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Entosthodon elimbatus (Bryophyta, Funariaceae), a new species from the sub-alpine region in Yunnan and *E. physcomitrioides* new for mainland China

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Abstract

Entosthodon elimbatus W.Z. Ma, Shevock & S. He, a new species from a sub-alpine area in Yunnan, China, is described here based on morphological and molecular data. Morphologically, the new species resembles the widespread species *E. buseanus*, but differs most notably in having significantly shortened setae, smaller capsules and nearly unbordered laminae. Images from the scanning electron microscope also show compound-papillose ornamentation over the spore surface, which is characteristic of this new species. The generic validation of the new species in *Entosthodon* is supported by a phylogenetic analysis based on the plastid *rps4* sequence. *Entosthodon physcomitrioides*, characterized by its well-developed apophysis and peristome, is confirmed as a new record for mainland China. Detailed descriptions and illustrations for both species are provided.

Keywords: Mt. Gaoligongshan, moss taxonomy, new species, range extension, *rps4*, SEM

Introduction

Entosthodon Schwägrichen (1823: 44) is a cosmopolitan genus generally characterized by small plants with leaves crowded at the stem apex, exhibiting a rosette-like appearance when moist. The genus is likely to be confused with *Funaria* Hedwig (1801: 172) when plants are infertile. In addition, the generic type of *Entosthodon*, *E. templetonii* (Smith) (1813: 2524) Schwägrichen (1823: 44), was originally described as *F. templetonii* Smith (1813: 2524). However, *Entosthodon* differs from *Funaria* in having 1) typically longitudinally symmetrical capsules that are mostly erect or inclined, but rarely horizontal; 2) simple, non-revoluble rather than compound, revoluble annulus; and 3) intermediate to large sized, variably ornamented spores.

While Crosby *et al*. (2000) listed 83 acceptable species worldwide, only three species were treated in China by Li *et al*. (2003). They are: *E. buseanus* Dozy & Molkenboer (1855: 31), *E. gracilis* Hooker f. & Wilson (1854: 91), and *E. wichurae* M. Fleischer (1904: 481).

Admittedly, the shape of leaves, laminal cells, and costae vary a lot within species as well as between species, making these features less useful in taxonomy. Thus, identifications of *Entosthodon* species mostly depend on characters of the sporophyte (e.g. Fife 1985, Frey *et al*. 2006). Usually, an accurate species identification depends on the availability of fertile material. As revealed in most literature, useful taxonomic features are the shape of the capsule, the proportional length of the neck (apophysis) (Ochi 1968), the presence or absence of peristome teeth (e.g., Fife 1985), and the shape of exothecial cells (Enroth 1991).

In 2013, an unusual, pottiaceous-like moss (*W.Z. Ma & Shevock 13-5097*) was collected by the first two authors in the sub-alpine region in the Mt. Gaoligongshan, NW Yunnan, China. Subsequently, this interesting specimen attracted

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our immense attention for its tiny size, delicate pyriform-shaped capsules, verrucose spores, and undifferentiated leaf margins. After a careful study of the plants, the third author suggested that it might be a new member of *Entosthodon*. The first author then pursued a series of studies and comparisons of other *Entosthodon* specimens in the following herbaria: Kunming Institute of Botany, Chinese Academy of Sciences (KUN), California Academy of Sciences (CAS), University of California (UC), Harvard University (FH), Missouri Botanical Garden (MO), and Institute of Botany, Chinese Academy of Sciences (PE). Unfortunately, no additional collection was found from all of the unidentified backlog specimens and no match to any known species was revealed. Our collection possesses a unique combination of characters that fit well with the generic concept of *Entosthodon*. In order to validate its generic identity, the corresponding author recently carried out a molecular phylogenetic analysis using *rps4* sequence data. In this paper, we present our morphological and molecular studies for describing a species new to science; meanwhile during the course of our study *E. physcomitrioides* (Montagne 1842: 253) Mitten (1859: 55) was confirmed as a new record for mainland China.

