



## *Mawenzhangia thamnobryoides* (Bryophyta, Lembophyllaceae), a new moss genus and species from the Shangri-la region of Yunnan Province, China

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### Abstract

*Mawenzhangia thamnobryoides* gen. et sp. nov. (Bryophyta, Lembophyllaceae) is described based on collections from Yunnan, China. It is known from the Niru River watershed and grows on boulders just above the high water zone but can also be seasonally inundated. According to ITS sequences it is closely related to *Nogopterium gracile* (*Pterogonium* g.), but can be distinguished by the loosely patent and elliptic vs. imbricate and ovate leaves; much longer, single costa; thinner-walled and less strongly prorate laminal cells; and much smaller group of alar cells. *Mawenzhangia thamnobryoides* is illustrated in line drawings and an identification key to Chinese genera of the Lembophyllaceae is provided.

**Key words:** Morphology, new taxa, phylogeny, pleurocarpous mosses, systematics, taxonomy

### Introduction

During field work in Shangri-la County in the fall of 2016 by Wen Zhang Ma and the second author, an interesting moss was collected on metamorphic boulders along the banks of the Niru River in a high-quality mixed hardwood-conifer forest dominated by *Quercus* Linnaeus (1753: 994) and *Pinus* Linnaeus (1753:1000). At the time of collection, this specimen resembled *Thamnobryum* Nieuwland (1917:50) and that was the field name placed on the draft label. Later that fall, a duplicate set of Neckeraceae *s.l.* was sent to the first author. As soon as a microscope slide was prepared of this plant, the cellular architecture across the lamina was not consistent with members of the Neckeraceae. We determined that the plant has some resemblance to *Isothecium* (Bridel 1827: 355) so that genus was next investigated. It quickly was determined that these plants did not match any Asian species of *Isothecium* or other genera in the Lembophyllaceae (Gangulee 1976, Quandt *et al.* 2009, Wu 2011). We concluded that this moss was new to science. With this new discovery, another expedition was planned for late August 2017 for a return visit to the Niru River to relocate this moss and to document additional populations.

### Materials and Methods

Before microscopic examination the specimens or fragments thereof were soaked in 95% vol. denaturated ethanol for about 10 seconds and then in tap water for about 20 seconds. Microscopic examination and measurements were taken with Leica Biomed compound microscope and Leica GZ4 stereomicroscope.

Molecular studies of the Yunnan specimen (paratype *Shevock & W.Z. Ma 48904*, see below) includes two gene regions, especially variable and widely used and copiously represented in GenBank, namely nuclear ITS and chloroplastic *trnL-F*. The sequences are deposited in GenBank with accession numbers MG515239 (ITS) and MG515240 (*trnL-F*). 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). After BLAST found species with the most similar sequences, all from Lembophyllaceae (Hypnales), the dataset was formed from Genbank sequences. *Pterogonium gracile* (Hedwig 1801: 80) Smith (1802: pl. 1085) was found to be most closely related, so we added to the dataset some Leucodontaceae, where most authors place it (Goffinet *et al.* 2009). The currently accepted name of *Pterogonium gracile* is *Nogopterium gracile* (Hedw.) Crosby & Buck (2011: 424). The tree was rooted on *Hypopterygium* Bridel (1827: 709–718), a representative of Hookeriales, the order sister to Hypnales.

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 as selected by MrModeltest2.3 (Nylander 2004). It was run for 20 000 000 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).

## Results

### *Phylogenetic analysis*

The ITS tree (Fig. 1), rooted on *Hypopterygium* (Hookeriales), comprises a grade formed of species from, sequentially, Hylocomiaceae, Leucodontaceae, an as yet formally unnamed OPP-clade (Olsson *et al.* 2009), Heterocladiaceae, Neckeraceae, Thamnobryaceae, and Lembophyllaceae. The Lembophyllaceae-clade is highly supported (PP=0.96), and is composed of two subclades, both only moderately supported. The larger subclade (PP=0.76) includes one species of *Dolichomitra* Brotherus (1907: 867) and main part of Lembophyllaceae (20 species, 7 genera), the latter clade highly supported (PP=0.99). The smallest clade (PP=0.69) is composed of the enigmatic Yunnan moss plus clade of four accessions of *Nogopterium* (PP=0.85).

