

ON THE SYSTEMATIC POSITION OF *HYMENOLOMA* (BRYOPHYTA)

О СИСТЕМАТИЧЕСКОМ ПОЛОЖЕНИИ РОДА *HYMENOLOMA* (BRYOPHYTA)

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Abstract

Molecular phylogenetic analyses based on *nad5*, *rps4* and *trnL*-F sequences suggest the position of the genus *Hymenoloma* in the order Scouleriales. This order includes two monogeneric families: Scouleriaceae, with aquatic plants, and Drummondiaaceae that includes mostly epiphytic mosses. Mainly epilithic *Hymenoloma* strongly differs from both of them, as well as they differ from each other, thus a new family Hymenolomataceae is suggested for this genus. Morphologically, *Hymenoloma* has no unique characters, although its outstanding plasticity provides some explanation of its relationships with *Scouleria* and *Drummondia*.

Резюме

Согласно данным молекулярно-филогенетического анализа, основанного на последовательностях генов *nad5*, *rps4* и *trnL*-F, род *Hymenoloma* следует относить к порядку Scouleriales. Этот порядок включает два семейства, в каждом из которых по одному роду: Scouleriaceae, представители которого растут в воде, и Drummondiaaceae, куда входят эпифитные виды. Виды рода *Hymenoloma* растут преимущественно на каменистых субстратах и резко отличаются от видов из обоих других семейств порядка, которые, в свою очередь, совершенно не похожи друг на друга. Поскольку род *Hymenoloma* не представляется возможным включить ни в одно из двух семейств порядка по морфологическим признакам, то описывается новое семейство Hymenolomataceae. Этот род не имеет каких-либо уникальных морфологических признаков; с другой стороны, его выдающаяся морфологическая пластичность позволяет объяснить родство со *Scouleria* и *Drummondia*.

KEYWORDS: Dicranidae, mosses, *nad5*, *rps4*, *trnL*-F, Scouleriales, molecular phylogenetics

INTRODUCTION

The genus *Hymenoloma* has been installed by Dusén (1905) for one Patagonian species, *H. nordenskjöldii* Dusén. Brotherus (1924) accepted it in the Seligeriaceae and also added to this genus three other species from Subantarctic islands and southern South America, described originally in the genera *Blindia* Bruch et al., *Dicranoweisia* Milde, and *Verrucidens* Cardot. Reimers (1936) synonymized *Hymenoloma* with *Dicranoweisia* (fam. Dicranaceae), bringing the former genus to the long oblivion. This decision had been invariably followed by bryologists up to 1990s.

The transfer from Seligeriaceae to Dicranaceae was not considered conspicuous, as these two families were placed one by one within the same order Dicranales in

the system of Brotherus (1924). Reimers argued his decision by complex costa structure with guide cells and stereid bands, as well as non Seligerioid peristome, as the latter was described by Brotherus.

However, when the attention to structural botany revived in 1970s, it resulted, among others, in the discovery of considerable distinction between *Seligeria*-type and *Dicranum*-type of peristome (Edwards, 1979, 1984). As a consequence, the position of Seligeriaceae was reevaluated and a separate order was established for its accommodation (Nyholm, 1987; Anderson *et al.*, 1990).

Ochyra (1993, 1998) was the first who noticed a disagreement between the peristome structure of *Hymenoloma crispulum* (Hedw.) Ochyra, that time called *Dicranoweisia crispula* (Hedw.) Milde, with its position in

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Dicranaceae, and placed Antarctic species, including *D. crispula*, in the Seligeriaceae. Subsequent studies revealed that the type of the genus *Dicranoweisia*, Mediterranean *D. cirrata* (Hedw.) Lindb., is so different from the cosmopolitan *D. crispula*, that another generic name is required for the group of taxa around *D. crispula*. This problem was solved later by Ochyra, as he resurrected the genus *Hymenoloma*, accepting it in the Seligeriaceae (Ochyra *et al.*, 2003).

Since the beginning of molecular phylogenetic studies, the close position of *Hymenoloma* to the Scouleriaceae and Drummondaceae was revealed, while *Dicranoweisia* was found within Rhabdoweisiaceae (Hedderston *et al.*, 2004; Tsubota *et al.*, 2003, 2004). The most comprehensive molecular phylogenetic studies of the genus *Hymenoloma* by Werner *et al.* (2013) also supported a remote position of this genus from *Dicranoweisia*. Their analysis placed *Hymenoloma* within the strongly supported clade with *Scouleria* and *Drummondia*. However, their set did not include any Seligeriaceae. An important point of this study was the inclusion of *H. antarcticum* (Müll. Hal.) Ochyra, because *H. nordenskjöldii*, the type of the genus *Hymenoloma*, was synonymized with this species by Ochyra *et al.* (2008a).

Subsequent wider analyses of haplolepidous mosses constantly resolved *Hymenoloma* in a clade with Scouleriaceae or/and Drummondaceae (Stech *et al.*, 2012; Inoue & Tsubota, 2014; Fedosov *et al.*, 2016). However, no taxonomic implications were made so far, thus *Hymenoloma* has been treated either in the Seligeriaceae (Ochyra *et al.*, 2008a, b), or in the Rhabdoweisiaceae (Goffinet *et al.*, 2009; Ignatov *et al.*, 2006; Ros *et al.*, 2013), or in the Oncoporidae (Frye & Stech, 2009).

In the present paper we revisit the problem, invoking both molecular and morphological data on the genus *Hymenoloma*.