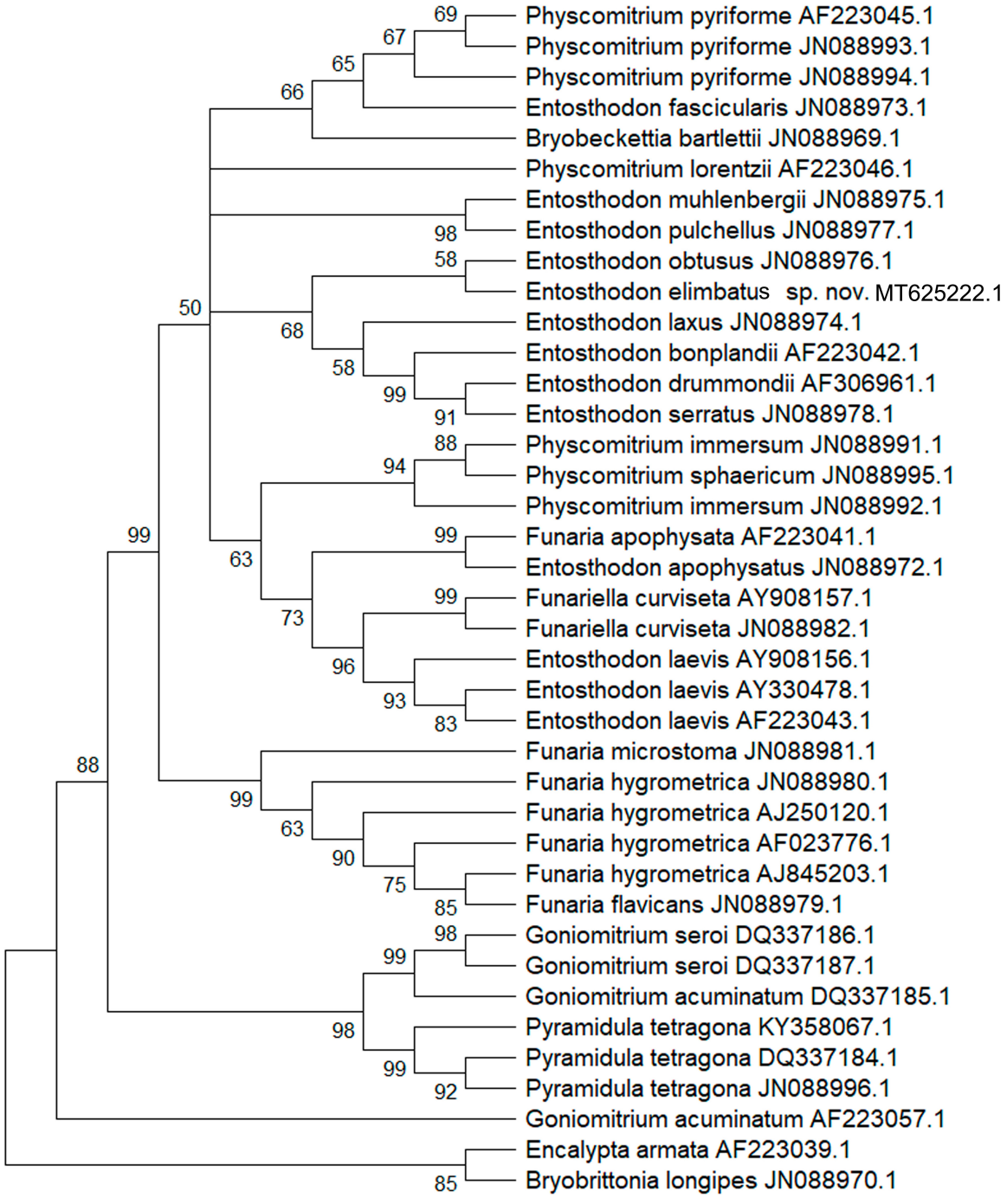

FIGURE 1 Maximum-parsimony phylogram based on the plastid *rps4* gene. Bootstrap values (≥50%) are given above the branches. Accession numbers of *rpss4* sequences obtained from GenBank and generated in the present study are following the species name.

