

NEW ASIAN LOCALITIES OF *HYMENOSTYLIUM XEROPHILUM* AND *H. GRACILLIMUM*

О НОВЫХ НАХОДКАХ *HYMENOSTYLIUM XEROPHILUM* И *H. GRACILLIMUM* В АЗИИ

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Abstract

We report here the new, highly disjunct Asian occurrences for two *Hymenostylium* species, which have so far only been known from Europe. *Hymenostylium xerophilum* is newly reported from Oman and eastern Yakutia, while the other species, *H. gracillimum* was found among herbarium material collected at the shore of Lake Baikal in southern Siberia. While the new *H. xerophilum* records matched both the earlier described morphology of the plants and ecological preferences, the plants of *H. gracillimum* were morphologically and probably also ecologically rather distinct from the known European material. Molecular phylogenetic analysis of Pottiaceae trib. Pleuroweisiae based on nuclear ITS region and two chloroplast loci was performed to assess newly the phylogenetic affinities of the two species and to confirm the identity with the European plants. The lineage formed by accessions of these two species was confirmed as rather distinct from the rest of analyzed *Hymenostylium* taxa and pointed to the necessity of a large-scaled study of *Hymenostylium* and related genera. We discuss the ecological and phytogeographic characteristics of the new Asian occurrences.

резюме

Приводятся новые находки в Азии широко дизъюнктивных видов из рода *Hymenostylium*, которые до настоящего времени были известны только из Европы. *Hymenostylium xerophilum* впервые найден в Омане и в восточной Якутии, а *H. gracillimum* выявлен в гербарных коллекциях с побережья озера Байкал на юге Сибири. В то время как новый образец *H. xerophilum* полностью соответствует опубликованному описанию этого вида и его экологическим предпочтениям, то растения *H. gracillimum* из местонахождения на Байкале оказались существенно отличающимися по морфологии и, возможно, экологическим характеристикам от известных европейских популяций. Молекулярно-филогенетический анализ Pottiaceae trib. Pleuroweisiae, основанный на ядерном участке ITS и двух хлоропластных маркерах, *rps4* и *trnM-trnV*, был выполнен с целью подтверждения филогенетического родства этих двух видов и соответствия их новых находок европейскому материалу. Анализ последовательностей ДНК изученных маркеров *H. xerophilum* и *H. gracillimum* подтвердил их ограниченность от остальных видов рода *Hymenostylium* и указал на необходимость широкомасштабного изучения комплекса близких к нему родов. В статье также обсуждаются экологические и фитогеографические характеристики азиатских популяций.

KEYWORDS: *Hymenostylium*, Pleuroweisiae, Asia, Siberia, disjunct distribution, metalliferous rocks, molecular phylogeny, ITS, *rps4*, *trnM-trnV*

INTRODUCTION

Hymenostylium xerophilum Kőckinger & J.Kučera and *H. gracillimum* (Nees & Hornsch.) Kőckinger & J.Kučera are two closely related species, which were recently described, respectively re-established as species from European material in the treatment by Kőckinger & Kučera (2011). Both species escaped nearly completely the attention of European bryologists despite the fact that at least *H. xerophilum* cannot be regarded a rare

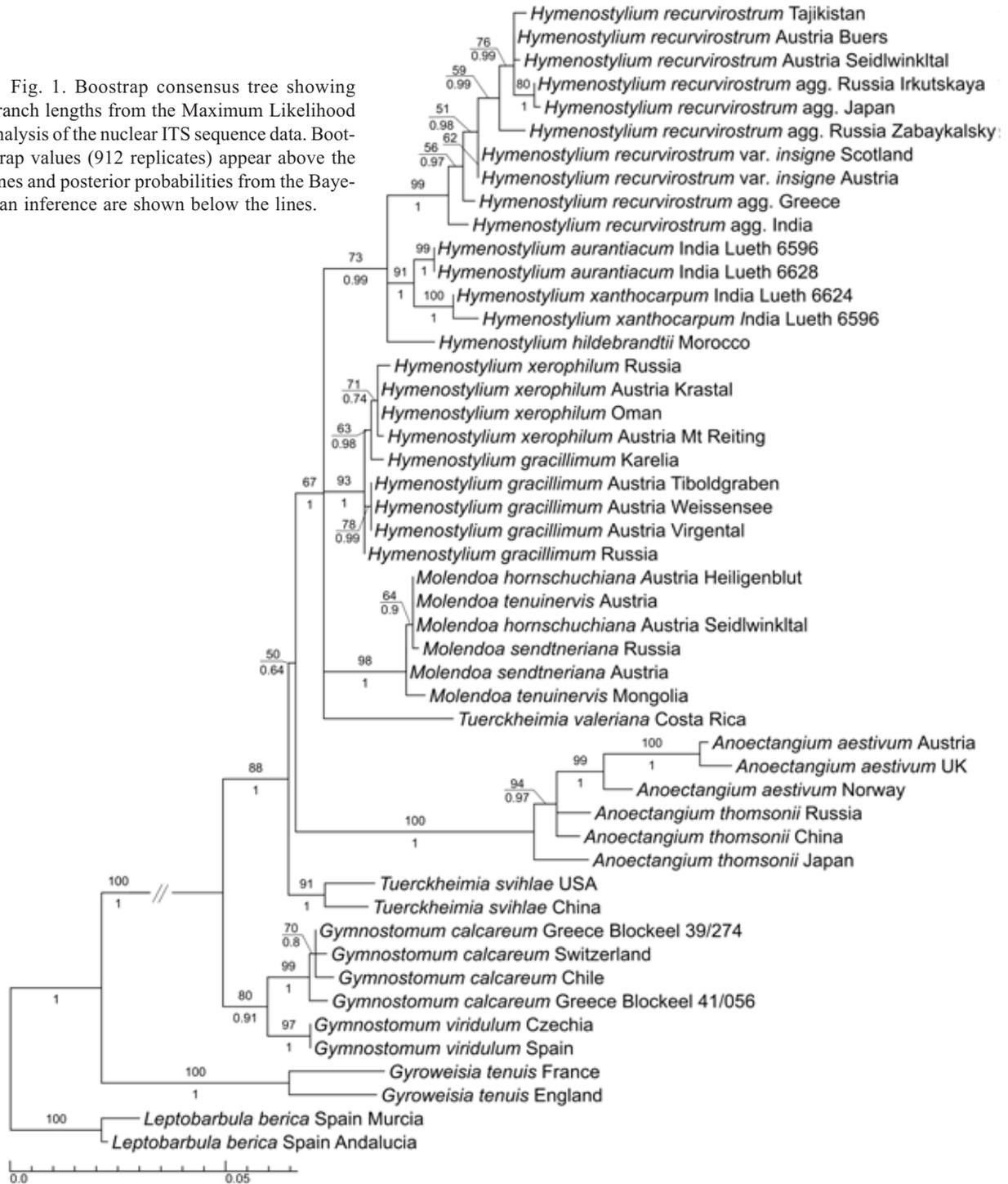
species. Indeed, it was discovered at about 35 localities in the Austrian Alps in course of a focused search conducted by H. Kőckinger between 1994 and 2009. The second species, *H. gracillimum*, was described three times from Europe even though it is a smaller, less apparent species, which seems to be significantly rarer than *H. xerophilum*. On the other hand, the two historical descriptions of *H. gracillimum* fell in oblivion soon after their publication, and the modern re-description of the

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Fig. 1. Bootstrap consensus tree showing branch lengths from the Maximum Likelihood analysis of the nuclear ITS sequence data. Bootstrap values (912 replicates) appear above the lines and posterior probabilities from the Bayesian inference are shown below the lines.