Chloroplastic *trnL-F* region is much less variable compared to nrITS, thus the analysis yielded no trees with reasonable statistical support. However, the maximum parsimony analysis resulted in a strict consensus tree where the terminal clade includes a trichotomy of (1) *Thamnobryum*; (2) other Neckeraceae species, and (3) Lembophyllaceae *s.l.* The latter clade also included a trichotomy of (1) the new Yunnan moss; (2) *Nogopterium*, and (3) all other Lembophyllaceae, comprised by 25 species of eight genera (Supplementary material 1). Thus, the topology of this tree is fully consistent with that obtained in the ITS analysis, albeit without support. Both analyses indicate a highly isolated position of the Yunnan moss, which obviously merits recognition as a new genus in Lembophyllaceae.

## Taxonomy

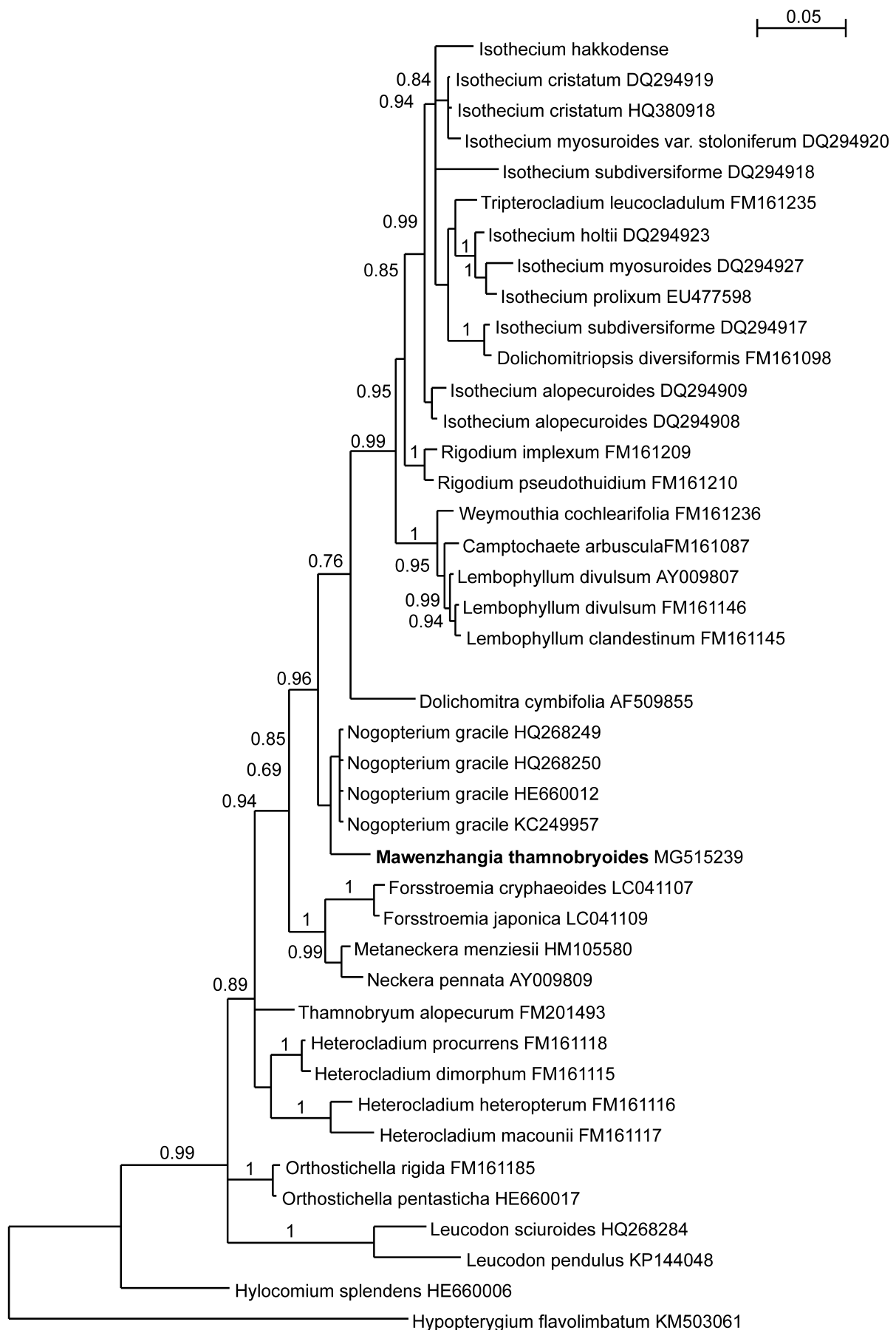
*Mawenzhangia* Enroth, Shevock & Ignatov, *gen. nov.*