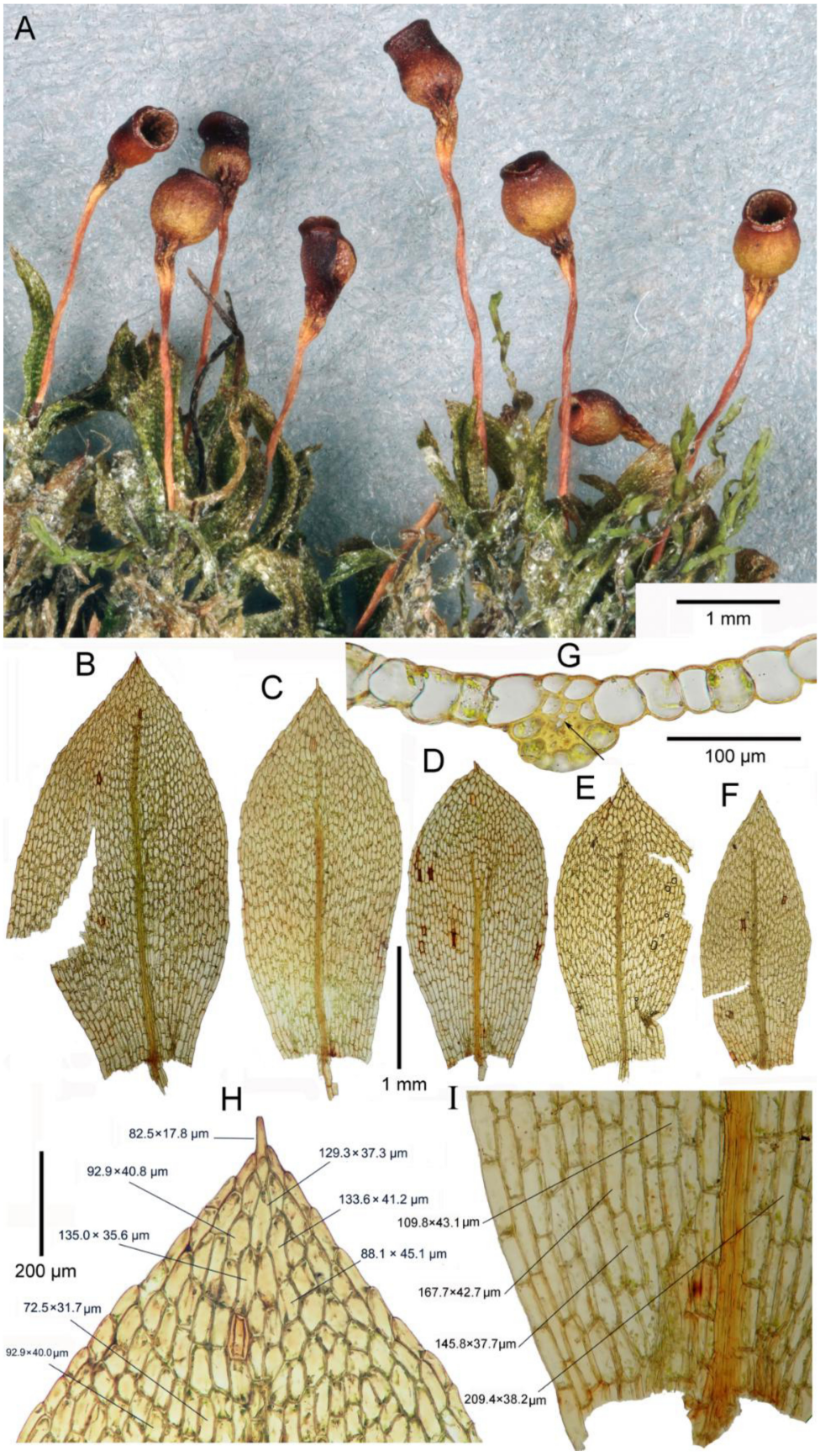

FIGURE 2 *Entosthodon elimbatus* W.Z. Ma, Shevock & S. He. **A**, dry plants with sporophytes. **B–C**, upper leaves. **D–F**, lower leaves. **G**, transverse section of a leaf. **H**, laminal cells in the leaf apex. **I**, laminal cells at leaf base (all prepared from the holotype, *W.Z. Ma & Shevock 13-5097*, KUN).

