar, Laos and Vietnam.

**Ecology.** A rheophytic species restricted to cold, clear cascading streams and rivulets affixed to boulders and rockslabs in high-quality mountain forest areas.

**Selected specimens examined:** CHINA: **Yunnan Province:** Jin-ping County, Wu-Tai Mountain, along trail from Shi-Dong Village to the deserted radar station, 21 Feb 2017, 2430 m, 22°45'19" N, 103°27'19" E, *W.Z. Ma et al. 17-8912* (CAS, GSUB, HSNU, KUN, SZG); Teng-chong County: Hou-Qiao Zhen, Dan-Zha Forest Farm, on seeping granitic rock along stream channel, 2754 m, 25 Apr 2015, 25°34'30" N, 98°11'53" E, *W.Z. Ma, Shevock & Y.L. Yao 15-6121* (CAS, KUN); Ming Guan Xiang, Zi-Zhi community near No. 6 Sino-Burma boundary, on metamorphic rock in intermittent stream channel, 6 May 2015, 2850 m, 25°45'14" N, 98°28'24" E., *W.Z. Ma, Shevock & Y.L. Yao 15-6319* (CAS, KUN); headwaters of the Linjia-

tang River, Mingguang Forest Farm, on road 17.5 km above Zizhi Village near No. 6 Pass at border with Myanmar, on wet granitic rockslab bedrock of intermittent rivulet, 6 May 2015, 2800 m, 25°45'05" N, 98°28'33" E, *Shevock, W.Z. Ma & Y.L. Yao 46633* (CAS, E, KRAM, KUN, MHA, PE, SZG, TAIE); headwaters of Linjiatang River on fire access road near junction of road 2 km below No. 6 Pass, 7 May 2015, 2750 m, 25°44'23" N, 98°28'04" E, *Shevock, W.Z. Ma & Y.L. Yao 46660* (BOL, CAS, F, FH, H, HYO, KRAM, KUN, MHA, MO, NY, S, UBC, VBG1), 7.3 km above the Danzha Forestry Farm headquarters and Zhaobitan River Bridge and 5.2 km below Changdifang Pass, 27 Apr 2015, 2950 m, 25°35'30" N, 98°10'10" E, *Shevock, W.Z. Ma & Y.L. Yao 46478* (CAS, E, H, KRAM, KUN, MHA, MO, NY, PE, SZG, VBG1).

**Differentiation.** At the first glance in the field, *Rheoshevoekia* may look like *Hygrohypnum* s.l., some species of which lack a long single costa. However a multilayered sclerodermis of cells with so small a lumen never occurs in species of that genus. The stem looks dark brown and only around young branch primordia a pellucid areas occur. Lamina areolation is also very tight due to narrow cell lumens, and this pattern (along with entire leaves) is similar to that of former *Glossadelphus* species, especially *G. ogatae*, which, however, was found to be not closely related to most species of the genus, i.e. *Phyllodon*, and appeared completely unrelated to them, clustering with *Taxiphyllum* and separated to the genus *Filibryum*.

In addition, the branching in *Hygrohypnum* s.l. is also more or less pinnate, whereas *Rheoshevoekia* is characterized by fasciculate branching, indicating its affinity with “Isobryalean” families, where “primary” and “secondary” stems are differentiated. Interestingly, the Yunnan moss flora has some other examples of rheophytic mosses from otherwise principally epiphytic families, i.e. from monospecific *Yunnanobryon*, Regmatodontaceae, and oligospecific *Handeliobryum*, Neckeraaceae (Shevock *et al.*, 2011, 2017).