MATERIAL AND METHODS

Molecular phylogenetic study The material used in the present study was sampled from MW and MHA and supplemented by sequences available in GenBank. As most species of *Hymenoloma* occur in Antarctic region, only two of them, *H. crispulum* and *H. mulahaceni* (Höhn.) Ochyra (= *H. intermedium* (J.J. Amann) Ochyra) were involved in present study. For molecular study we used three markers, mitochondrial *nad5*, chloroplastic *rps4* and *trnL-F* regions, commonly used for molecular-phylogenetic studies at generic and familial levels. The dataset was compiled with special attention to former Dicranaceae, Seligeriaceae, and several lineages of proto-haplolepidous mosses related to *Hymenoloma* according to recent molecular-phylogenetic studies (Distichiaceae, Timmiellaceae, Drummondaceae, Scouleriaceae, Catosciaceae, *etc.*). *Diphyscium foliosum* (Hedw.) D. Mohr (Diphysciaceae), several representatives of lineages with diplolepidous-opposite (*Encalypta streptocarpa* Hedw., *Funaria hygrometrica* Hedw.) and diplolepi-

deous-alternate (*Bryum pseudotriquetrum* (Hedw.) P.Gaertn., B. Mey. & Scherb., *Hedwigia ciliata* (Hedw.) P. Beauv., *Hookeria lucens* (Hedw.) Sm.) peristomes were involved as outgroups. Totally 110 sequences from 37 specimens representing 35 species were involved into the analysis, including 25 sequences of 12 species obtained de novo. Vouchers of newly sequenced specimens and GenBank accession numbers of all used sequences are compiled in Appendix 1. Laboratory protocol was essentially the same as in previous moss studies, described in detail by, *e.g.*, Fedosov *et al.* (2016).

Sequences were aligned manually in Bioedit (Hall, 1999). Four dataset were built. The first three of them correspond to the individual gene alignments, *nad5* (1130 bp), *rps4* (568 bp) and *trnL* (624 bp); they were analyzed separately to check their congruence. The fourth dataset represented concatenated *rps4 – nad5 – trnL* sequences (2322 positions). Best-fit substitution models were identified for each gene separately using Partitionfinder V1.1.1 (Lanfear *et al.*, 2012). Best-scoring Maximum Likelihood (ML) trees were estimated using RaxML (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. RaxML was performed on the Cipres Science Gateway (<http://www.phylo.org/portal2>), using RAXML-HPC2 on XSEDE.

Bayesian Analyses (BA) were performed running two parallel analyses in MrBayes (Huelsenbeck & Ronquist, 2001). For single gene analyses each run consisted of six Markov chains and 5,000,000 generations with default number of swaps chains. For *rps4 – nad5 – trnL* dataset the analysis consisted of eight Markov chains and 50.000.000 generations with the number of swaps chains set to five was performed. A sampling frequency was one tree each 10.000 generations, and the chain temperature was set at 0.02 in all analyses. Convergence of each analysis was evaluated using Tracer1.4.1 (Rambaut & Drummond, 2007) to check that all ESS values exceeded 200. Consensus tree were calculated after omitting the first 25% trees as burn-in. The combined dataset was separated into eight unlinked partitions respectively: three codon positions of *rps4* gene, three codon positions for coding portion of the *nad5* gene, the intron in the *nad5* gene and *trnL-F* gene. Analyses were performed on the Cipres Science Gateway (<http://www.phylo.org/portal2>), using tool MrBayes 3.2.2 on XSEDE.

Morphological studies Three structures were in the main focus of the study: (1) cuticular papillae on the leaf, (2) the surface structure of calyptra and (3) the peristome structure.

Observations of leaf surface structure were done with the SEM Jeol 6380 for specimens coated by gold without additional preparation and with LSCM Olympus FV1000 for alive plants, stained by berberine.