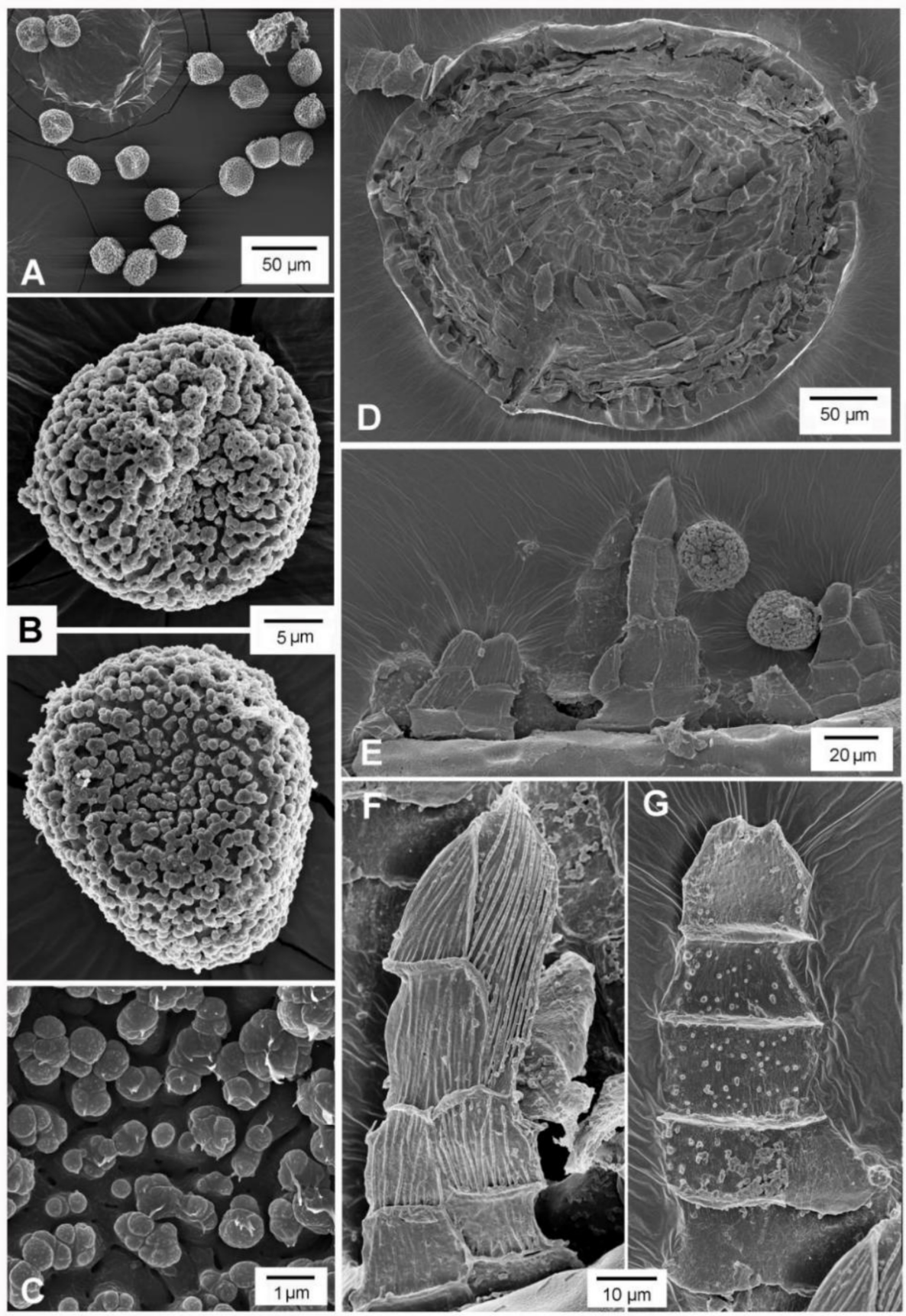

FIGURE3 SEM images of *Entosthodon elimbatus* W.Z. Ma, Shevock & S. He. **A–B**, spores under different magnifications. **C**, the verrucose papillae over the surface of spore. **D**, inner surface of an operculum with broken endostome. **E**, close-up on capsule mouth. **F**, external surface of an endostome segment. **G**, inner surface of an endostome (all prepared from the holotype, *W.Z. Ma & Shevock 13-5097*, KUN).