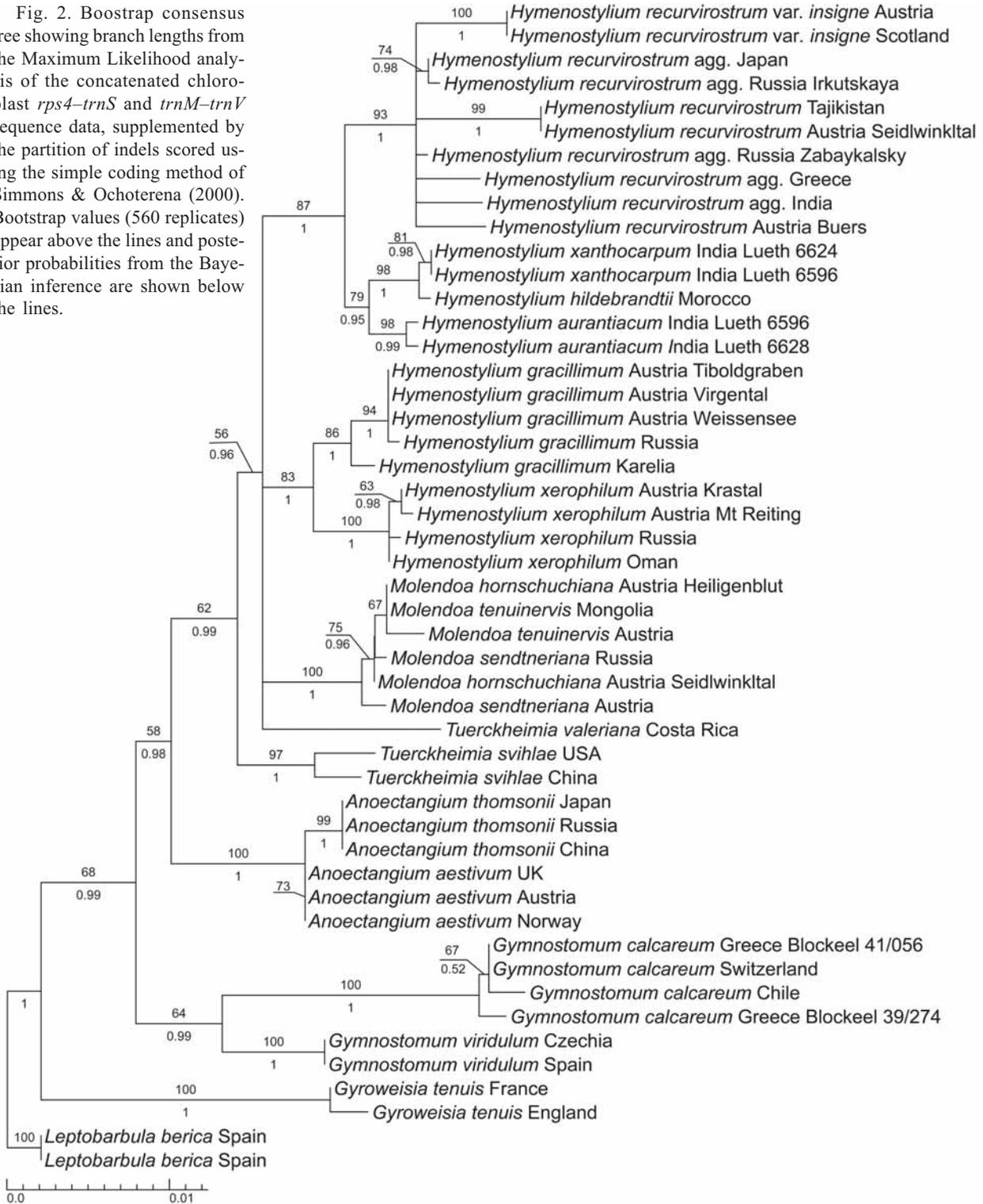
species (as *Gymnostomum boreale* Nyholm & Hedenäs) has not been understood correctly by most authors, which resulted in the common confusion of that species with *Gymnostomum aeruginosum* Sm.

Both species have so far only been known from Europe (Austrian and Bavarian Alps plus one Fennoscandian outpost of *H. gracillimum* in Karelia; the earlier reports of *H. gracillimum* (as *Gymnostomum boreale*) from Norway, Slovakia, North America (Québec, Canada) and northern Siberia (Taimyr) were proven wrong by

Köckinger & Kučera (2011), although the authors speculated that the real distribution of both species is likely much wider, despite their search in herbaria was not successful.

The first records of *H. xerophilum* outside the distribution area presented by Köckinger & Kučera (2011) succeeded independently in course of the revision of selected genera of Pottiaceae during a SYNTHESYS-funded stay of J. Kučera in the herbarium of Royal Botanic Garden Edinburgh, and during the treatment of material col-

Fig. 2. Bootstrap consensus tree showing branch lengths from the Maximum Likelihood analysis of the concatenated chloroplast *rps4-trnS* and *trnM-trnV* sequence data, supplemented by the partition of indels scored using the simple coding method of Simmons & Ochoterena (2000). Bootstrap values (560 replicates) appear above the lines and posterior probabilities from the Bayesian inference are shown below the lines.



lected in course of the expedition to the Sette-Daban mountain range in the Russian Yakutia (Ignatova & al., 2018). In the former case, one specimen collected by W. Frey & H. Kürschner in Oman and labelled *Gymnostomum mosis* (Lorentz) Jur. & Milde, in the latter case a specimen collected in the Kuraanakh River valley drew our attention as the possible specimens of *H. xerophilum*. The third *Hymenostylium* specimen, which EAI and

MSI were unable to name according to morphological characters, was collected in Olkhon Island surroundings at the NW shore of Lake Baikal. In order to be assured about the identification of the two *H. xerophilum* specimens which would represent a significant range extension of the species with respect to the current knowledge, and to barcode the unnamed specimen from Olkhon Island, we decided to obtain DNA sequence data for loci