Plants stipitate-frondose, habit resembling *Isothecium* and other genera in the Lembophyllaceae, but can be distinguished by a combination of morphological features: distant, not overlapping stipe leaves; mostly elliptic stem leaves distinctly narrowed at base; relatively long, single costa; leaf margins serrate at leaf tip and often down to midleaf, teeth often reflexed near leaf apex; thin-walled, usually abaxially prorate laminal cells; and often abundant flagelliform branchlets.

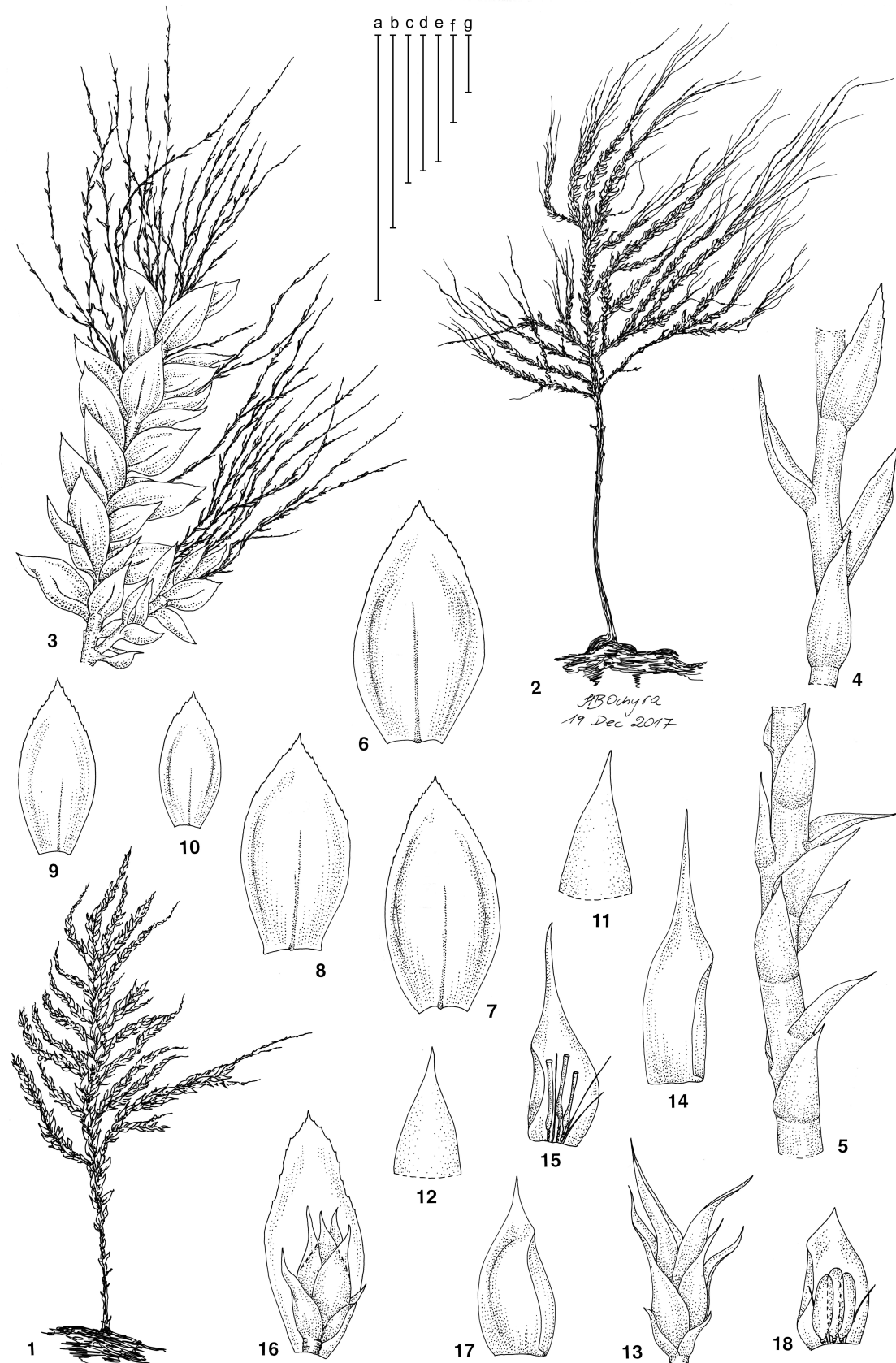
Generitype: *Mawenzhangia thamnobryoides* Enroth, Shevock & Ignatov