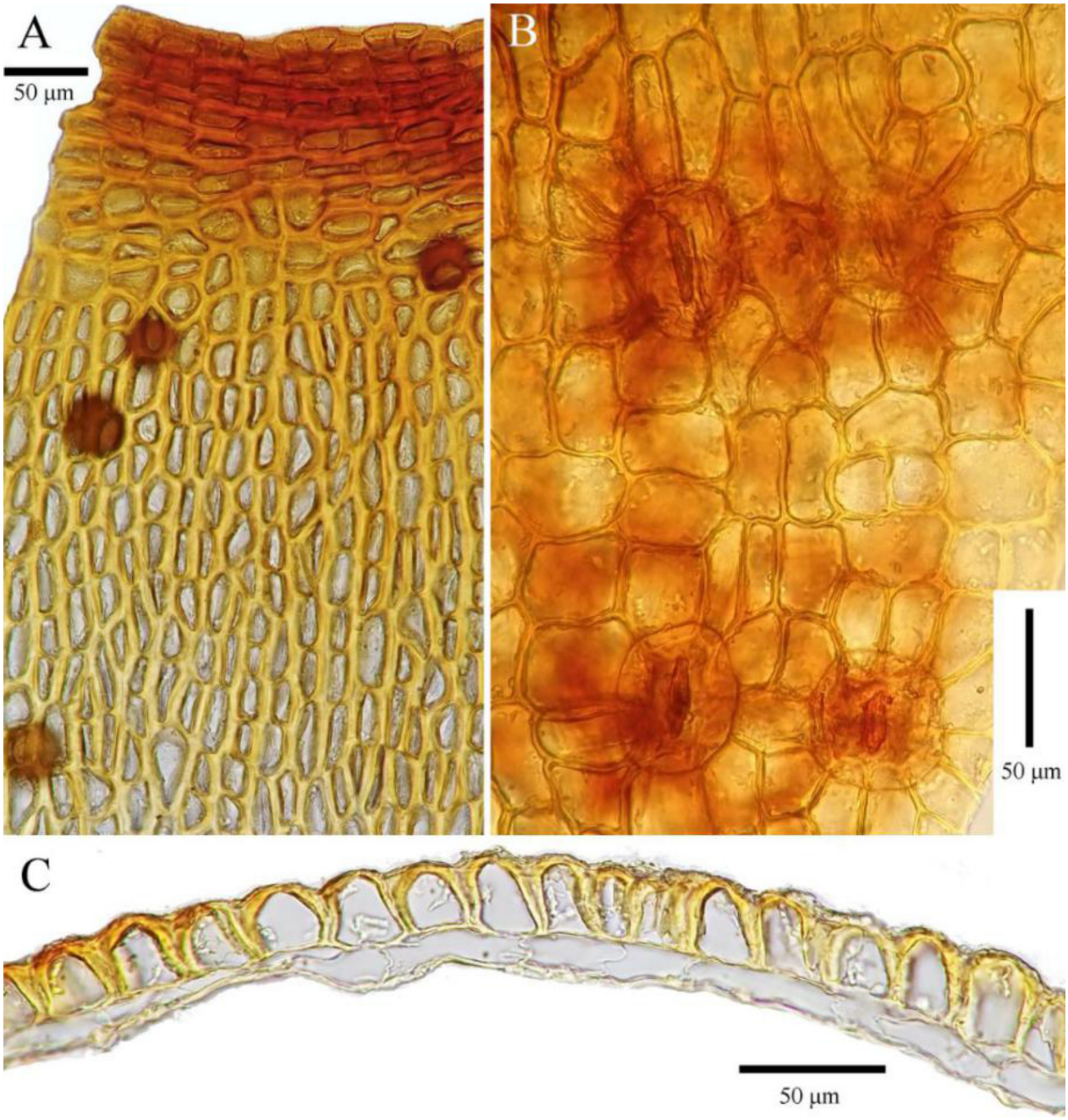

FIGURE4 *Entosthodon elimbatus* W.Z. Ma, Shevock & S. He. **A**. Exothecial cells in upper capsule. **B**. Stomata in apophysis. **C**. Transverse section of exothecial cells at mid capsule. (all prepared from the holotype, *W.Z. Ma & Shevock 13-5097*, KUN).

Material and methods

Morphological examinations

Plants of *W.Z. Ma & Shevock 13-5097* (KUN) and relevant specimens were dissected under a stereo zoom scope (Phenix) and examined with a compound microscope (Leica, DM2500). Microscopic images of morphological importance were captured by a digital camera (Olympus, TG-5) over the above mentioned microscope. Scanning Electron Microscope (SEM) images were obtained through a Zeiss machine (Sigma 300). Measurements were done using ImageJ (Schneider *et al.* 2012).