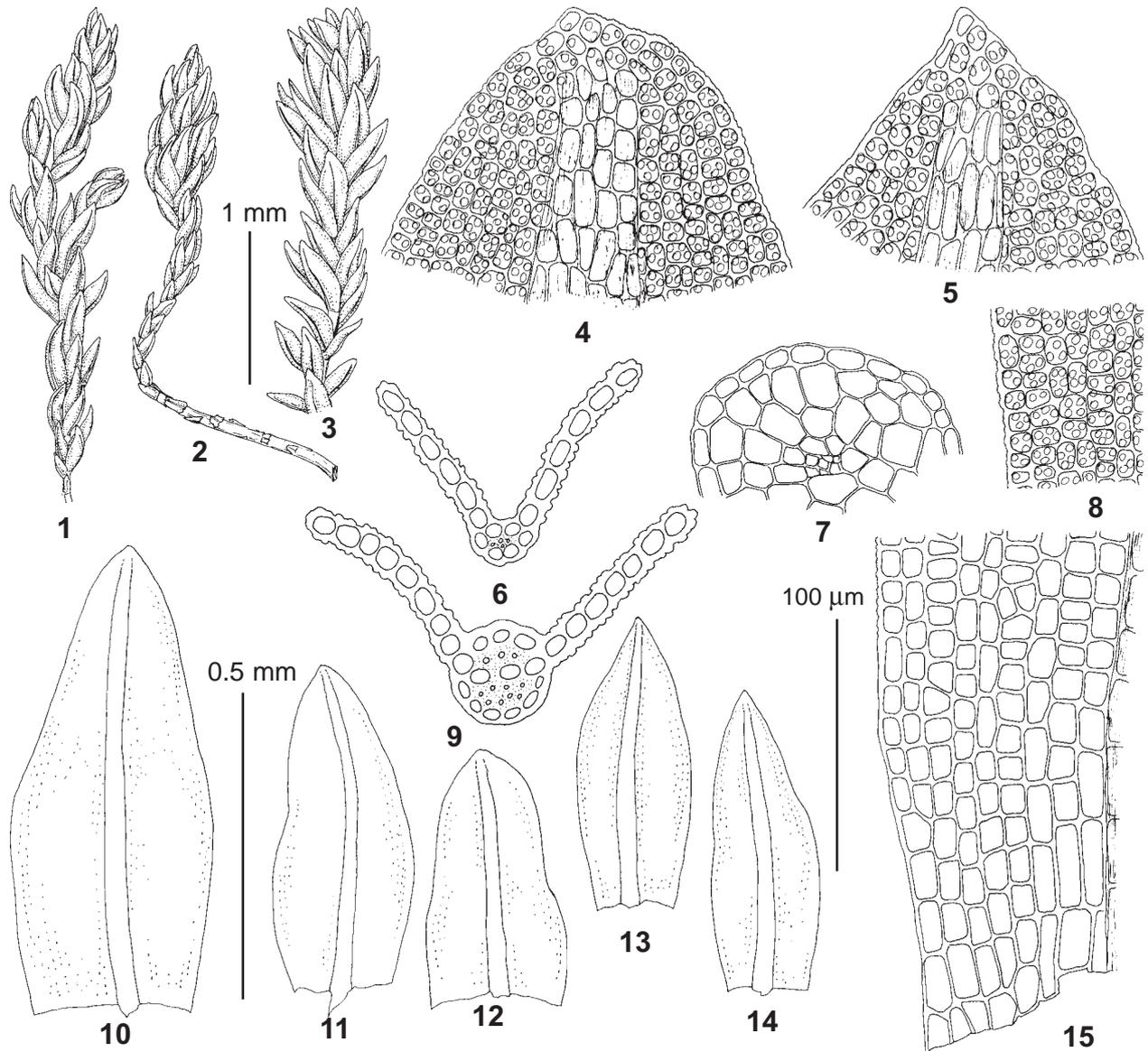


Fig. 3. *Hymenostylium xerophilum* (Republic of Sakha/Yakutia, Tomponsky Distr., Sette-Daban Mt. Range, 21.VII.2015, Ignatov & Ignatova 15-851, MHA): 1–2 – habit, dry; 3 – habit, wet; 4–5 – upper laminal cells; 6, 9 – leaf transverse sections; 7 – stem transverse section; 8 – mid-leaf cells; 10–14 – leaves; 15 – basal laminal cells. Scale bars: 1 mm for 1–3; 0.5 mm for 10–14; 100 µm for 4–8, 15.

well-represented in the library of Pleurowesiaceae Limpr. species, i.e., nuclear ITS region, and chloroplast *rps4* and *trnM–trnV*. Moreover, as the dataset of Pleurowesiaceae was rather limited in the study of Köckinger & Kučera (2011) and only one chloroplast locus was employed in that study, this was a welcome chance to explore the phylogenetic affinities of *H. xerophilum* and *H. gracillimum* within *Hymenostylium* Brid. and closely related taxa.

MATERIAL AND METHODS

Specimens *Frey & Kürschner 1-3949* from herbarium E (Oman, Jabal Al Akhdar bei Wakhan, Felsspalten, Kalk, 2050 m, 18.2.1983 coll. W. Frey & H. Kürschner) and *Ignatov & Ignatova 15-851* from herbarium MHA (Russia, Yakutia, Tomponsky District, Sette-Daban Mountain Range, right slope of Kuraanakh River valley in its lower

course, 63°02'N 138°23'E, 765 m, rock outcrops in small shallow depression on rocky slope, rock fissure, 21.7.2015 coll. M.S. Ignatov & E.A. Ignatova) morphologically matching *Hymenostylium xerophilum* and the specimen *Bardunov s.n.* from herbarium MW, duplicate from IRK (Russia, Irkutsk Province, Olkhon District, Sakhyurta [Sakhyurte in label] Settlement, 53°01'N 106°53'E, limestone cliffs, in fissures, 31.8.1997 coll. L.V. Bardunov) morphologically only attributable to genus *Hymenostylium* were isolated for DNA and amplified for three loci: nuclear ribosomal ITS spanning ITS1, 5.8 rRNA and ITS2, chloroplast *rps4* gene with the adjacent spacer towards *trnS* tRNA gene, and the *trnM–trnV* spacer. The specimen *Frey & Kürschner 1-3949* was extracted using the Qiagene Extractor in the facilities of Royal Botanic Gardens Edin-