*Mawenzhangia thamnobryoides* Enroth, Shevock & Ignatov, *sp. nov.* (Figs. 2, 3)

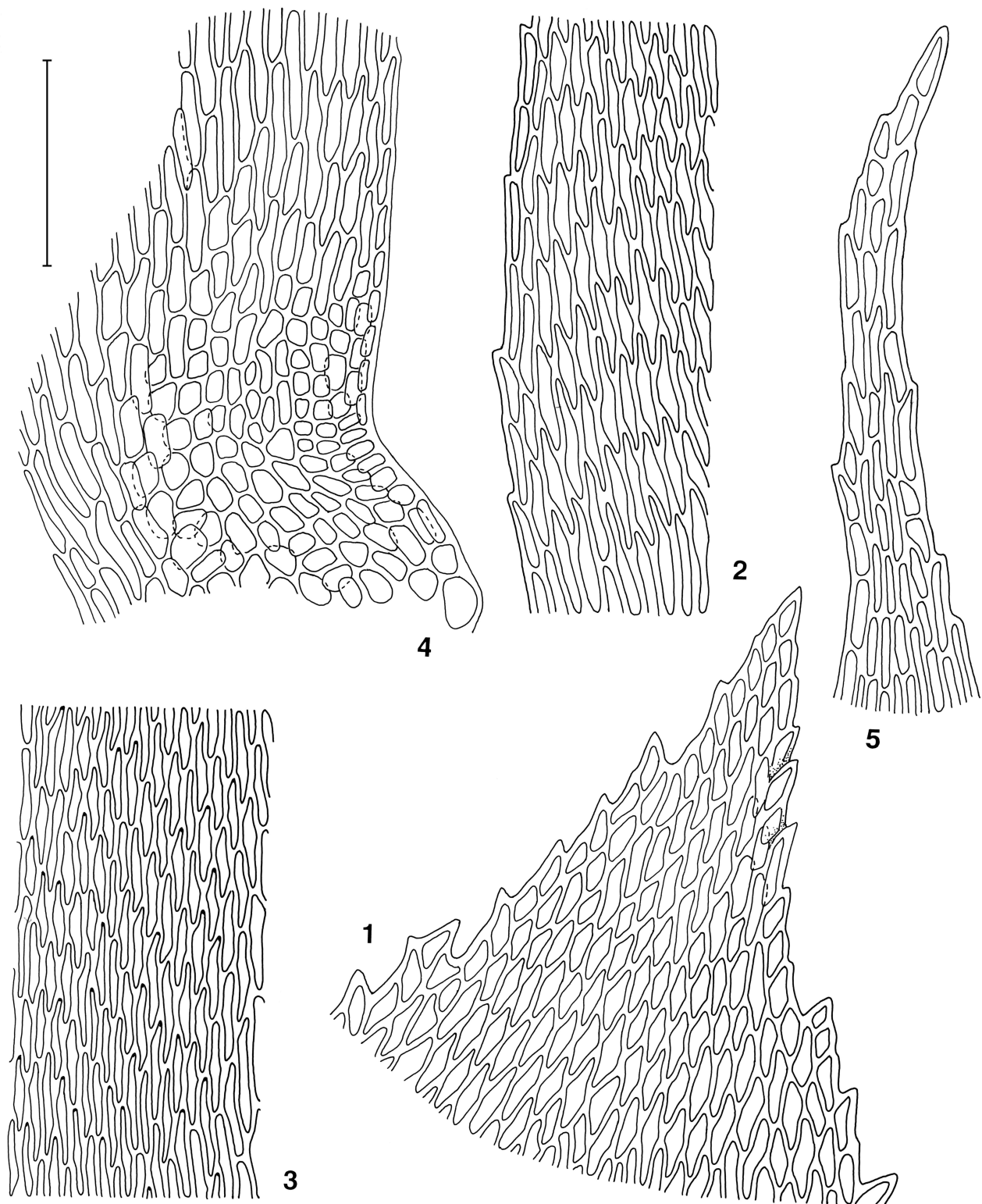
**Type:**—CHINA. YUNNAN PROVINCE: Shangri-la County, along the trail about 5 km from the upper end of Niru Village to Colorful Rainbow Waterfall, slopes of the Pielong Shan above the Niru River, on base of *Picea* trunk, 27°57'42"N, 100°02'11"E, 3170 m, 29 August 2017, *W.Z. Ma 17-9217* (holotype KUN!, isotypes CAS!, E!, H!, KRAM!, MHA!, MO!, NY!, PE!, SZG!, TAIE!, TNS!).