For the study of peristome development, the mate-

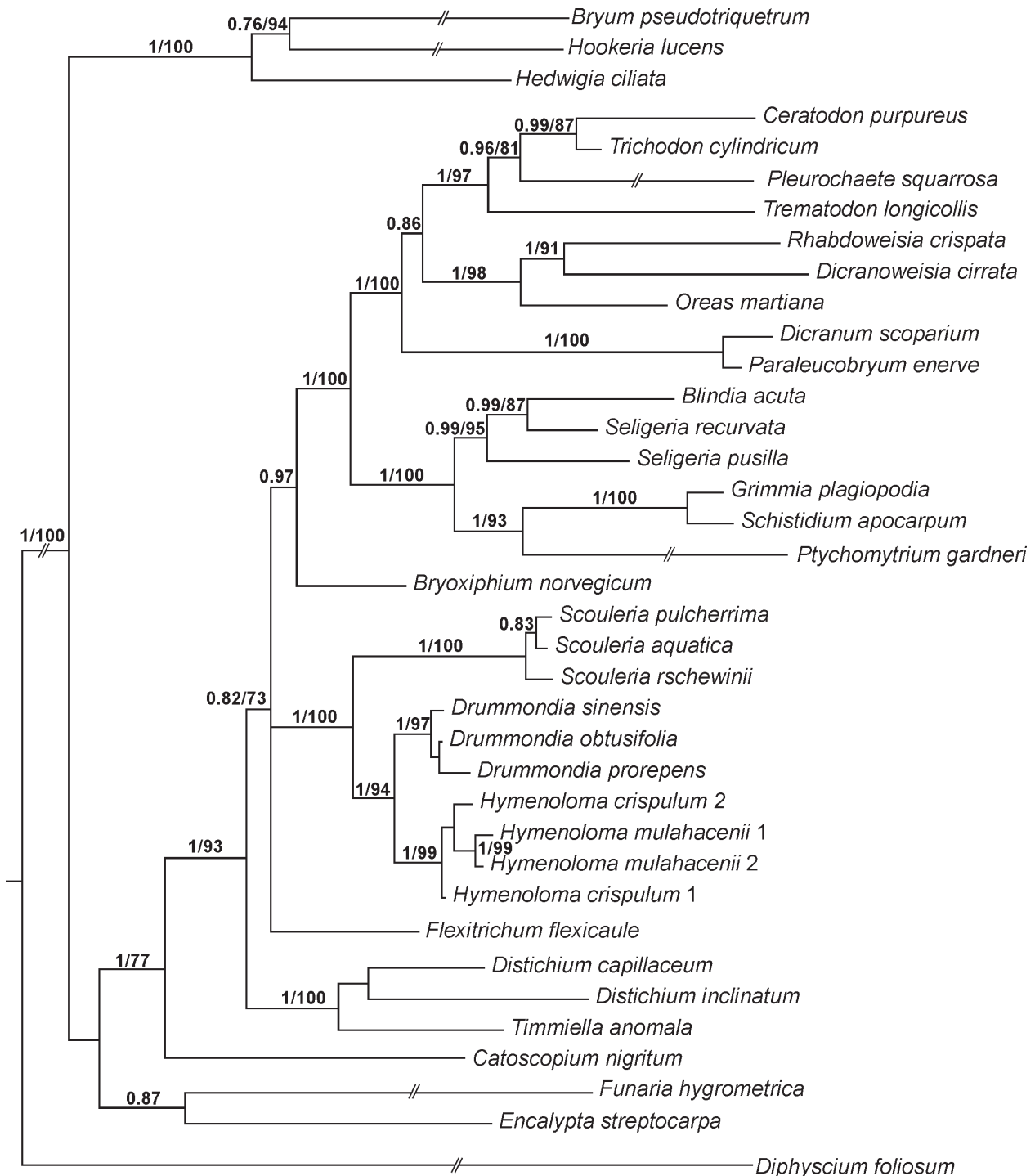


Fig. 1. Bayesian phylogenetic tree based on *nad5*, *rps4* and *trnL-F*. Bayesian posterior probabilities (>0.7) and / bootstrap supports (>70) from maximum likelihood analysis are shown above branches.

rial from Kola Peninsula, Khibiny Mts., 11.IX.2015 *Ignatov s.n.* (MHA), was used. The protocol of sample preparation was the same as described in Ignatov *et al.* (2015), and observations in LSCM Olympus FW-1000 were made with the berberin stained sections.

Mature peristomes of *H. crispulum* were studied from two specimens: (1) Russia, Yakutia, Chersky Mt. system, 2.VIII.2015, *Ignatov & Ignatova 15-1232* (MHA); (2)

Russia, Kola Peninsula, Khibiny Mts, 11.IX.2015 *Ignatov s.n.* (MHA); peristomes of *H. mulahaceni* were studied from: Russia, Altai Mts., 31.VII.1993, *Ignatov* (MHA).

Supplementary observation of peristome were done for *Drummondia sinensis* (Russia, Primorsky Territory, *Ignatov, Ignatova & Malashkina 13-1792*, MW). Peristomes were observed under SEM Jeol 6380 for specimens coated by gold without additional preparation.

RESULTS

Phylogenetic analyses. The preliminary trees obtained in the Bayesian analyses of *nad5*, *rps4* and *trnL-F* had nearly identical topologies without supported conflicts. The topologies of the consensus trees obtained from BA and ML were largely congruent. The topology from BA is shown in Fig. 1, with ML bootstrap support added to the supported clades.

The overall topology of the tree rooted on *Diphyscium* includes three supported clades of Diplolepideous opposite, diplolepeidous alternate and haplolepeidous mosses. The latter clade, corresponding to Dicranidae, is well supported (PP=1, BS=77). The most basal position within it has *Catoscopium* (Catoscopiaceae), followed by the grade of Distichiaceae + Timmiellaceae. The next tritomy (PP=0.82, BS=73) includes individual specimen of *Flexitrichum* (Flexitrichaceae) and two clades: (1) *Scouleria* (Scouleriaceae) + *Drummondia* (Drummondiaaceae) + *Hymenoloma* (PP=1, BS=100), and (2) *Bryoxiphium* (Bryoxiphaceae) + Grimmiaceae + Dicranales (PP=0.97, BS=56). Within the latter clade, Dicranales s.l. and Grimmiaceae s.l. have maximal support. In the first clade, the maximally supported subclade of *Scouleria* (PP=1, BS=100) is a sister to the strongly supported subclade of *Drummondia* + *Hymenoloma* (PP=1, BS=93), and each of these genera form their own well supported clades (PP=1, BS=97 and PP=1, BS=100 correspondingly).

Morphology

Leaf surface. Light microscopy observation of leaves in ordinary water slides revealed striolation in almost all leaves of *Hymenoloma crispulum*, although at places it was seen better than in others. In *H. mulahaceni*, longitudinal striolation was seen only at places in some individual leaves.

SEM observations on dry herbarium specimens without additional preparation showed a fairly diverse surface structure (Figs. 2–9). The ridges of cuticular material above cell walls appeared less collapsed, as compared to the central parts of cells (Figs. 2–6), on both dorsal and ventral sides of lamina. The cuticular papillae upon the lumen were more conspicuous on the ventral surface in acumen and their shape was more variable (Figs. 2, 3, 5), comparatively with dorsal side of acumen (Figs. 4, 6).

In the basal part of leaf, cuticular ridges have a distinct striolation upon their surface (Fig. 7), which is well expressed in some places, though less conspicuous in others. Longitudinal ridges are arranged at the same distance one from another, ca. 1 µm in average. Low cuticular ridges at the same distance one from another occur in the distal part of leaf as well (Fig. 8), but there they are low and unlikely discernible with light microscope.

LSCM observations in living plants of *H. crispulum* (Figs. 10–13) illustrate striolation of cell walls with the period of 1.5 µm in the distal part of leaves, which relates to the surface striolation of cell walls extending along

many cells. At the same time, cuticular papillae upon cell walls and partly on lumen cover them, obscuring the cell wall striolation in Z-stack images (Fig. 10). In *H. mulahaceni* the above mentioned striolation with the period of 1.5 µm can be found at places (Figs. 12–13), otherwise only a small cuticular papillae can be discerned in transverse leaf section (Fig. 18).