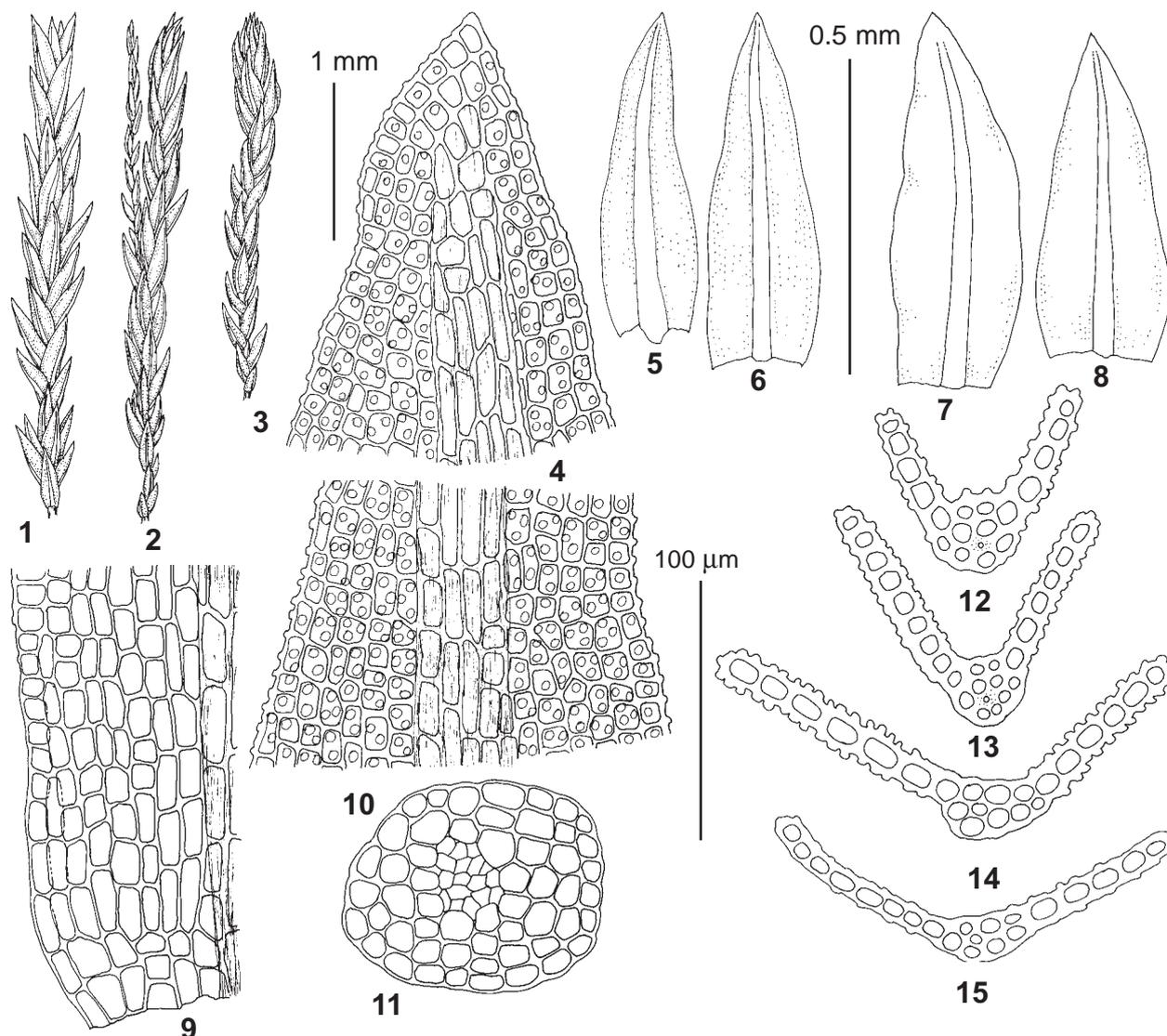


Fig. 4. *Hymenostylium gracillimum* (Irkutsk Province, Sakhyurta, 31.VIII.1997, *Bardunov s.n.*, IRK, MW): 1 – habit, wet; 2–3 – habit, dry; 4 – upper laminal cells; 5–8 – leaves; 9 – basal laminal cells; 10 – mid-leaf cells; 11 – stem transverse section; 12–15 – leaf transverse sections. Scale bars: 1 mm for 1–3; 0.5 mm for 5–8; 100 µm for 4, 9–15.

burgh, and further processed with other samples specified in the Appendix in the molecular laboratory of the Department of Botany at University of South Bohemia following the protocols described in Kučera & al. (2019). The laboratory protocols for specimens *Ignatov & Ignatova 15-851* and *Bardunov s.n.* were processed at the Tsitsin Main Botanical Garden following the protocols described in Fedosov *et al.* (2016).

The choice of analysed taxa was guided by the previously published account by Köckinger & Kučera (2011), and restricted to the tribe Pleuroseisae. The earlier selection of taxa was supplemented by accessions of *Anoctangium thomsonii* Mitt., *Tuerckheimia valeriana* (E.B.Bartram) R.H.Zander, *Hymenostylium xanthocarpum* (Hook.) Brid. and *H. aurantiacum* Mitt., and the species already used in that study were complemented with additional material in cases when the original selection included only one accession per species.

Sequences were aligned using MAFFT v. 7.402 (Kato & Standley, 2013) using the E-INS-i strategy and fine-tuned manually. ITS and concatenated chloroplast matrix included accessions from the same isolates but were analysed separately. For chloroplast matrix, indels were scored using the simple indel coding method (Simmons & Ochoterena, 2000), whereas indel data were not used for ITS matrix due to the complexity of the alignment. The analyses included Bayesian Inference (BI) and Maximum Likelihood (ML), using the MrBayes 3.2.6 (Ronquist *et al.*, 2012) and RAxML v. 8.2.8 (Stamatakis, 2014) software packages run at the cluster computer facilities of MetaCentrum VO (see Acknowledgement). For the parameters of the analyses, see Kučera & al. (2019). TreeGraph 2 (Stöver & Müller, 2010) was finally used to summarise the topology and support from different analyses, and the resulting trees were then further edited graphically in the Inkscape v.0.92 program under the GPLv2+ licence. Trees were rooted with *Leptobarbula berica*.

RESULTS

Barcoding. Only ITS2 was obtained from the Omani specimen *Frey & Kürschner 1-3949*, the two Russian samples yielded the complete ITS region and chloroplast data were obtained from all three samples and loci. The Omani sample of the morphologically putative *H. xerophilum* was fully identical in ITS2 (ITS1 could not be obtained) and *trnM-trnV* with the European samples of that species and differed in one substitution in *rps4*. The Russian sample of the morphologically putative *H. xerophilum* was identical in *trnM-trnV* with the European samples, differed in two substitutions in *rps4* (one shared with the Omani sample), and also differed in one ITS1 and one ITS2 substitutions from the European samples. The unnamed Baikal *Hymenostylium* shared fully the *rps4* sequences of European samples of *H. gracillimum* and differed in one substitution from the Austrian samples of *trnM-trnV* from that species (the Karelian sample of *H. gracillimum* from the paratype of *Gymnostomum boreale* differed in 3 substitutions and 3 T-deletions in one poly-T region of the *trnM-trnV* spacer from the remaining samples of that species). The ITS region of the Baikal sample agreed with the Austrian samples of *H. gracillimum* except for one single-base insert in ITS1 and one substitution in ITS2, shared with the Karelian sample (which differed in 3 additional substitutions in ITS2 from the other *H. gracillimum* samples). Within the whole trees, both putative plants of *H. xerophilum* were included in the lineages formed by the other accessions of that species (PP1 in chloroplast dataset and PP=0.74 in the ITS dataset), the Baikal *Hymenostylium* was nested in the *H. gracillimum* lineage with full support in the chloroplast dataset and it formed one leaf tip in the paraphyletic grade representing that species in the ITS phylogeny (Figs. 1, 2).