**FIGURE 1.** Phylogenetic position of *Mawenzhangia thamnobryoides* (in bold face) based on nrITS sequences. The numbers are PP-values from Bayesian inference. GenBank accession numbers are given after the taxon names.



**FIGURE 2.** *Mawenzhangia thamnobryoides*. 1–2. Habit. 3. Portion of flagelliform branch. 4. Portion of leafy flagella. 5. Portion of leaf stipe. 6–8. Main branch leaves. 9–10. Secondary branch leaves. 11–12. Stipe leaves. 13. Perichaetium. 14. Inner perichaetial leaf. 15. Innermost perichaetial leaf with archegonia. 16. Perigonium. 17. Inner perigonial bract. 18. Innermost perigonial bract with antheridia. 1–5, 8, 13–15 from *Ma 17-9217* (KRAM, isotype); 6–7, 10–12, 16–18 from *Ma 17-9184* (KRAM, paratype). Scale bars: a – 0.5 cm (3); b – 1 mm (14–15, 17–18); c – 1 mm (11–12); d – 1 cm (1–2) and 1 mm (13, 16); e – 1 mm (6–10); f – 1 mm (5); g – 100  $\mu$ m (4).



**FIGURE 3.** *Mawenzhangia thamnobryoides*. 1. Leaf apex. 2. Upper marginal cells. 3. Mid-leaf cells. 4. Basal cells. 5. Apex of innermost perichaetial leaf. 1–2, 5 from *Ma 17-9217* (KRAM, isotype); 3–4 from *Ma 17-9184* (KRAM, paratype). Scale bar 100  $\mu$ m.