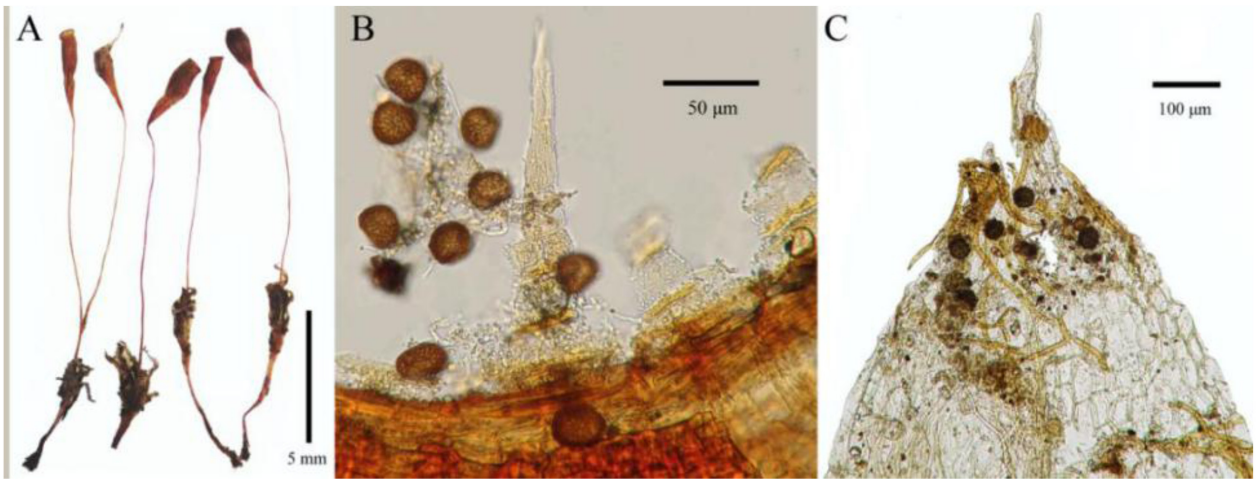

FIGURE5 *Entosthodon physcomitrioides* (Mont.) Mitt. **A**, dry plants with sporophytes. **B**, endostome and spores. **C**, leaf apex (*M. Zang 31681*, KUN).

DNA extraction, amplification and phylogenetic analysis

The plastid gene *rps4* sequences of the Funariaceae available from GenBank were used as a barcode to uncover the systematic position of this new moss. The corresponding sequence of the new species was obtained from the holotype with the voucher number *W.Z. Ma & Shevock 13-5097* (KUN) in the present study. The systematic concept of the family Funariaceae followed "Classification of the Bryophyta" (http://bryology.uconn.edu/classification/). The genomic DNA extraction, PCR amplification, and sequencing methods followed the protocols as described by Liu *et al.* (2010). The obtained sequences were aligned with CLUSTAL X2.1 (Thompson *et al.* 1997). A phylogenetic tree based on maximum parsimony (MP) was constructed using PAUP* version 4.0b10 (Swofford 2003). Heuristic searches were performed with 1000 replicates of each 100 random sequence addition, tree-bisection-reconnection (TBR) branch swapping, and MulTrees option on. Bootstrap analyses were computed with 1000 heuristic replicates (Felsenstein 1985). *Encalypta armata* (Dusén 1906: 32) and *Bryobrittonia longipes* (Mitten 1864: 29) D.G. Horton **(**1978: 19) both belonging to the Encalyptaceae were used as outgroup.

Results

The new species is nested within the clade with *Entosthodon bonplandii* (Hooker 1816: 245) Mitten (1869: 245), *E. drummondii* Sullivant (1856: 651), *E. laxus* (Hooker & Wilson 1847: 399) Mitten (1856: 259), *E. obtusus* (Hedwig 1801: 54) Lindberg (1864: 221) and *E. serratus* (Bridel 1817: 70) Fife (1985: 192) with relatively low bootstrap support (BS=68, Fig. 1). However, the phylogenetic relationships among these species are consistent with previous studies based on more loci from three different genomes (Liu *et al.* 2012, Wilding 2015; Medina *et al*. 2018). The present phylogenetic inference from plastid *rps4* gene shows that the new species described in this paper most closely aligns to *E. obtusus*.

Taxonomic treatment

Entosthodon elimbatus W.Z. Ma, Shevock & S. He *sp. nov.* (Fig. 2–4)

Diagnosis:—The new species is similar to *Entosthodon buseanus* in China, but differs by possessing much shorter setae (less than 5 mm long), larger upper laminal cells (wider than 35 μm vs 25–32 μm), nearly undifferentiated marginal cells, and relatively better-developed endostome.

Type:—CHINA. Yunnan: Gong-shan County, east side of the Mt. Gaoligongshan, about 2km from Nan-Mo-Wang-Shan Pass along trail, on soil in rock fissure, 3590m, 27°41'43" N, 98°27'25" E, 18 Sep., 2013 *W.Z. Ma & Shevock 13-5097* (holotype, KUN; isotypes, CAS, CONN, KRAM, MO).