Morphology. Morphology of the Russian plants of *H. xerophilum* (Fig. 3) agrees well with the description provided by Köckinger & Kučera (2011), although in small plants the leaves are only indistinctly twisted when dry. The Omani sample is unusually stunted, with leaves ovate, only 0.35–0.55 mm long and rounded at apex, although the costa remains typically wide, 45–50 µm, hardly narrowed towards the apex. The general shape markedly approaches the morphology of *Gymnostomum mosis* (which was the original identification, confirmed even by Whitehouse & Crundwell in their treatment (1991)); it differs, however, in the elongate adaxial superficial costa cells, less papillose lamina cells and completely unistratose leaf lamina. Morphology of the sample of *Hymenostylium* from Irkutsk Province (Fig. 4) differs from the description of *H. gracillimum* as indicated by Köckinger & Kučera (2011) in the leaves being rather short and slightly wider, 0.5–0.6 mm long and 0.15–0.20 mm wide, with length:width ratio 2.75–3.5:1 (vs. usually 4–12:1, rarely 3:1); the costa smooth on ventral surface or rarely with few papillae in distal portion (vs. usually papillose), poorly differentiated in cross section, with-

out or with single dorsal stereid cells. The plant is rather similar to *H. xerophilum* in general appearance in the small size of plants, not or weakly branched stems, whitish subterranean rhizoids, stem transverse section with clearly differentiated central strand and almost undifferentiated sclerodermis, lanceolate keeled leaves with rather broadly acute apices, subpercurrent costa and distal laminal cells covered with rounded papillae. The plants from Irkutsk Province differ from both *H. xerophilum* and the common phenotype of *H. gracillimum* in the leaf lamina flat to slightly convex vs. often concave from abaxial view, costa slightly narrowed from leaf base to apex vs. costa hardly becoming narrower towards the apex, and in the transverse section of the costa weakly differentiated, without or with single stereids vs. well differentiated, with two stereid bands and dorsal and ventral epidermis, and in the costa surface cells smooth on both sides vs. papillose on adaxial side and smooth to variously papillose on abaxial side.

Ecology. The Yakutian locality of *Hymenostylium xerophilum* (Fig. 5) is a bald hill composed of peculiar rock, which probably contains a toxic concentration of heavy metals. The neighbouring slopes are covered with light *Larix* forests, at places open, with large stands of *Cladonia stellaris* (Opiz) Pouzar & Vězda and screes in the steepest places. The foothill at 625 m harboured, among others, *Aloina rigida* (Hedw.) Limpr., *Anomobryum bavaricum* (Warnst.) Holyoak & Köckinger, *A. concinatum* (Spruce) Lindb., and *Pterygoneurum ovatum* (Hedw.) Dixon, which is rare in the boreal zone. In the vicinity of *H. xerophilum* population at 765 m we recorded *Encalypta mutica* I. Hagen, *Timmia sibirica* Lindb. & Arnell, *Molendoa sendtneriana* (Bruch & Schimp.) Limpr., *Flexitrichum gracile* (Mitt.) Ignatov & Fedosov, *Buckia vaucheri* (Lesq.) D. Ríos, M.T. Gallego & J. Guerra, *Cyrtomnium hymenophylloides* (Huebener) T.J. Kop., *Dicranum elongatum* Schleich. ex Schwägr., *D. bardunovii* Tubanova & Ignatova, and two especially interesting species, *Tortella densa* (Lorentz & Molendo) Crundw. & Nyholm and *Indusiella thianschanica* Broth. & Müll. Hal. We have no other information than the label data for the Omani specimen of *H. xerophilum*, which reads “rock fissures, calcareous, at 2050 m”. Al Jabal Al Akhdar or Jebel Akhdar (“Green Mountains” is a mainly limestone mountain range ca 100–150 km inland from the coast of the Gulf of Oman, which receives about 300 mm of precipitation at higher altitudes, which support some shrubby and tree vegetation and agriculture. The locality of *H. gracillimum* in Irkutsk Province is situated at the NW shore of Baikal Lake, at ca. 460 m a.s.l., in a place which is known as extremely dry and sunny; annual precipitation does not exceed 140 mm and mean annual number of cloudy days is only 48 (Voloshin *et al.*, 2009). According to label data, the specimen of *H. gracillimum* was collected in fissures of a limestone cliff.



Fig. 5. Habitat of *Hymenostylium xerophilum* in Yakutia.

Phylogeny of Pleurowesiaceae. The trees resulting from the analysis of nuclear (Fig. 1) and chloroplast loci (Fig. 2) agree in the basal position of *Gyroweisia tenuis* (Hedw.) Schimp. lineage with respect to the well supported crown group of Pleurowesiaceae taxa, represented by accessions of *Anoectangium*, *Tuerckheimia*, *Molendia* and *Hymenostylium*. They also agree in the supported monophyly of analysed taxa of *Gymnostomum*, *Anoectangium* and *Molendia*, and non-monophyly of *Tuerckheimia* and *Hymenostylium*, as well as the monophyly of *Hymenostylium xerophilum*+*gracillimum* lineage, being a sister to the rest of *Hymenostylium* taxa, which constitute another moderately- to well-supported lineage. Analyses based on the nuclear and chloroplast loci differ, however, in the mutual position of *Gymnostomum*, *Anoectangium* and *Tuerckheimia* lineages, as well as among the taxa of the main *Hymenostylium* lineage, which includes the type of the genus, *H. xanthocarpum*. Therefore, we have not concatenated the data from the different genomic compartments and present the results from the analyses of nuclear and chloroplast datasets separately.

The additional accessions of *Hymenostylium recurvirostrum* s.lat. suggest a large genetic differentiation of this group and the potential for delimitation of addition-

al taxa, particularly if var. *insigne* should continue to be recognised. The two analysed accessions of that taxon form a well-supported lineage according to the chloroplast data but not according to ITS, which is however nested among accessions with the general morphology of var. *recurvirostrum*. *Hymenostylium xanthocarpum* which has been recognised as the sole member of this genus upon the recognition of genus *Ardeuma* R.H. Zander & Hedd. by Zander (2016) was found nested in the lineage containing *H. (Ardeuma) aurantiacum* and *H. (Ardeuma) hildebrandtii* (Müll. Hal.) R.H. Zander (chloroplast data).

Within the clade of *H. xerophilum*+*H. gracillimum*, the current analyses confirmed the uncertain position of the Karelian specimen of *H. gracillimum* (paratype of *G. boreale*), which clusters with a weak support (BS 60/PP 0.98) with the lineage of *H. xerophilum* in the ITS analysis. The results of concatenated analysis (not shown), however, agree with the results of chloroplast analysis.

