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ENROTHIA, A NEW GENUS OF NECKERACEAE (BRYOPHYTA) FROM EAST ASIA
ENROTHIA, НОВЫЙ РОД NECKERACEAE (BRYOPHYTA) ИЗ ВОСТОЧНОЙ АЗИИ

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Abstract

Molecular phylogenetic analysis supported an isolated position of *Neckera polyclada* within the "*Pinnatella*–clade" of the family Neckeraceae, thus it is segregated in a new monospecific genus *Enrothia*. The closest of relatives of *Enrothia* the obtained reconstructions, *Neckera warburgii, N. himalayana*, and *Curvicladum kurzii* never form a supported clade with it, and morphologically are very distinct as well.

Резюме

Молекулярно-филогенетический анализ подтвердил изолированное положение вида, известного как *Neckera polyclada* в пределах клады мхов из родства *Pinnatella* семейства Neckeraceae, так что этот вид выделен в отдельный монотипный род *Enrothia*. Его ближайшими родственными группами являются *Neckera warburgii, N. himalayana* и *Curvicladum kurzii*, однако они не образуют клады с Enrothia, и каждый из них имеет значительные морфологические отличия от *Neckera polyclada*.

KEYWORDS: mosses, *Neckera*, taxonomy, East Asia

INTRODUCTION

Molecular phylogenetic revolution has caused significant changes in pleurocarpous moss taxonomy in the two most recent decades. These changes inferred from DNA sequence analysis broke the ordinal subdivision of pleurocarps, and especially strongly modified the systematics at both family and genera levels (Goffinet *et al.*, 2009; Frey & Stech, 2009). Pleurocarp systematics from allochthonous northern floras appears to be somewhat less complicated, and in general the classification of Amblystegiaceae (Vanderpoorten *et al.*, 2002), Brachytheciaceae (Ignatov & Huttunen, 2002), and Plagiotheciaceae (Huttunen *et al.*, 2013) were completed in general in the course of earlier treatments, although additional amendments, of course, continue.

Pleurocarp families of south-temperate to tropical regions have appeared to be more difficult, obviously representing, in addition to recently diversified groups, also relic lineages, surviving in restricted areas and in specific environments and having small population size. The molecular phylogenetic analysis of such groups, including Neckeraceae, appear to be complicated due to greater differences in sequences among species compared to northern families.

Great progress in Neckeraceae classification was achieved in the last decades. The genus *Neckera* itself was rectified from similar-looking but unrelated species (Olsson *et al.* 2011), and subtropical taxa were grouped in a number of natural genera (Enroth *et al.*, 2010; Olsson *et al.* 2009a, b, 2010, 2016). However, some problematic taxa still remain 'hanging', hampering the usage of generally revised new classification in floras and manuals. One of such species is the East Asiatic *Neckera polyclada* Müll. Hal., whose position in the *Pinnatella*-clade became apparent from earlier analyses (Olsson *et al.* 2009a), but it was always found in a grade near different, usually also 'hanging' taxa. The immediate aim of the present paper is mainly to solve the puzzling situation with the generic placement of *Neckera polyclada*, recently discovered in Russia (Ignatova *et al.*, 2009), by means of comparison with most similar taxa.

MATERIAL AND METHODS

The material used in the present study was sampled from MW and MHA and supplemented by sequences available in GenBank. For the molecular-phylogenetic study we used three markers, nuclear ITS1,2 and 5.8 rRNA gene, plastid region trnS-F, and mitochondrial Nad5, which were successfully used by Olsson *et al.* (2009a,b, 2011) in the backbone molecular phylogenetic studies of Neckeraceae and thus are available in Genbank for most lineages of the family. Vouchers of newly sequenced specimens and GenBank accession numbers of all used sequences are compiled in Appendix 1.

