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 Neckeraеae, 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 Neckeraеae, так что этот вид выделен в отдельный монотипический 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 classication 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 Neckeraеae, appear to be complicated due to greater differences in sequences among species compared to northern families.

Progress in Neckeraеae 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 Neckeraеae 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 Neckeraeaceae as monophyletic (considering Heterocladium heteropterum (Brid.) Bruch, Schimp. & W. Gümbel and H. macounii Best as members of Neckeraeaceae, which was already found in previous analyses). The Neckeraeaceae 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 genericity of the genus Neckera, and the clade, corresponding to Neckera s.str. (see Olson 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 Neckeraeaceae systematics.

Diagnosis: Combination of overall Neckeroide 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 throughout. 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 distinctly 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 Neckeraeaceae genera, e.g. Forstroemia, Homalia, and Pinnatella, but the character state combination mentioned in diagnosis is not known to us in other Neckeraeaceae. Molecular phylogenetic results point most clade 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 exerted on setae many times longer than capsules. The species resembles Taiwano Bryum (e.g., T. undulatifoli um; 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.
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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.
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.


**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 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 mid-leaf, subtire 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 po-role 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. Dioicus. Capsules immersed (according to Wu (2011) and BM image of BM000844653654).

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


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**LITERATURE CITED**


Appendix 1. Species used in the molecular phylogenetic analysis, ITS / trnS-F / Nad5 (for newly generated ones, the specimen voucher information is added).

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