Transverse sections of leaves of *H. crispulum* demonstrated bulgings above the cell walls throughout the leaf, while in the upper leaf portion a rounded structures also appeared above the lumens on both dorsal and ventral sides (Figs. 14, 17), and on both sides of costa (Fig. 19).

Summing up, the surface sculpture in leaves of *Hymenoloma* includes longitudinal ridges above cell walls, low to moderate cuticular papillae and ridges upon cuticular papillae. Depending on a method of observation, they are more or less well seen.

Calyptra surface has a superficially similar pattern of variation as the leaf (Figs. 20–25). Closer to its top, calyptra has conspicuous longitudinal ridges mixed with bulgings, and the ridges themselves are finely striolate. Closer to the calyptra base, the ridges become low and sometimes outer surface of calyptra is totally smooth. However, the similarity with leaf lamina surface is only superficial, papillae on the surface of calyptra occur above the cell lumen, not above cell walls, as it is in the case of leaf.

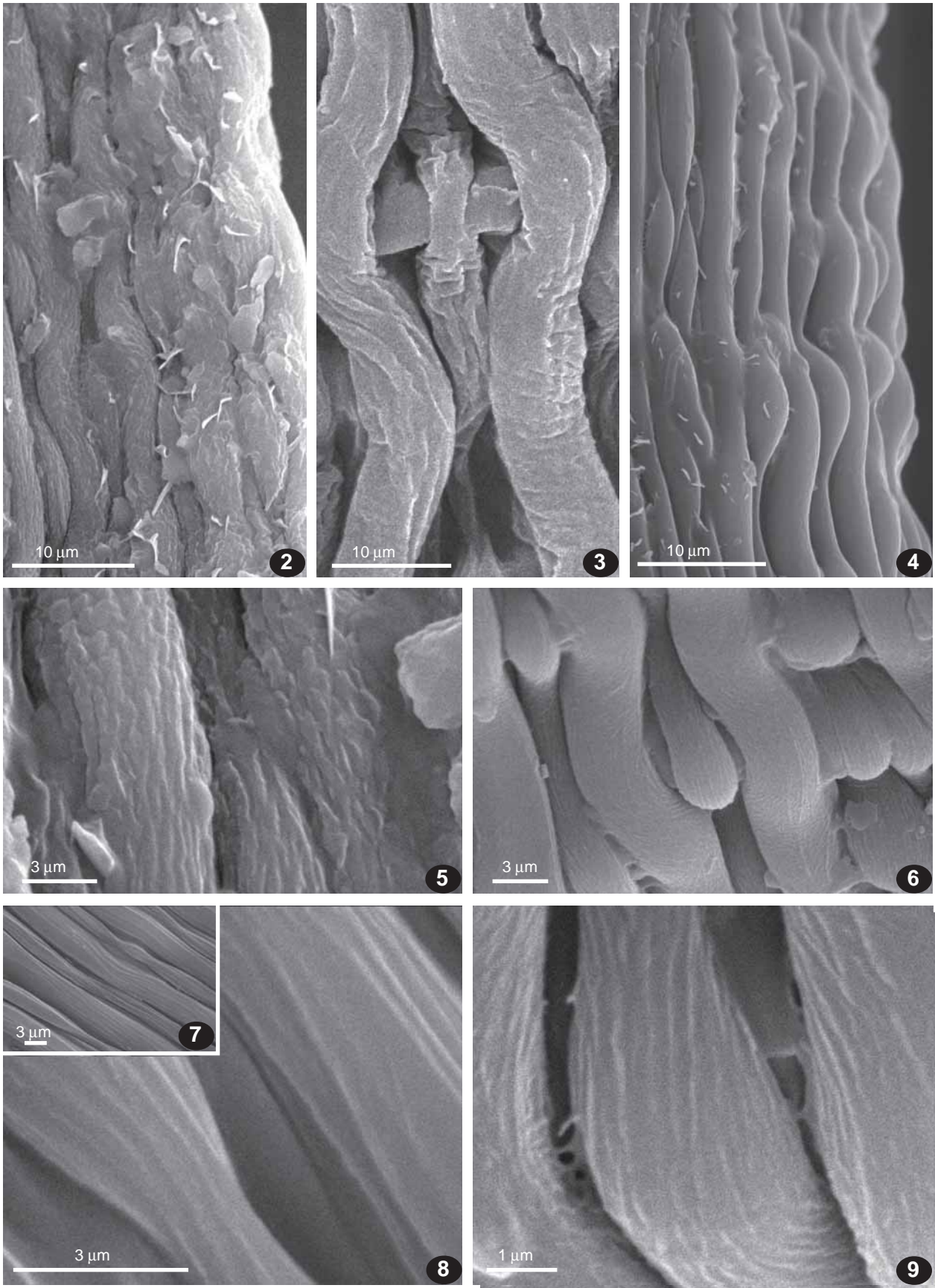
Peristome structure in *Hymenoloma crispulum* is of haplolepeidous type, e.g., single and homologous of endostome, based on 4:2:3 peristomial formula, developed from 2:2:2 stage (Fig. 26) due to unequal cell division in the inner peristomial layer (Fig. 27).

The haplolepeidous '2:1 /1:2' pattern, as defined by Edwards (1979, 1984) and Fedosov *et al.* (2016), is not apparent in all teeth of *Hymenoloma*. The teeth shape in the latter is often somewhat irregular, and the number of teeth occasionally 15 or 17 (e.g., Fig. 28).