#### **On the generic content of the Symphyodontaceae**

The present analysis also confirmed the close systematic position of the genera *Bryocrumia*, *Chaetomitriopsis*, *Chaetomitrium*, *Dimorphostegium*, *Phyllodon*, and *Symphyodon*, as was found by Cox *et al.* (2010), suggesting a somewhat broader definition of the family. The main morphological traits in the family include a papillose seta, conspicuously prorate lamina cells and usually obtuse leaves and a more or less modified peristome. Some taxa, e.g. *Bryocrumia* (Ma *et al.*, 2016b) have a smooth seta, which is however characteristically hooked, as in e.g. many *Chaetomitrium* (Suleiman & Akiyama, 2015) and *Phyllodon* (Tixier, 1988) species.

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Table 1. GenBank accessions of the species used in phylogenetic analysis. Newly generated sequences include voucher data .

Species	ITS	nad5	rps4	trnL
<i>Anomodon giraldii</i> 1	KF770680	-----	KF770572	KF770518
<i>Anomodon giraldii</i> 2	FM161075	FM161240	AM990342	AM990342
<i>Bryocrumia vivicolor</i>	-----	AY908437	AY908196	xxxxxxxx
<i>Chaetomitriopsis glaucocarpa</i>	HQ443721	AY908681	AY908603	HQ443858
<i>Chaetomitrium orthorrhynchum</i>	HQ443722	HQ443789	HQ443821	HQ443859
<i>Chaetomitrium dusenii</i>	HQ613414	AY452334	AY306881	AY306715
<i>Chaetomitrium borneense</i>	HQ613413	AY452333	AY306880	AY306714
<i>Chileobryon callicostelloides</i>	FM161088	FM882226	FM882222	FM210283
<i>Dimorphostegium borneense</i>	HQ443732	AY452348	AY306898	AY306732
<i>Echinodiopsis hispida</i>	FM161099	-----	-----	FM210286
<i>Echinodiopsis umbrosa</i>	AY999172	AY908680	AF143044	-----
<i>Homalia glabella</i>	FM161123	FM161277	AM990382	AM990382
<i>Homalia trichomanoides</i> 1	DQ680150	-----	AY908276	AM990385
<i>Homalia trichomanoides</i> 2	FM161126	FM161280	-----	GQ428071
<i>Homaliodendron piniforme</i>	FM161134	FM161286	AM990391	AM990391
<i>Neckera himalayana</i>	FM161163	FM882223	882219	-----
<i>Neckera polyclada</i>	FM161170	FM882224	FM882220	FM210307
<i>Orthostichella pentasticha</i>	HE660017	AY908655	AY907962	AF543548
<i>Orthostichella rigida</i>	FM161185	FM161312	-----	AM990422
<i>Pendulothecium punctatum</i>	FM161187	FM161314	AM990421	AM990421
<i>Phyllocladon lingulatus</i> 1	FM161105	FM161262	AM990367	AM990367
<i>Phyllocladon lingulatus</i> 2	KT804691	-----	-----	-----
<i>Phyllocladon truncatulus</i>	HQ443764	AY908682	AY908604	HQ443880
<i>Porotrichum stipitatum</i>	HE660018	HE717040	JQ815892	AY010299
<i>Symphyodon imbricatifolius</i>	FM161214	-----	-----	-----
<i>Symphyodon imbricatifolius</i>	HQ443774	AY452387	AY306999	AY306833
<i>Thamnobryum tumidicaule</i>	FM161231	FM161337	AM990447	AM990447
Newly generated sequences				
<i>Rheoshevoekia fontana</i> 1				
Yunnan, Shevock 46478 (MHA ex CAS)	MK164022	MK279669	MK257801	MK257805
<i>Rheoshevoekia fontana</i> 2				
Yunnan, Shevock 46419 (MHA ex CAS)	MK164023	MK279670	MK257802	MK257806
<i>Symphyodon machrisianus</i>				
Brasil, Schaefer-Verwimp 13309 (MHA ex GOET)	MK164024	MK257809	MK257803	MK257807
<i>Symphyodon erinaceus</i>				
Thailand, Akiyama TH-74 (MHA ex HIRO)	MK164025	MK257810	MK257804	MK257808

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