Description

Plants small, less than 8.8 mm high, yellowish to light brown. Rhizoids abundant, reddish brown. Stems erect, branching frequently near the base, 3.6–7.2 mm long, in transverse section a central strand weakly developed. Leaves 8–10 per terminal stem, erect and contorted when dry, broadly ovate (Fig. 2B), ovate (Fig. 2F), obovate or oblong-obovate (Fig. 2C–E), gradually larger from the base to top stems. Upper leaves concave, apiculate at apex, ending with a single cell, widest part above leaf middle, becoming narrowed towards the base, 3.0–3.4 × 1.3–1.5 mm (Fig. 2B–C); margins plane, minutely crenulate above the midleaf, entire below, border not differentiated or with slightly narrower cells (Fig. 2H); lower leaves similar to the upper leaves, but smaller in size, 2.3–3.1 × 0.9–1.2 mm and less concave, sparsely scattered along basal stems (Fig. 2D–F). Costae single, strong, ending about 6–10 cells below leaf apex; in transverse section a dorsal stereid band present, 25–36 μm wide, a few enlarged and thin-walled cells present between the guide cells and stereid cells (Fig. 2G, see arrow points). Laminal cells thin-walled, lax, the single terminal cell of the apical cells much narrowed, median and upper laminal cells oblong-hexagonal, 88–135 × 36–45 μm in the upper, 109–135 × 45–56 μm in the middle; lower laminal cells becoming elongate, rectangular at base, 143–200 × 36–51 μm (Fig. 2I). Monoicous.

Setae straight, reddish brown, 2.4–4.5 mm long (averaged 2.9 mm, n=11). Capsules erect, yellowish brown to deep brownish, ovoid to obloid-ovoid (Fig. 2A), become globose-pyriform when wet, 1.2–1.4 × 0.6–0.8 mm, the urn abruptly constricted at the apophysis (40–47% the capsule length); mouth contracted when dry; exothecial cells rectangular to short pentagonal, 23–45 × 18–30 μm (Fig. 4A), incrassate below the capsule mouth, become rather thin-walled in the apophysis (Fig. 4B); in transverse section at the middle urn exothecial cells cuneate (Fig. 4C). Stomata in the apophysis, superficial (Fig. 4B). Operculum convex. Annulus somewhat differentiated, consisting of 5–6 rows of oblate-rectangular cells (Fig. 4A). Peristome single, endostome fragile, often with the basal part attached after operculum fell off, triangular-subulate, 68–99 μm long (averaged 88.7 μm, n=5), about 40 μm wide at base, longitudinally striolate on the outer surface (Fig. 3F), sparsely papillose on the inner surface (Fig. 3G). Calyptra cucullate. Spores spherical to subspherical, 24.3–30.0 μm in diameter (averaged 26.5 μm, n=19), densely papillose, with compounded papillae under a higher magnification (Fig. 3B–C).

Etymology:—The specific epithet *elimbatus* refers to the lamina without a differentiated border.

Vernacular name:—Wu Bian Li Shuo Xian (无边梨蒴藓).

Conservation Status:—According to IUCN criteria, the new species should best be included into the category "Data Deficient (DD)", given that it is only known from the type. Since the type locality is along a historic tea-horse trail which is hardly accessible nowadays, and it is also remote from any villages and townships, the population is less likely to be threatened by any human disturbances.

Distribution and habitat:—Endemic to China, only known from the type locality; in a sub-alpine zone in northwest Yunnan province; on soil over rock fissure under indirect sunlight.

Entosthodon physcomitrioides (Mont.) Mitt., Jour. Linn. Soc. Bot. Suppl. 1: 55. 1859. Basionym: *Funaria physcomitrioides* Mont., Ann. Sci. Nat., Bot., sér. 2, 17: 253. 1842. (Fig. 5)

Plants 4–8 mm high, loosely tufted, yellowish when dry, not glossy. Stems simple, scarcely branched, 2.6–4.8 mm long. Leaves about 6–10 per terminal stem, clustered at the top of stems, oblong-obovate, obovate-lanceolate to oblong-lanceolate, slightly concave, 2.0–2.5 × 0.9–1.1 mm, apex tapering into a long capillary point (Fig. 5C); margins nearly entire, sometimes crenulate near the apex; costae strong, ending a few cells below the leaf apex. Laminal cells thin-walled, oblong-hexagonal to elongate-hexagonal, 48–73 × 27–38 μm in the upper part, 59–89 × 16–24 μm in the middle, 140–170 × 38–55 μm near the base.