Plants gregarious, stipitate-frondose, to ca. 5 cm tall, yellowish green or partly brownish-gray, somewhat glossy. Stolons creeping, tiny-leaved, bearing tufts of intensely reddish-brown, verruculose, sparsely branching rhizoids. Fronds rather complanate, subpinnately to irregularly but densely branched above stipe, branches variously curved and with secondary branches, tapering towards tip, tips sometimes flagelliform and microphyllous; flagelliform, axillary, microphyllous branchlets often present, sometimes very abundant especially in older, more or less withered fronds, making them appear broom- or tassel-like. Stipe in cross-section elliptical, with small, thick-walled epidermal cells

and 4 or 5 layers of small, thick-walled cortical cells, medullary cells just inside cortex thicker-walled and smaller than inner medullary cells, central strand small but distinct, consisting of cells clearly smaller than surrounding medullary cells. Stipe leaves not overlapping, patent, to 1 mm long and 0.6 mm wide, apex narrowly acute to acuminate; costa absent or ill-defined and short; margins plane, entire. Branch primordia tightly covered by tiny, wide, arching embryonic leaves forming a dome-shaped structure; pseudoparaphyllia absent. Axillary hairs 5–8-celled, c. 140  $\mu$ m long, other cells thin-walled and hyaline except 1–2 basal cells brownish but translucent, and shorter than upper cells. Stem leaves ca. 1.6  $\times$  0.8 mm, elliptical or nearly oblong, concave, loosely appressed when dry, patent to spreading when wet, leaf apices acute. Branch leaves similar but smaller, sometimes nearly ovate and more strongly narrowed toward base; ultimate branch leaves sometimes ecostate or nearly so. Costa single but often spurred or bifurcate near base, tapering upwards, reaching to  $\frac{1}{2}$ – $\frac{2}{3}$  of leaf length; margins plane, serrate by uni- to tri-cellular teeth in upper parts of leaves, these teeth often reflexed, serrate to serrulate at midleaf, entire near leaf base. Leaf cells fairly thin-walled, with blunt ends, often prorate in median and upper parts of leaves, smooth or nearly so at apex and near base; apical cells somewhat sinuous, oblong to sub-rhomboid or vaguely penta- to hexagonal, 20–40  $\times$  8–10  $\mu$ m, median laminal cells oblong to linear, slightly sinuous, 40–60  $\times$  6–8  $\mu$ m, basal laminal cells similar to median cells in shape but to ca. 80  $\mu$ m long, marginal cells on both sides of leaves shorter than corresponding laminal cells; alar cells forming distinct groups, small, thick-walled, short-rectangular to isodiametric and quadrate or roundish, heavily chlorophyllose in fresh plants. Dioicous. Perichaetia and perigonia uncommon, scattered in upper parts of fronds. Perigonia bud-shaped, c. 1 mm tall; perigonial leaves ovate-lanceolate or oblong-lanceolate, ecostate, margins entire throughout or with few indistinct teeth near apex. Inner perichaetial leaves ca. 1 mm long, from an ovate base at ca. midleaf narrowed to a lanceolate-lingulate acumen, ecostate; margins entire below, with scattered but prominent and somewhat reflexed teeth in acumen; laminal cells fairly firm-walled, smooth, linear elsewhere but rectangular to oblong near leaf base, a discontinuous row of (long-)rectangular cells present along both leaf margins; archeogonia ca. 0.35 mm long, intermixed with hyaline, filiform paraphyses with elongate, narrow upper cells and wider, thinner-walled basal cells. Sporophytes unknown.

**Paratypes:**—CHINA. YUNNAN PROVINCE: Shangri-la County, along the banks of the Niru River, 0.25 km from the upper end of Niru Village, slopes of the Pielong Shan, 27°57'16.4"N, 100°03'39.8"E, 2770 m, 18 September 2016, *Shevock & W.Z. Ma 48904* (CAS!, H!, KUN!, MO!, MHA!); Niru River, 27°57'13"N, 100°03'41"E, 2750 m, 27 August 2017, *Shevock & W.Z. Ma 50590* (CAS!, E!, H!, KRAM!, KUN!, MO!); Niru River, 27°57'14"N, 100°03'42"E, 2790 m, 27 August 2017, *W.Z. Ma & Shevock 17-9184* (CAS!, H!, KRAM!, KUN!, NY!) and 100°57'18"N, 100°03'37"E, 2820 m, 27 August 2017, *17-9192* (CAS!, H!, KUN!, PE!, SZG!).