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The laboratory protocol was essentially the same as in previous moss studies, described in detail by, e.g., Gardiner *et al.* (2005). Sequences were aligned using MAFFT v. 7.402 (Katoh & Standley, 2013) with standard settings. At first ITS (1135 bp), trnS-F (1934 bp) and Nad5 (1098 bp) were analyzed separately to check their congruence. The fourth dataset represented concatenated ITS, trnS-F and Nad5 sequences (63 taxa, 4167 positions). Markers were divided in three partitions, one for nuclear, plastid and mitochondrial data. Best-scoring Maximum Likelihood (ML) trees were estimated using RaxML 8.2.10 (Stamatakis, 2006) from 1000 independent searches each starting from distinct random trees. Robustness of the nodes was assessed using the thorough bootstrapping algorithm (Felsenstein, 1985) with 1000 iterations. Bayesian Analyses were performed by running two parallel analyses in MrBayes 3.2.6 (Ronquist *et al.*, 2012). For the single gene ITS, trnS-F and Nad5 analyses each run consisted of six Markov chains, 10 000 000 generations with default number of swaps and sampling frequency one tree each 2500 generations. For the concatenated datasets the analysis consisted of eight Markov chains and 20 000 000 generations, with the default number of swaps and sampling frequency one tree each 5 000 generations was performed. The chain temperature was set at 0.02 in all analyses. Convergence of each analysis was evaluated using Tracer1.4.1 (Rambaut & Drummond, 2007). Consensus trees were calculated after omitting the first 25% trees as burn-in. Analyses were performed on the Cipres Science Gateway (http://www.phylo.org/portal2) on XSEDE.

Results

The tree inferred from the concatenated dataset (Fig. 1) resolves Neckeraceae as monophyletic (considering *Heterocladium heteropterum* (Brid.) Bruch, Schimp. & W. Gümbel and *H. macounii* Best as members of Neckeraceae, which was already found in previous analyses). The Neckeraceae is split into two large clades, the "*Neckera*–clade" and second clade further subdivided into "*Thamnobryum*–clade""and "*Pinnatella*–clade". *Neckera polyclada* was found in the *Pinnatella*–clade, far from *Neckera pennata*, the generitype of the genus *Neckera,* and the clade, corresponding to Neckera s.str. (see Olsson *et al.*, 2011). *Neckera polyclada* occurs in the middle part of grade within the *Pinnatella*–clade, being closest to *Curvicladium kurzii*, *Neckera himalayana*, and *N. warburgii.*

Discussion

The sequences of *Neckera polyclada* from the Russian Far East were found to be identical to those obtained earlier from Chinese material. However inclusion of all the possible relatives in the present analysis does not point any species related to *Neckera polyclada* sufficiently to be placed in one genus. Therefore we see no better solution than to describe it as new genus.

Enrothia Ignatov & Fedosov, gen. nov.

Type: *Enrothia polyclada* (Müll. Hal.) Ignatov & Fedosov (*Neckera polyclada* Müll. Hal.)

Etymology: In honour of Johannes Enroth (b. 1956), a bryologist from Helsinki, Finland, who with his colleagues has provided outstanding contributions to the Neckeraceae systematics.

Diagnosis: Combination of overall Neckeroid shoot architecture, erect-spreading leaves, attenuate and small-leaved at branch tips, the leaves at branch tips often caducous and immersed capsules differentiate the new genus from other genera.

Description: Medium-sized to robust plants, mostly on calcareous rocks. Primary stems creeping, very thin, with minute leaves. Secondary stems pinnately and moderately densely branched, the branches forming acute angles with the stem, densely subcomplanately foliate, branches partly with attenuate ends with minute leaves, these leaves often caducous; similar tiny shoots may also appear as reiterations on secondary stems; proximal branch leaves lanceolate and 1–2(–3) outermost somewhat distant from densely foliate, hemispheric branch primordium; axillary hairs conspicuous, numerous per leaf axil, of 4–6 cells, brown thoughout. Stem and large branch leaves ligulate, more or less broadly acute to apex, enlarging from basal parts of plant upwards, and accordingly with their size varying from hardly to distictly undulate, which is more expressed in large, better developed and fertile plants; margin serrulate to moderately serrate near apex, costa single, half-leaf up. Dioicous. Perichaetia narrow-tubulose. Seta short, capsule immersed.

Molecular phylogenetic analysis resolved *Enrothia* in the "*Pinnatella*-clade", far from *Neckera* s. str., the latter being also morphologically distinct in having a double, short or absent costa. A long single costa occurs in many Neckeraceae genera, e.g. *Forsstroemia, Homalia,* and *Pinnatella*, but the character state combination mentioned in diagnosis is not known to us in other Neckeraceae. Molecular phylogenetic results point most close relationship of *Enrothia* with *Curvicladium kurzii* (Kindb.) Enroth, *Neckera himalayana* Mitt., and *N. warburgii* Broth. Among these, the former has a much stronger costa, reaching 0.9 leaf length, leaves distally coarsely serrate with multistratose teeth. *Neckera himalayana* is a large distinctly complanate plant with broadly ovate leaves, the leaf axis forming with the stem an angle close to 90°. *Neckera warburgii* is the only species of these three, with occasionally attenuate branch tips bearing smaller leaves, but this species has a longer costa and leaves strongly serrate near apex, and its capsules are exserted on setae many times longer than capsules. The species resembles *Taiwanobryum* (e.g., *T. undulatifolium*; Ma *et al.*, 2018), but it does not belong in that clade. Despite *Neckera himalayana* and *N. warburgii* remain in current classification in the "default" genus *Neckera*,