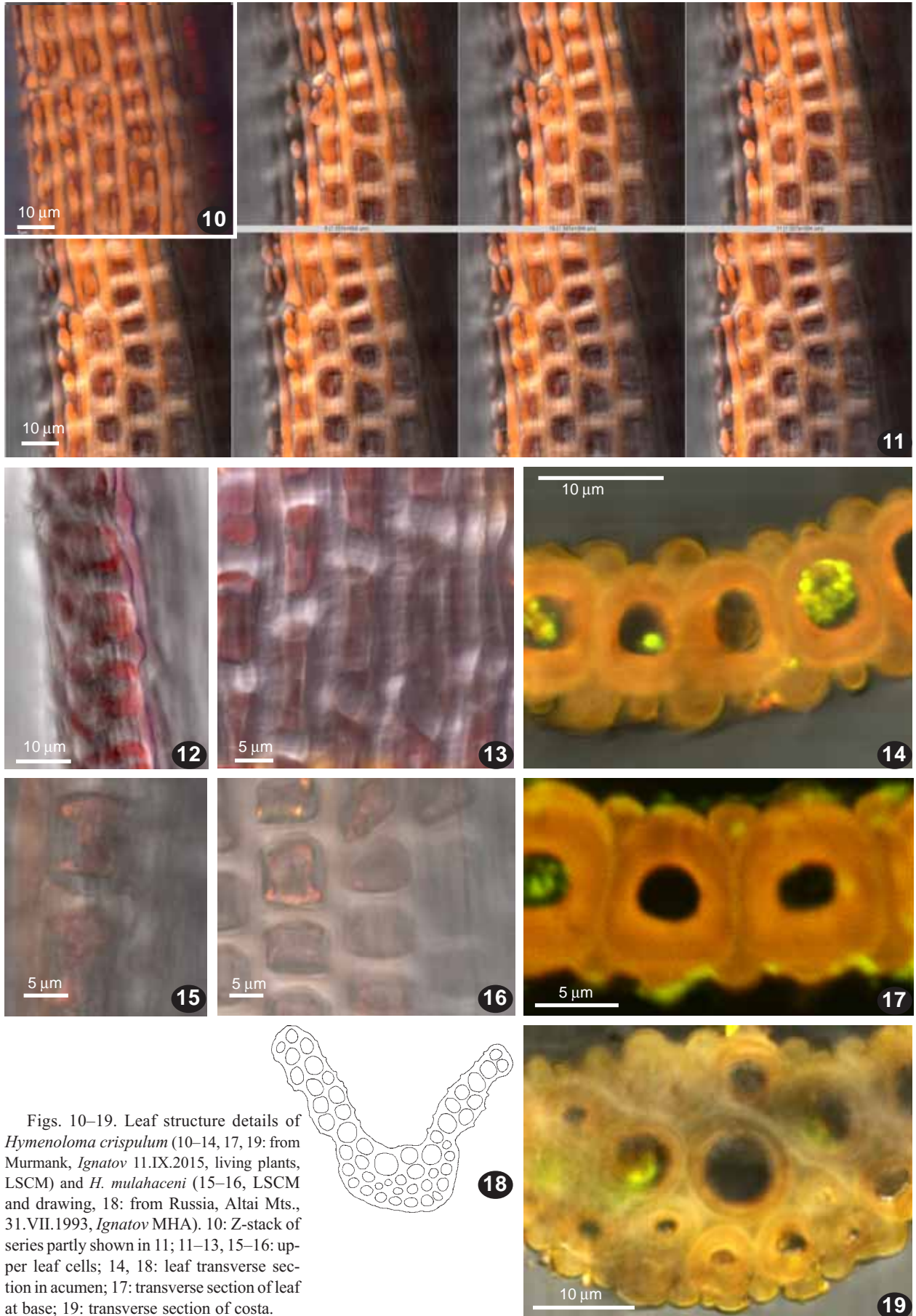
Some teeth have median line (arrowed in Fig. 29), however, judging from the ornamentation of the outer teeth surface, this median line is caused not by the 'pre-peristome' presence (a remnants of OPL, i.e., a strongly reduced exostome), but due to additional division in the primary peristomial layer (Fig. 29).

Peristome of *H. mulahaceni* differs in less papillose teeth that also often have oblique-longitudinal striolation (Fig. 30). Its elements are fused at base, being somewhat similar in this character to the peristome of *Drummondia*. In *Drummondia sinensis*, peristome is deeply inserted below the urn mouth, recurved, its elements are strongly fused laterally, so the number of 'teeth' is difficult to count, and it often exceeds 16 (Fig. 39).