DISCUSSION

Köckinger & Kučera (2011) anticipated that the real distribution of both the newly described *Hymenostylium xerophilum* and the resurrected *H. gracillimum* will be broader than the European occurrences known at the time

of publication. Particularly the mountain ranges of southern Siberia are the area with many elements common to European mountain ranges, including those known from the Alps, Carpathians and Dinaric mountain ranges. Olkhon Island is a very dry area despite it is an island in Lake Baikal. Western coastal ranges of Baikal provide the “wind shade” making Olkhon an almost cloudless area with only 140 mm of annual precipitation, vegetated by steppes which include endemics, such as *Deschampsia turczaninowii* Litv., *Festuca baicalensis* (Griseb.) V. Krecz., *F. olchonensis* E. Alexeev, *Papaver olchonensis* Peschkova, *Dracocephalum olchonensis* Peschkova, etc. (Zarubin & Lyakhova, 1999). The widespread Mongolian and Central Asian grass *Achnatherum sibiricum* (L.) Keng ex Tzvelev survives here at the northern limit of its range in this longitudinal sector (<https://www.gbif.org/species/4141276> accessed 1 Dec 2019). However, the surroundings of Olkhon Island are fairly diverse, with outcrops of both marble and acidic rocks. According to the label data, *H. gracillimum* was collected on limestone cliffs, but details of site conditions are unknown. In Europe, the species prefers moist and shady, easily disintegrating rocks (typically phyllite or other schists). Occurrences on hard rocks were known to be restricted to shaded N-facing crevices, which was probably also the case of the Karelian locality, and rarely the species was collected on artificial walls in Central Europe (Köckinger & Kučera, 2011).

The Yakutian locality of *H. xerophilum* was ecologically more like European occurrences. Although the species has not been recorded from metalliferous carbonate-containing rocks in the Alps, dolomite itself can be regarded toxic for many species occurring on limestone without higher magnesium contents. The toxic concentration of heavy metals apparent from the lack of vegetation on mountain slopes at the Yakutian locality and the co-occurrence of metallophytic *Coscinodon hartzii* C.E.O. Jensen and *Mielichhoferia* spp., which were seen at several sites nearby, might be the reason for its rarity at the site, as the species has not been re-found despite a targeted search afterwards. On the other hand, this environment can be considered stable, allowing the colonisation by tolerant species of different geographical elements, including pan-xeric (Frey & Kürschner, 1988) *Pterigonium* and *Aloina* species, cryoarid *Indusiella thianschanica*, *Leptopterigynandrum* spp., *Coscinodon hartzii*, or the more western *Tortella densa* and *Anomobryum bavaricum*. *Indusiella* was thought for a long time to be extremely rare in Russia, but recent exploration focused on its findings revealed ca. 10 localities in Yakutia (Ivanova et al., 2016, 2017, 2018; Ignatova et al., 2018). The locality of *Tortella densa* is one of only two localities known so far in Yakutia, the second being ca. 50 km apart. The bedrock in this mountain range is fairly complex, including large limestone outcrops about 5 km from this locality, with the occurrence of *Andreaebryum macrosporum* Steere & B.M. Murray (Ignatov et al., 2018).

The mixture of geographic elements among mesophytic and hygromesophytic species of the Sette-Daban is also conspicuous: Beringian *Andreaebryum macrosporum*, *Haplodontium macrocarpum* (Hook.) J.R. Spence, *Schistidium relictum* T.T. McIntosh, H.H. Blom & Ignatova or *Scouleria* spp. co-occur here with the Central Asian *Struckia enervis* (Broth.) Ignatov, T.J. Kop. & D.G. Long or the predominantly Sino-Himalayan *Didymodon leskeoides* K. Saito, *Hydrogonium gregarium* (Mitt.) Jan Kučera and *H. amplexifolium* (Mitt.) P.C. Chen.

While even the new, highly disjunct Asian records of *H. xerophilum* do not significantly add to known molecular diversity of the species, the Omani population extends the known morphological plasticity considerably, showing that round-leaved forms similar to *Gymnostomum mosis* and *G. viridulum* must be expected under particularly arid and warm site conditions. Similarly, the Siberian population of *H. gracillimum* represents a significant addition to the known morphological variability of this species, making the distally vanishing costa in the latter species one of the few rather safe diagnostic characters between the species. The molecular divergence of the new Asian record, on the other hand, does not seem to be too high, being considerably smaller than that of the Karelian plants.

The phylogenetic affinities of *Hymenostylium xerophilum* and *H. gracillimum* with other *Hymenostylium* taxa have not changed significantly as compared to Köckinger & Kučera (2011), despite the addition of several taxa of the genus, the generally better representation of Pleuroweisiae and the addition of one chloroplast locus. This study does not confirm Zander’s arguments for segregating *H. xanthocarpum* into a monotypic *Hymenostylium* while moving all other species to a newly established genus, *Ardeuma* (Zander 2016). *H. xanthocarpum* is clearly nested (on a relatively short branch, showing a little genetic divergence from the other species) inside a lineage that contains most other *Hymenostylium* species, including the generitype of *Ardeuma*, *H. recurvirostrum*. *Ardeuma* was proposed as a new genus mainly based on the absence of the stem central strand, which might not be enough phylogenetically informative in this group of Pottiaceae. Interestingly, Zander has not proposed the generic affinity of *H. xerophilum* and *H. gracillimum*, which also possess the stem central strand, although he discussed the species. Despite *Ardeuma* can safely be merged with *Hymenostylium* based on the available molecular data, it is less clear whether the lineage containing *H. xerophilum* and *H. gracillimum* should be maintained within *Hymenostylium* or segregated to another morphologically weakly delimited genus of Pleuroweisiae. The conservative solution which we retain here might however result in the later necessity of merging the whole genus *Molendoa*, which is principally recognised by the lateral position of perichaetia, with *Hymenostylium*. The final decision needs in our opinion a much wider representation of *Hymenostylium* and *Hymenostylium*-related taxa.