**Etymology:**—The genus is named after bryologist Dr. Wen Zhang Ma (1981–), curator of bryophytes, Kunming Institute of Botany, Chinese Academy of Sciences (KUN). The species epithet refers to the appearance of the thickened dendroid erect shoots and branches of younger plants.

**Distribution and Habitat:**—At present, *Mawenzhangia thamnobryoides* is known only from the Niru River watershed within Shangri-la County of De-qin Prefecture. All occurrences lie within the broader Hengduan Mountains, the easternmost extension of the Himalayas comprising large areas of both Sichuan and Yunnan provinces. *Mawenzhangia* apparently prefers boulders just above the high water zone although it can also be seasonally inundated. The forest cover along the river corridor likely provides both filtered light to shade and more mesic conditions than the drier upland slopes. *Mawenzhangia thamnobryoides* also occurs on the base of large *Picea* Dietrich (1824:794) trunks in steeper shaded canyons within the river corridor. Populations are mixed among other mosses, particularly species of *Plagiomnium* Koponen (1968:145) and *Thuidium* Schimper (1852:157). However, these habitat parameters are based on only two separate encounters in a rather limited geographical area, and therefore, should be viewed as preliminary observations. The likelihood of discovering additional populations of *Mawenzhangia* is relatively high. Much of the surrounding area of the type locality is rugged mountainous terrain above 3000 m and comprised of natural pristine-appearing forests with few roads or trail access.

## Discussion

The Lembophyllaceae, containing 13 genera, is a sister group of the Neckeraceae, and the clade formed by the two families is sister to an “OPP-clade” (Quandt *et al.* 2009). The Lembophyllaceae are best represented in temperate regions of both hemispheres, especially in Australasia, and have fewer tropical genera. Although Tangney (1997) recognized only five genera in the Lembophyllaceae, most of the character states he used to jointly define the family are

still valid, such as weft-forming or pendent growth form; commonly epiphytic habitat; ovate-oblong, concave leaves; rhombic upper laminal cells; prorate mid-laminal cells; and exserted capsules. According to our observations, most genera and species in the Lembophyllaceae also have flagelliform branch tips and/or flagelliform axillary branchlets.

According to Wu (2011), in China the Lembophyllaceae are represented by *Isothecium*, *Dolichomitra* Brotherus (1907: 867) and *Dolichomitriopsis* Okamura (1911: 66). Wu (2011) also mentioned the unispecific genus *Dixonia* Horikawa & Ando (1964: 23), which has been placed in the Lembophyllaceae but actually belongs in the aforementioned OPP-clade (Olsson *et al.* 2009) and is known from China (He *et al.* 2009). Redfearn *et al.* (1996) cited *Pterogonium gracile* var. *tsinlingense* P.-C. Chen *ex* Zhang (“Chang”, 1974: 347) (Lembophyllaceae) from two provinces (Shaanxi, Sichuan), but according to Wu (2011) that was based on misidentified specimens of *Pterogoniadelphus esquirolii* (Thériot 1907: 22) Ochyra & Zijlstra (2004: 811) of the Leucodontaceae. The variety has been considered to be a distinct species, *Pterogonium tsinlingense* (Zhang) Suzuki & Iwatsuki (2012: 59), which was subsequently given a name in *Nogopterium* Suzuki (2016: 138). However, that combination was not validly published and consequently Ochyra & Bednarek-Ochyra (2017) validated the name *N. tsinlingense* (Zhang) Tad. Suzuki *ex* Ochyra & Bednarek-Ochyra (2017: 89, “*tsilingense*”). *Nogopterium gracile* is distributed in the south-western half of Europe (reaching to the North and East Sweden, Estonia, the Ukraine and Russian part of the Caucasus), Macaronesia, Middle East, Africa and its adjacent islands to the east, and in western North America (e.g. Lawton 1971, O’Shea 2003, Smith 2004, Ignatov *et al.* 2006, Ros *et al.* 2013, Bourell 2014). *Nogopterium* (as *Pterogonium*) is usually placed in the Leucodontaceae (Goffinet *et al.* 2009, Frey & Stech 2009). Alternatively it was earlier placed in its own family Pterogoniaceae (cf. Ignatov *et al.* 2006), which has a complicated nomenclatural history (Ochyra & Bednarek Ochyra 2017). Molecular phylogenetic analysis indicates its position in Lembophyllaceae (Troitsky *et al.* 2007, Cox *et al.* 2010, Huttunen *et al.* 2012). Additional evidence for its position in Lembophyllaceae rather than Leucodontaceae were provided by Spirina & Ignatov (2011) based on the structure and arrangement of the proximal branch leaves.