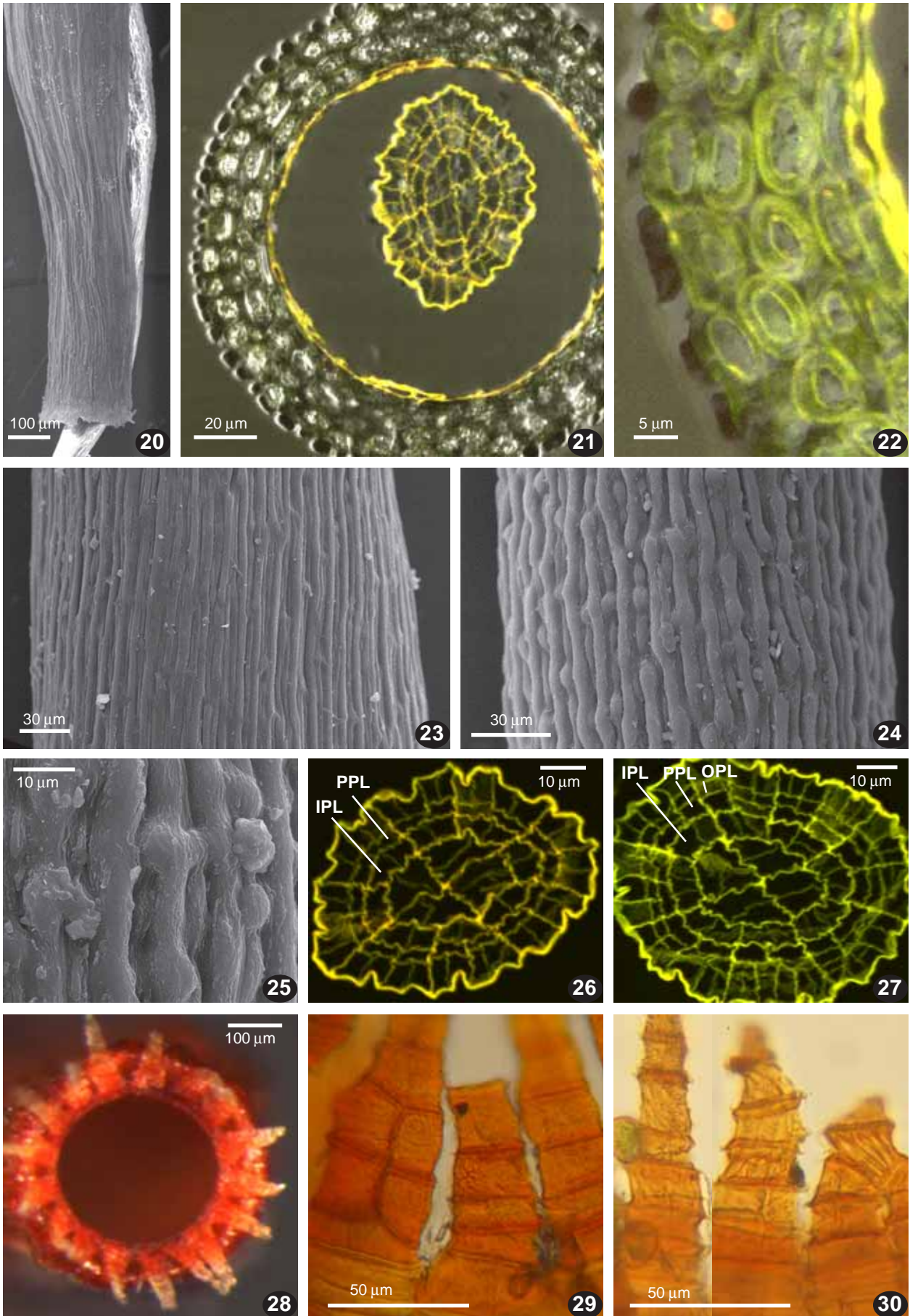
Hymenoloma crispulum peristome appeared to be quite variable in the degree of papillosity (Figs. 34–35 and 37–38). The sporophytes from even the same collection are quite variable in some cases, ranging from pale and slightly papillose to reddish and more strongly pap-