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LITERATURE CITED

- FEDOSOV, V.E., A.V. FEDOROVA, A.E. FEDOSOV & M.S. IGNATOV. 2016. Phylogenetic inference and peristome evolution in haplolepidous mosses, focusing on Pseudoditrichaceae and Ditrichaceae s. l. – *Botanical Journal of the Linnean Society* **181**(2): 139–155. <https://doi.org/10.1093/sysbio/49.2.369>
- FEDOSOV, V.E., E.A. IGNATOVA, M.S. IGNATOV & A.I. MAKSIMOV. 2011. Rare species and preliminary list of mosses of the Anabar Plateau (Subarctic Siberia). – *Arctoa* **20**: 153–174.
- FREY, W. & H. KÜRSCHNER. 1988. Bryophytes of the Arabian Peninsula and Socotra. Floristics, phytogeography and definition of the xerothermic Pangean element. Studies in Arabian bryophytes 12. – *Nova Hedwigia* **46**: 37–120.
- IGNATOV, M.S., O.M. AFONINA, E.A. IGNATOVA et al. 2006. Checklist of mosses of East Europe and North Asia. – *Arctoa* **15**: 1–130.
- IGNATOV, M.S., E.A. IGNATOVA, E.I. IVANOVA, O.V. IVANOV & N.A. BYSYIN. 2018. On the distribution of *Andreaobryum* in Russia. – *Arctoa* **27**(2): 112–118.
- IGNATOVA, E.A., E.I. IVANOVA & M.S. IGNATOV. 2018. Moss flora of Sette-Daban Range (Yakutia). – *Arctoa* **27**(2): 119–130.
- IVANOVA, E.I., E.A. IGNATOVA, V.G. ISAKOVA, I.A. BALAKIREV, O.I. KUZNETSOVA & M.S. IGNATOV. 2018. Moss flora of the Ust-Nera region in the upper course of Indigirka River, East Yakutia. – *Arctoa* **27**(1): 18–28. DOI 10.15298/arctoa.27.02

- IVANOVA, E.I., V.G. ISAKOVA, M.S. IGNATOV & E.A. IGNATOVA. 2017. On the moss flora of Khangalassky District (Republic of Sakha/Yakutia, East Siberia). – *Arctoa* **26**(1): 47–57. DOI: 10.15298/arctoa.26.04
- IVANOVA, E.I., E.A. IGNATOVA & M.S. IGNATOV. 2016. Moss flora of the Suntar-Khayata Reserve, Yakutia). – *Arctoa* **25**: 131–140. DOI: 10.15298/arctoa
- KATO, K. & D.M. STANDLEY. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. – *Molecular Biology and Evolution* **30**: 772–780. DOI: 10.1093/molbev/mst010
- KÖCKINGER, H. & J. KUČERA. 2011. *Hymenostylium xerophilum*, sp. nov., and *H. gracillimum*, comb. nov., two neglected European mosses and their molecular affinities. – *Journal of Bryology* **33**(3): 195–209.
- KUČERA, J., O.I. KUZNETSOVA, A. MANUKJANOVA & M.S. IGNATOV. 2019. A phylogenetic revision of the genus *Hypnum*: Towards completion. – *Taxon* DOI: 10.1002/tax.12095
- RONQUIST, F., M. TESLENKO, P. MARK. VAN DER, D.L. AYRES, A. DARLING, S. HÖHNA, B. LARGET, L. LIU, M.A. SUCHARD & J.P. HUELSENBECK. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. – *Systematic Biology* **61**: 539–542. DOI: 10.1093/sysbio/sys029
- SIMMONS, M.P. & H. OCHOTERENA. 2000. Gaps as Characters in Sequence-based Phylogenetic Analyses. – *Systematic Biology* **49**: 349–381.
- STAMATAKIS, A. 2014. RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. – *Bioinformatics* **30**(9): 1312–1313, DOI: 10.1093/bioinformatics/btu033
- STÖVER, B.C. & K.F. MÜLLER. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. – *B. M. C. Bioinformatics* **11**: 7. DOI: 10.1186/1471-2105-11-7
- [VOLOSHIN, A.L., S.G. ANDREEV & V.N. PRONIN] ВОЛОШИН А.Л., С.Г. АНДРЕЕВ, В.Н. ПРОНИН. 2009. Климатические особенности. – [Climate patterns] В кн.: Тулохонов, А.К. (ред.) Байкал. Природа и люди [In: Tulokhonov, A.K. (ed.) Baikal. Nature and people] Улан-Удэ: ЭКОС, Изд-во БИЦ СО РАН [Ulan-Ude, EKOS publishing house of BSC SB of RAS], 606 pp.
- WHITEHOUSE, H.L.K. & A.C. CRUNDWELL. 1991. *Gymnostomum calcareum* Nees & Hornsch. and allied plants in Europe, North Africa and the Middle East. – *Journal of Bryology* **16**: 561–579.
- ZANDER, R.H. 2016. Re-evaluation of *Hymenostylium xanthocarpum* (Hook.) Brid., and *Ardeuma* R.H.Zander & Hedd., a new name for all other species of *Hymenostylium* (Pottiaceae, Bryophyta). – *Journal of Bryology* **38**: 295–301.

Appendix 1. Specimen used in the molecular analyses, with GenBank accession numbers. Accessions printed in bold were newly obtained for this study.

Species	Specimen voucher	Isolate	Genbank code (ITS/rps4/trnM-trnV)
<i>Anoetangium aestivum</i>	Austria, Heiligenblut, Kučera 12848 (CBFS)	JK104	HM147801/ HM147774/ JX679910
<i>Anoetangium aestivum</i>	UK, Ben Earb, Kučera 10128 (CBFS)	JK658	MN817235/ MN815933/ MN815965
<i>Anoetangium aestivum</i>	Norway, Divielva valley, Kučera 15892 (CBFS)	AM682	MN817236/ MN815934/ MN815966
<i>Anoetangium thomsonii</i>	Japan, Honshu, Okawara, Y. Inoue 1281 (CBFS)	AM678	MN817237/ MN815935/ MN815967
<i>Anoetangium thomsonii</i>	Russia, Zabaykalsky, Afonina 6612 (CBFS)	AM698	MN817238/ MN815936/ MN815968
<i>Anoetangium thomsonii</i>	China, Yunnan, Bai Ma Shan, Long 24221 (E)	RBGE885	MN817239/ MN815937/ MN815969
<i>Gymnostomum calcareum</i>	Switzerland, Muzzano, Kučera 6508 (CBFS)	JK177	HM147812/ HM147786/ MN815970
<i>Gymnostomum calcareum</i>	Greece, Ourlias River, Blockeel 39/274	AM704	MN817240/ MN815938/ MN815971
<i>Gymnostomum calcareum</i>	Greece, Samos, Blockeel 41/056	AM705	MN817241/ MN815939/ MN815972
<i>Gymnostomum calcareum</i>	Chile, XII, Cueva del Milodón, Sérgio 12804 (LISU)	AM718	MN817242/ MN815940/ MN815973