*Mawenzhangia* is sister to *Nogopterium* (Fig. 1) and these two genera share several morphological character states. Both are distinctly stipitate-frondose, often with a long stipe relative to the length of the frond, which branches irregularly to (sub)pinnately. The branches are typically arcuate, albeit more strongly so in *Nogopterium*, and many of them have gradually tapering and flexuose, flagelliform tips with minuscule leaves. Flagelliform, axillary and easily detachable branchlets may also be produced in variable quantities. The leaf apices are serrate, the laminal cells prorate (although sometimes only indistinctly so in *Mawenzhangia*), and the small alar cells well differentiated. The morphological distinctions between *Mawenzhangia* and *Nogopterium* are however clear. The stipe leaves in *Mawenzhangia* are distant and patent, while those of *Nogopterium* are overlapping and closely appressed, with just the acumen somewhat spreading. The stem and branch leaves in the former are loosely appressed rather than imbricate as in *Nogopterium*, they (especially stem leaves) are elliptic rather than ovate, the costa is much stronger and longer, the leaf cells are less strongly prorate and have much thinner walls, and the alar cell group is much smaller. For illustrations of *Nogopterium gracile* (as *Pterogonium g.*), see Schimper (1851: pl. 467), Lawton (1971: pl. 131, figs. 12–17), Magill & van Rooy (1998: 555, fig. 154, 13–22), Smith (2004: 716, fig. 236, 1–3), Malcolm *et al.* (2009: 305), Suzuki & Iwatsuki (2012: 63, fig. 2; 66, fig. 5c), Bourell (2014: 596) or Fuertes & Oliván (2014: 199, pl. 65).

*Mawenzhangia* should also be compared with two East Asian genera in the Lembophyllaceae, *viz.* *Dolichomitra* and *Dolichomitriopsis*. *Dolichomitra* is unispecific while *Dolichomitriopsis* has three or four species (Noguchi 1950, 1989; Wu 2011); both genera are distributed in China, Korea and Japan. The leaves of *Dolichomitra* are imbricate, strongly concave and have a rounded apex, rendering the shoots appear turgid, and the leaf cells are smooth. *Dolichomitriopsis* differs from *Mawenzhangia* in the much less branched shoots (thus the plants do not appear frondose) and imbricate leaves with smooth leaf cells and margins entire throughout or serrulate near leaf apex. The dioicous *Mawenzhangia* resorts to vegetative reproduction by easily detachable, flagelliform branchlets since we observed very few plants with perigonia, perichaetia are uncommon, and sporophytes remain unknown.

### Key to the Lembophyllaceae in China

1. Leaf apices rounded..... *Dolichomitra cymbifolia*
- Leaf apices acute to subacuminate .....2.
2. Leaf margins serrulate at apex..... *Dolichomitriopsis diversiformis*
- Leaf margins serrate at apex .....3.
3. Leaf apex subacuminate, often twisted; median laminal cells fairly thick-walled, not prorate ..... *Isothecium subdiversiforme*
- Leaf apex acute, not twisted; median laminal cells thin-walled, usually abaxially prorate ..... *Mawenzhangia thamnobryoides*

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