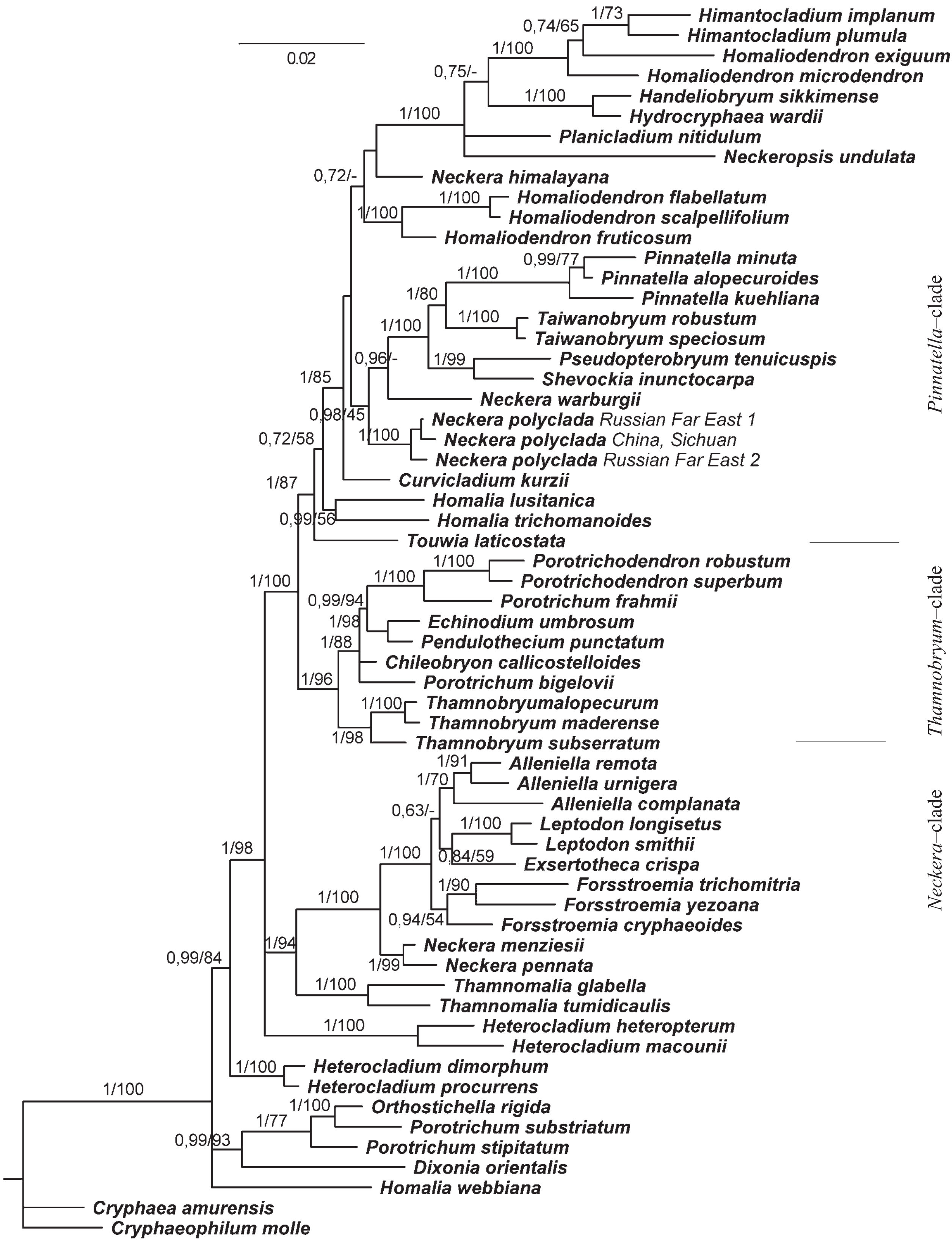

Fig. 1. Bayesian molecular phylogenetic tree inferred from the combined ITS-trnS-F-Nad dataset, showing isolated position of *Neckera polyclada*. Posterior probabilities from Bayesian analysis (>70) and / bootstrap support from ML analysis (>45) are shown at nodes.