<i>Gymnostomum viridulum</i>	Czechia, Lukov, <i>Hradílek s.n.</i> (CBFS)	JK99	HM147797/ HM147770/ JQ890412
<i>Gymnostomum viridulum</i>	Spain, Grazaema, <i>Kučera 17493</i> (CBFS)	AM1067	MN817243/ MN815941/ MN815974
<i>Gyroweisia tenuis</i>	France, Mont-Dore, <i>Kučera 10748</i> (CBFS)	JK176	HM147799/ HM147772/ JX679908
<i>Gyroweisia tenuis</i>	UK, Derbyshire, <i>Blockeel 42/036</i>	AM734	MN817244/ MN815942/ MN815975
<i>Hymenostylium aurantiacum</i>	India, Mussoorie, <i>Lüth 6596</i>	JK611	MN817245/ MN815943/ MN815976
<i>Hymenostylium aurantiacum</i>	India, Mussoorie, <i>Lüth 6628</i>	JK615	MN817246/ MN815944/ MN815977
<i>Hymenostylium gracillimum</i>	Austria, Tiboldgraben, <i>Köckinger 14264</i> (CBFS)	JK165	HM147809/ HM147782/ JQ890413
<i>Hymenostylium gracillimum</i>	Austria, Virgental, Obermauern, <i>Köckinger 14267</i> (CBFS)	JK173	HM147811/ HM147784/ MN815978
<i>Hymenostylium gracillimum</i>	Russia, Karelia, Kulmakkapuro, <i>Hülphers s.n.</i> (S)	JK304	HM147815/ HM147789/ MN815979
<i>Hymenostylium gracillimum</i>	Austria, Weißensee lake <i>Köckinger 14650</i> (CBFS)	JK317	HM147818/ HM147792/ MN815980
<i>Hymenostylium gracillimum</i>	Russia, Irkutsk Province, Sakhyurta Settlement, <i>Bardunov s.n.</i> , 31.8.1997 (MW)	AF1208	MN817247/ MN815945/ MN815981
<i>Hymenostylium hildebrandtii</i>	Morocco, Jbel Saghro, <i>Ros s.n.</i> (MUB)	JK322	AY796282/ HM147793/ MN815982
<i>Hymenostylium recurvirostrum</i> var. <i>recurvirostrum</i>	Austria, Seidlwinkltal valley, <i>Kučera 12780</i> (CBFS)	JK103	HM147800/ HM147773/ JX679909
<i>Hymenostylium recurvirostrum</i> var. <i>recurvirostrum</i>	Tajikistan, Iskanderkul, <i>Plášek s.n.</i> (CBFS)	JK646	MN817249/ MN815947/ MN815984
<i>Hymenostylium recurvirostrum</i> var. <i>recurvirostrum</i>	Austria, Bürser Schlucht, <i>H. Köckinger 14935</i> (CBFS)	AM970	MN817253/ MN815951/ MN815988
<i>Hymenostylium recurvirostrum</i> var. <i>insigne</i>	Austria, Haselschlucht ravine, <i>Schlüsslmayr s.n.</i> (CBFS)	JK167	HM147810/ HM147783/ MN815990
<i>Hymenostylium recurvirostrum</i> var. <i>insigne</i>	UK, VC108, Inchnadamph, <i>Long 29181</i> (E)	AM934	MN817255/ MN815953/ MN815991
<i>Hymenostylium recurvirostrum</i> s.lat.	India, Mussoorie, <i>Lüth 6625</i>	JK614	MN817248/ MN815946/ MN815983
<i>Hymenostylium recurvirostrum</i> s.lat.	Japan, Honshu, Kamimura, <i>Y. Inoue 1323</i> (CBFS)	AM681	MN817250/ MN815948/ MN815985
<i>Hymenostylium recurvirostrum</i> s.lat.	Greece, Mt Timfi, <i>Blockeel 37/253</i>	AM702	MN817251/ MN815949/ MN815986
<i>Hymenostylium recurvirostrum</i> s.lat.	Russia, Zabaykalsky, Koira creek, <i>Afonina 6612</i> (CBFS)	AM728	MN817252/ MN815950/ MN815987
<i>Hymenostylium recurvirostrum</i> s.lat.	Russia, Irkutskaya, Slyudyanka, <i>Kučera 20804</i> (CBFS)	AM1571	MN817254/ MN815952/ MN815989
<i>Hymenostylium xanthocarpum</i>	India, Mussoorie, <i>Lüth 6624</i>	JK613	MN817256/ MN815954/ MN815992
<i>Hymenostylium xanthocarpum</i>	India, Mussoorie, <i>Lüth 6596</i>	JK616	MN817257/ MN815955/ MN815993
<i>Hymenostylium xerophilum</i>	Austria, Seitz, Kaisertal valley, <i>Köckinger 05-954</i> (CBFS)	JK62	HM147796/ HM147769/ JQ890415
<i>Hymenostylium xerophilum</i>	Austria, Carinthia, Krastal valley, <i>Köckinger 14243</i> (CBFS)	JK100	HM147798/ HM147771/ MN815994
<i>Hymenostylium xerophilum</i>	Oman, Jabal Al Akhdar, <i>Frey & Kürschner 1-3949</i> (E)	AM902	MN817258/ MN815956/ MN815995
<i>Hymenostylium xerophilum</i>	Russia, Sakha, <i>Ignatov & Ignatova 15-851</i> (MHA)	AF1142	MN817259/ MN815957/ MN815996
<i>Leptobarbula berica</i>	Spain, Murcia, <i>Kučera 13640</i> (CBFS)	JK380	MN817260/ MN815958/ MN815997
<i>Leptobarbula berica</i>	Spain, Andalucía, <i>Kučera 17430</i> (CBFS)	AM1050	MN817261/ MN815959/ MN815998
<i>Molendoa hornschuchiana</i>	Austria, Seidlwinkltal valley, <i>Kučera 12790</i> (CBFS)	JK106	HM147802/ HM147775/ MN815999
<i>Molendoa hornschuchiana</i>	Austria, Heiligenblut, <i>Kučera 6341</i> (CBFS)	JK663	MN817262/ MN815960/ MN816000
<i>Molendoa sendtneriana</i>	Russia, Sakha, Lenskiye Stolby, <i>Ignatov 00-258</i> (CBFS)	JK181	HM147813/ HM147787/ MN816002
<i>Molendoa sendtneriana</i>	Austria, Heiligenblut, <i>Kučera 12838</i> (CBFS)	JK107	MN817263/ MN815961/ MN816001
<i>Molendoa tenuinervis</i>	Mongolia, Mt. Ikh-Bogd, <i>Ignatov 01-789</i> (CBFS)	JK134	JQ890531/ JQ890478/ JQ890417
<i>Molendoa tenuinervis</i>	Austria, Mt Hohe Dock, <i>Kučera 12768</i> (CBFS)	JK108	MN817264/ MN815962/ MN816003
<i>Tuerckheimia svihlae</i>	U.S.A., Florida, Marianna Caverns, <i>Cash & Rapp M193</i> (DUKE)	JK312	HM147817/ HM147791/ JX679914
<i>Tuerckheimia svihlae</i>	China, Yunnan, Shabadi, <i>Long 32566</i> (E)	RBGE822	MN817265/ MN815963/ MN816004
<i>Tuerckheimia valeriana</i>	Costa Rica, Río Savegre, <i>Holz & Schäfer-Verwimp CR 99-1178</i> (E)	RBGE821	AY854431/ MN815964/ MN816005