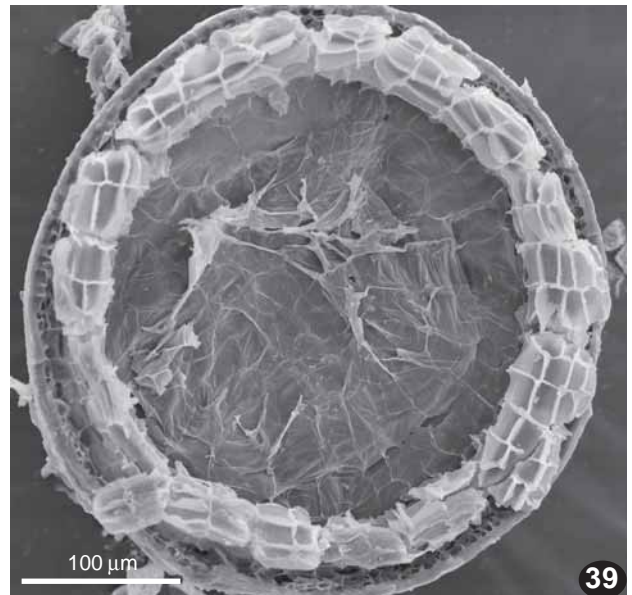
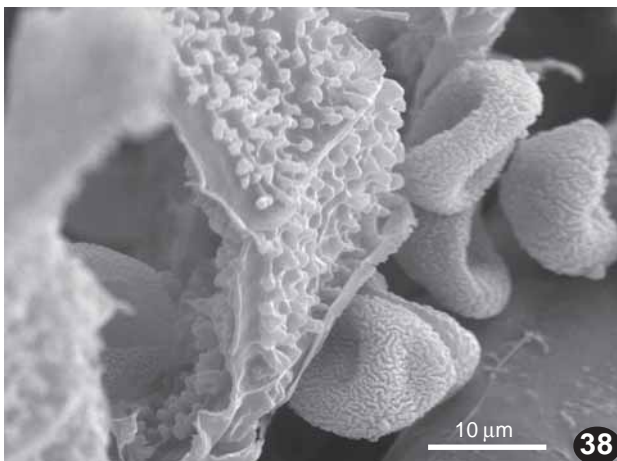
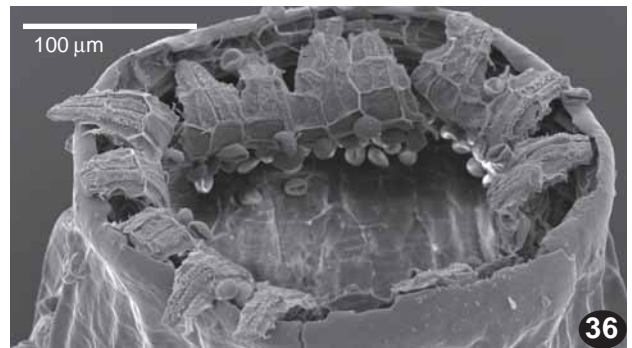
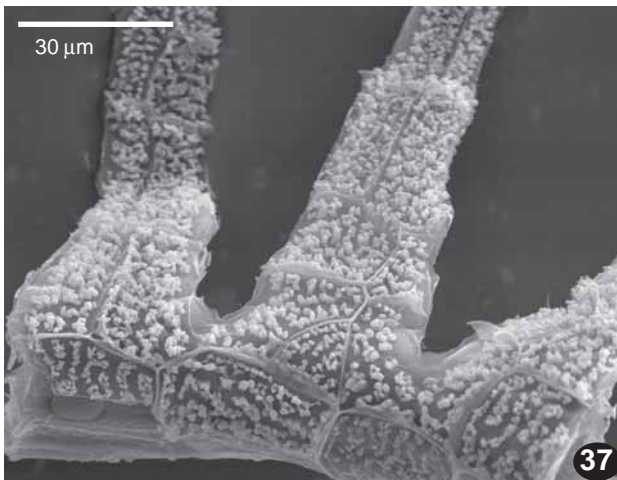
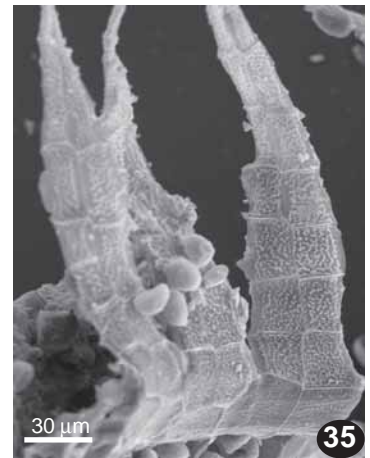
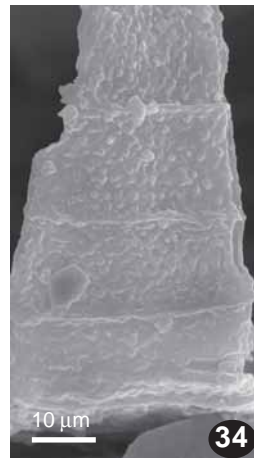
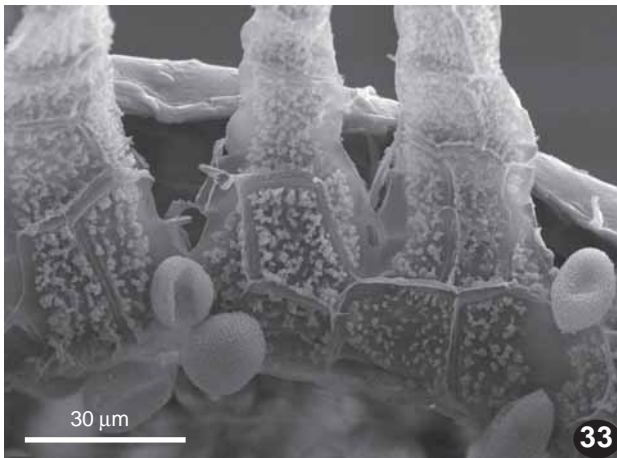
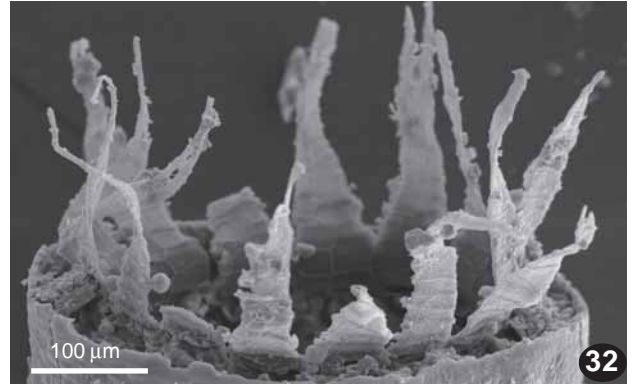
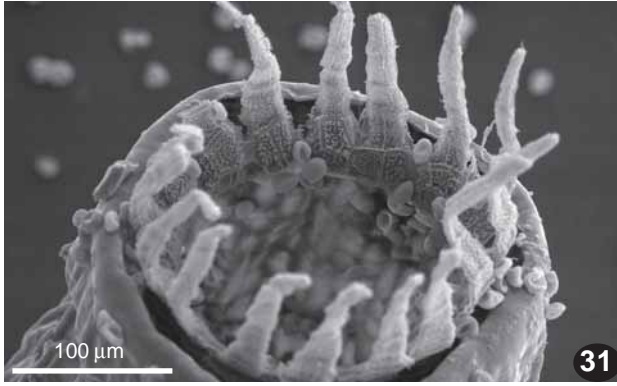


Figs. 2–9. SEM micrographs of leaf surface of *Hymenoloma crispulum* (from Yakutia, Ignatov & Ignatova 15-1232, MW). 2–3, 5, 7–8: ventral surface; 4, 6, 9: dorsal surface; 2–6, 9: from acumen; 7–8 from the leaf base.



Figs. 10–19. Leaf structure details of *Hymenoloma crispulum* (10–14, 17, 19: from Murmank, Ignatov 11.IX.2015, living plants, LSCM) and *H. mulhaceni* (15–16, LSCM and drawing, 18: from Russia, Altai Mts., 31.VII.1993, Ignatov MHA). 10: Z-stack of series partly shown in 11; 11–13, 15–16: upper leaf cells; 14, 18: leaf transverse section in acumen; 17: transverse section of leaf at base; 19: transverse section of costa.





illose. In general, the higher and more dense papillae occur in the recently opened capsules, while older peristomes tend to become less strongly papillose and paler in colour.