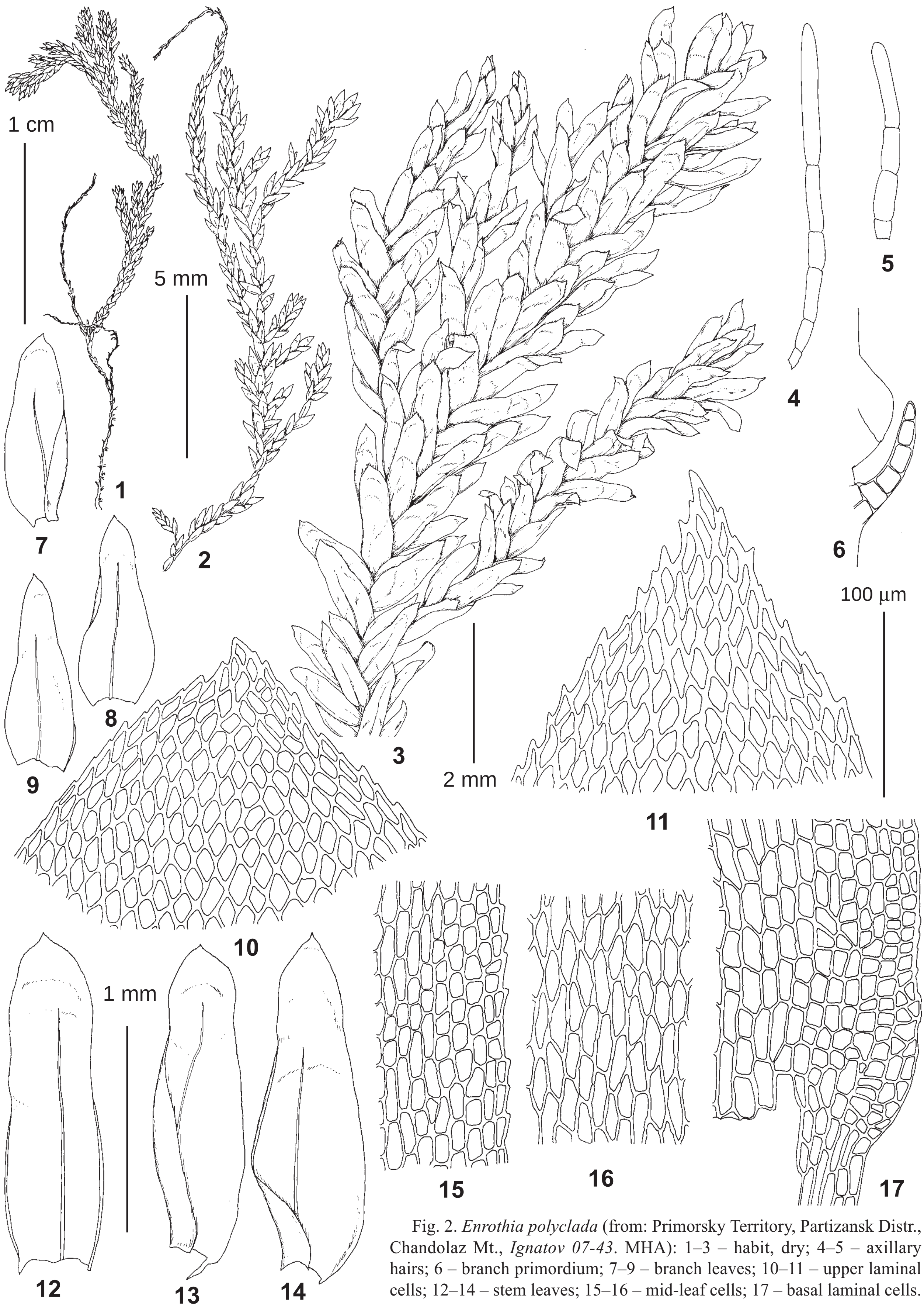

Fig. 2. *Enrothia polyclada* (from: Primorsky Territory, Partizansk Distr., Chandolaz Mt., *Ignatov 07-43*. MHA): 1–3 – habit, dry; 4–5 – axillary hairs; 6 – branch primordium; 7–9 – branch leaves; 10–11 – upper laminal cells; 12–14 – stem leaves; 15–16 – mid-leaf cells; 17 – basal laminal cells. Scale bars: 1 cm for 1; 5 mm for 2; 2 mm for 3; 1 mm for 7–9, 12–14; 100 µm for 4–6, 10–11, 15–17.