Setae slender, slightly flexuose, yellowish to reddish brown, 6.0–8.9 (–11) mm long. Capsules erect to suberect, yellowish brown, (1.8–) 2.8–3.2 × 0.7–1.0 mm, elongated pyriform, with a long neck about 50% or more the length of capsule, gradually tapering towards seta (Fig. 5A). Operculum small, convex. Annulus scarcely differentiated. Peristome single, with only endostome, fragile, subulate-linear (Fig. 5B), often attached to the operculum when

dissected, 110–180 μm long. Spores sub-spherical, 20.8–25.0 μm in diameter (averaged 23.1 μm, n=15), coarsely papillose.

This is a new moss record for mainland China. It was originally described from India (Montagne 1842), but later was discovered in other regions of Asia, such as Taiwan (Ochi 1968), the Philippines (Tan & Koponen 1989), and Thailand (Noguchi 1973). The sporophyte features of the Yunnan collection match very well with those that were described by Ochi (1968), but gametophytically, the Yunnan specimens generally have slightly larger leaves and laminal cells.

Specimen examined: CHINA, Yunnan, Bin-Chuan Co., Mt. Ji-Zu-Shan, on rock, 3200m, 28 May, 1992, *M. Zang 31681* (KUN).

Discussion

Based on the molecular phylogenetic analysis, *Entosthodon elimbatus* is most closely related to *E. obtusus* (Fig. 1), a largely European species; however, the latter has ovate-lanceolate leaves that are distinctly bordered with elongated cells (Nyholm 1989). As reflected in the phylogenetic tree, the relatively low support may result from insufficient sequence data available for the current analysis. Nevertheless, the generic placement of the new species in *Entosthodon* is justifiable.

With the significantly shortened seta, the tiny, swelling capsule without a well-developed apophysis (Fig. 2A), and the near-entire, non-differentiated marginal border (Fig. 2H), *E. elimbatus* differs from all other *Entosthodon* species currently documented in China. All other species have more or less border development with serrate or dentate margins and have capsules neither visibly swelling nor contracted below the mouth.

Similar to *Enthosthodon elimbatus*, *E. duriaei* Montagne (1849: 33) also lacks a differentiated laminal broader (see illustrations in Brugués 1998), but the latter has baculate-insulate type of ornamentation on spore surface which is quite different from that seen in *E. elimbatus* (Fig. 3B–C). Furthermore, the endostome in *E. elimbatus* is triangular-subulate-shaped, while it is rudimentary in *E. duriaei*.

Interestingly, the leaf morphology and the exothecial cell walls of *E. elimbatus* are similar to those of *E. laxus*, since the latter is mostly a Southern Hemisphere species. Fife (1986) described *E. laxus* as having lingulate, broadly acute, unbordered, and entire leaves with costae ending well below the apex and having "non-cuneate anticlinal [exothecial] cell walls. All of these features match well with those of *E. elimbatus*. The two species differ mainly in some sporophytic characters, such as much shorter setae (less than 5 mm long) and single peristome (endostome segments *ca.* 100 μm high) in *E. elimbatus*, while the setae are usually 20 mm long and the peristomes are double with teeth to 300 μm high in *E. laxus*.

Both *Entosthodon eberhardtii* Broth. & Paris in Paris (1907: 43) and *E. planifolius* Thwaites & Mitten ex Mitten (1873: 304) reported from India have entire leaf margins, showing some resemblance to *E. elimbatus*; however, *E. eberhardtii* has distinctly sinuous upper leaf margins (Schwarz 2016) and *E. planifolius* has acuminate leaves with a vivid limbidium.

Entosthodon elimbatus is likely to be found in the sub-alpine and alpine habitats in the nearby regions in Yunnan and Myanmar, if future detail-oriented expeditions to those areas are carried out in a right season. The sporophytes of this species seem to be produced late in the summer after an extended monsoon season. Other alpine ephemeral Funariaceae in the Sino-Himalayan region, such as *Brachymeniopsis gymnostoma* Brotherus in Handel-Mazzetti (1929: 13) also appears to bear sporophytes late in the growing season after summer. This may explain why so few specimens of certain ephemeral mosses have been collected in alpine areas of China.

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