Fig. 3. Lectotype of *Neckera polyclada*.

their position in the "*Pinnatella*-clade" obviously indicate a necessity of their placement in other, likely still undescribed genera (Olsson *et al.*, 2016).

Enrothia polyclada (Müll. Hal.) Ignatov & Fedosov, comb. nov.

Basionym: Neckera polyclada Müll. Hal., Nuovo Giorn. Bot. Ital., 1896. n. s. 3: 114. 1896.

Protologue: [China. Shaanxi], Zu-lu, in medio monte Kuan-tou-san, Jul. sterilis [1894, Giraldi].

Lectotype: Bryotheca E. Levier, *Neckera polyclada* C. Müll., Nuovo Giornale Botanico Italiano, 1986 p. 114, China interior, Provincia Schen-si sept., Zu-lu in valle Lao-y-san, Aug. 1894 legit Rev. Jos. Giraldi, determ. Prof. C. Müller sub. n° 897" (FI FI055514). Fig. 3.

Isolectotypes: FI FI055515!, FI055523!, H-BR2874002! and LE!.

Notes of lectotypification: FI herbarium has three syntypes from Zu-lu, C. Müller n° 897 and small differences in label content; FI055514, is selected as a best developed large plant.

Plants medium-sized to robust, in loose mats, pale or yellowish-green, slightly glossy. Primary stems creeping. Secondary stems ascending, 2–5(–10) cm long, 2–4 mm wide with leaves, obtuse at the apex, pinnately branched, branches ca. 5–7 mm long, sometimes flagelliform at apex and then being 5–10(–20) mm long, often naked due to fallen caducous leaves, but leaves at the very ends of such tiny shoots undetached; secondary stem and branches subcomplanately foliate; central strand absent, proximal branch leaves somewhat spaced from the branch initials; axillary hairs up to 180 mm, 12 mm, brown throughout. Leaves on secondary stems spreading oblong-lingulate, 1.6–2.0×0.6–0.8 mm, weakly concave, undulate distally, in smaller leaves indistinctly so, almost symmetric, widely acute to subobtuse, margins serrulate from apex to midleaf, subentire or minutely crenulate at leaf base, incurved at base on one side; costa single, extending to 2/3–3/4 of leaf length; laminal cells with moderately thickened porose walls, upper laminal cells ovate-rhomboidal, 15–20×10–14 µm, median laminal cells oblong, irregular in size, 20–40×8–15 µm, basal juxtacostal laminal cells linear, 35–60×10–14 µm, basal marginal cells subquadrate to short rectangular, forming small alar group. Dioicous. Capsules immersed (according to Wu (2011) and BM image of BM000844653654).

Images available on-line: Schen-si Giraldi Jul. 1894. Liku tsui, C. Müller # 945 (BM000987870; BM000844653). Sichuan, Pratt , Dec. 1890 (BM000844653654, the specimen with numerous perichaetia).

Specimens examined: Japan (Honshu, Oct. 1908, U. Mizushima, MHA9065824), Russia (Primorsky Territory, Czandolaz/Lozovyj: Gambaryan s.n. 10.VIII.1980 VLA, MHA9017182; *Ignatov* 07-43, MW, MHA9017181; Ignatov & Ignatova 13-1929, MHA9017180; MW), China: Shaanxi: coll. *Giraldi*, det. C. Müller 945 (LE, H-BR2874008).

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Appendix 1. Specimens used in the molecular phylogenetic analysis, ITS / trnS-F / Nad5 (for newly generated ones, the specimen voucher information is added). *Alleniella complanata* FM161158 / AM990413 / FM161305; *Alleniella remota* FM161171 / AM990415 / FM161307; *Alleniella urnigera* FM161174 / AM990416 / FM161308; *Chileobryon callicostelloides* FM161088 / FM210283 / FM882226; *Cryphaea amurensis* FM161090 / AM990413 / FM161251; *Cryphaeophilum molle* AJ862690 & AF509840 / HE717068 & AF509544 / HE717030; *Cryptoleptodon longisetus* FM161091 / AM990356 / FM161252; *Curvicladium kurzii* FM161093 / FM210285 / AY908670; *Dixonia thamnioides* FM161097 / AM990361 / FM161256; *Echinodium umbrosum* AY999172 / AF143044 & AF161137 / AY908680; *Forsstroemia cryphaeoides* LC041107 / LC041066 & LC041093 / -; *Forsstroemia trichomitria* FM161103 / AM990365 / FM161260; *Handeliobryum sikkimense* FM161110 / FM210287 / AY908672; *Heterocladium dimorphum* FM161115 / AM990376 / FM161271; *Heterocladium heteropterum* FM161116 / AM990377 / FM161272; *Heterocladium macounii* FM161117 / AM990378 / FM161273; *Heterocladium procurrens* FM161118 / AM990379 / FM161274; *Himantocladium implanum* FM161121 / FM210289 / -; *Himantocladium plumula* FM161122 / AM990381 / FM161276; *Homalia glabella* FM161123 / AM990382 / FM161277; *Homalia lusitanica* FM161124 / AM990383 / FM161278; *Homalia trichomanoides* FM161126 / AM990385 / FM161280; *Homalia webbiana* FM161127 / AM990387 / FM161282; *Homaliodendron exiguum* FM161130 / AM990389 / FM161284; *Homaliodendron flabellatum* FM161131 / FM210290 / AY908671; *Homaliodendron fruticosum* FM161202 / AM990430 / FM161322; *Homaliodendron microdendron* FM161133 / AM990390 / FM161285; *Homaliodendron scalpellifolium* FM161135 / FM210292 / HQ607391; *Hydrocryphaea wardii* FM161139 / FM210293 / HQ607392; *Leptodon smithii* FM161147 / AM990403 / FM161297; *Neckera crispa* FM161160 / FM210298 / -; *Neckera himalayana* FM161163 / FM210301 / FM882223; *Neckera menziesii* FM161167 / FM210305 / -; *Neckera pennata* (Austria, Kučera. SBFS 16367, isolate NF45) MN010515 / MN031368 / MN031371; *Neckera polyclada* (Russia, Primorskiy Territory, 26.VIII.2007 Ignatov. MW 9049910, isolate NF65) MN010517 / MN031370 / MN031373; *Neckera polyclada* (Russia, Primorskiy Territory, 9.IX.2013 Ignatov & Ignatova. MW 9049909, isolate NF64) MN010516 / MN031369 / MN031372; *Neckera polyclada* FM161170 / FM882220 & FM210307 / FM882224; *Neckera warburgii* FM161176 / FM210311 / -; *Neckera yezoana* FM161177 / FM210312 / -; *Neckeropsis nitidula* FM161183 / AM990419 / FM161311; *Neckeropsis undulata* FM161184 / FM210316 / -; *Orthostichella rigida* FM161185 / AM990422 / FM161312; *Pendulothecium punctatum* FM161187 / AM990421 / FM161314; *Pinnatella alopecuroides* FM161188 / AM990423 / FM161315; *Pinnatella kuehliana* FM161192 / FM201505 / -;*Pinnatella minuta* FM161194 / AM990424 / FM161316; *Porotrichodendron robustum* FM161197 / AM990426 / FM161318; *Porotrichodendron superbum* FM161198 / AM990427 / FM161319; *Porotrichum bigelovii* FM161200 / AM990428 / LR215208; *Porotrichum frahmii* FM161201 / AM990429 / FM161321; *Porotrichum stipitatum* HE660018 / LR215197 / LS999062; *Porotrichum substriatum* FM161204 / AM990431 / FM161323; *Pseudopterobryum tenuicuspis* LC041114 / LC041073 & LC041100 / AY908668; *Shevockia inunctocarpa* LC041117 / FM210323 / -; *Taiwanobryum robustum* FM161215 / AM990441 / FM161331; *Taiwanobryum speciosum* FM161216 / AM990442 / FM161332; *Thamnobryum alopecurum* FM161218 / AM990444 / FM161334; *Thamnobryum maderense* FM161223 / AM990445 / FM161335; *Thamnobryum subserratum* FM161230 / AM990446 / FM161336; *Thamnobryum tumidicaule* FM161231 / AM990447 / FM161337; *Touwia laticostata* FM161233 / FM210330 / FM882225.