DISCUSSION

Results of our molecular phylogenetic reconstructions confirmed the previously published phylogenies (Tsubota *et al.*, 2003, 2004; Hedderson *et al.*, 2004; Stech *et al.*, 2012; Werner *et al.*, 2013; Inoue & Tsubota, 2014; Fedosov *et al.*, 2016), both in general topology of the main lineages of Bryopsida, and, particularly, of the phylogenetic position of *Hymenoloma*. The latter genus was found outside Dicranaceae, Rhabdoweisiaceae, Oncophoraceae, and Seligeriaceae, in a supported clade with *Scouleria* and *Drummondia*.

Scouleriaceae Churchill (Churchill, 1981) and Drummondiaaceae B. Goffinet (Buck & Goffinet, 2000), were recently established for these two genera previously assigned to Grimmiaceae and Orthotrichaceae correspondingly. Moreover, they were united in one order Scouleriales B. Goffinet & W.R. Buck because of a strong phylogenetic signal from the DNA sequences (Goffinet & Buck, 2004), despite the lack of any morphological synapomorphies. Thus, the placement of *Hymenoloma* in this order is not supported by morphology, as well as do not contradict it.

No one character of *Hymenoloma* is unique, although some features are uncommon in other mosses and their combination is diagnostic for the genus. Some comments on these characters are required.

The leaf surface striolation in *Hymenoloma* is well known. This handbook' character is seen at places, but not everywhere, which usually is referred to the difficulty of observation rather than to its real variation. There is a discrepancy in descriptions of this striolation by different authors. Leaf surface of *H. crispulum* is described as follow:

Limpricht (1894): only on dorsal surface with low papillae above the cell lumen, and also with still lower papillae above cell walls (translation from German, MI);

Nyholm (1987): finely longitudinally striated;

Ochyra *et al.* (2008a): appearing papillose in transverse section because of the long cuticular ridges (their illustrations are especially similar to our Figs. 14, 17, 19).

Schofield (2007): distal cells usually longitudinally striolate (appearing papillose in transverse section);

Smith (2004): smooth.

The present observation showed that likely a number of characters contribute to the result commonly seen under the light microscope. Cuticular ridges and papillae

in the upper part of leaf are both upon lumens and upon cell walls. The striolation in this leaf portion most likely corresponds to these structures. However, at the base of leaf, striolation looks even denser (ca. 1.5 μm in average from one 'ridge' to another); at the same time, in this part of leaf, the cuticular papillae above the lumen are absent. The explanation of striolation in the transitional zone from upper lamina to the base and within the base cannot refer to the cuticular ridges upon cell walls only, as the cell width here is no less than 10 μm . SEM images illustrate the ridges upon cuticle at ca. 1 μm one from another. We presume that in wet condition these striae may become slightly more spaced, to ca. 1.5 μm , thus fitting the pattern observed in light microscope.

Summing up, a specific character of *Hymenoloma* comprises cuticular structures arranged along the leaf length, and also striolation upon the cuticular surface itself.

Calyptra surface has principally the same sculpture pattern as the leaves. Longitudinal striolation is conspicuous due to the cuticular ridges, which position above cell walls and above cell lumens are quite indefinite. Similarly to the leaf surface, in the lower part of calyptra, the cuticular ridges are fewer and lower.

Peristome. In general, the overall structure of the peristome of *Hymenoloma* is haplolepidous, with the 4:2:3 peristomial formula, but numerous irregular divisions result in occasionally different number of teeth: 15 to 17 instead of 16 (Fig. 28), lack of haplolepidous '2:1 /1:2' pattern, irregular shape and additional divisions in primary peristomial layer (Fig. 29).

These exceptions might probably be considered not important, but the related groups, *i.e.*, *Drummondia* and *Scouleria* are known to have more than 16 teeth. In *Drummondia*, the basal membrane of laterally fused teeth bases produce short teeth; despite they are somewhat ambiguous for counting, we saw 17 in several studied capsules (Fig. 39). The ultimately increase in teeth number is seen in *Scouleria*, where the number was evaluated as 32, with the formula, omitting 'preperistomes' is 4:8 (Edwards, 1979; Churchill, 2007; Ignatova *et al.*, 2015). Further studies are needed to understand the variation in peristome element number in the Scouleriales.

Although the genus is rather small, the peristome structure in different species represents different types. In *H. mulahaceni* the longitudinal, though somewhat oblique, ridges occur on teeth from the outer side. This pattern is a characteristic of Dicranales and only rarely represented in Grimmiales (although there are exceptions, *e.g.*, in *Coscinodon yukonensis*, Ignatova *et al.*, 2008).

Figs. 20–38 (two previous pages). Calyptra and peristome structure of *Hymenoloma crispulum* (20–29, 31–35, 37–38), *H. mulahaceni* (30: Allen 1054OA, 36: Ignatov 31.VII.1993) and *Drummondia sinensis* (39): 20, 23–25 (SEM): calyptra, surface views. 21–22 (LSCM): transverse sections in upper part of the capsule and calyptra (22 is a close up of 21). 26–27 (LSCM): early stages of peristome development. 26: formula 2:2 with only slightly unequal divisions in IPL; 27: formula 4:2:3 in most sectors. 28 (stereomicroscope): living, peristome with 17 teeth. 29–30 (LM): teeth from outside: in 29 one tooth has median line, apparently due to additional division in PPL; in 30 some teeth have longitudinal ridges over some plates. 31–39 (SEM): presitome structure and ornamentation: 31–32, 36, 39: general view; 33, 35, 37: view from inside; 34, 38: view from outside.

Among the basal haplolepidous mosses it is well expressed in Distichaceae, but never reported from either *Drummondia*, or *Scouleria*.

The degree of peristome papillosity vary greatly, probably reflecting the time since capsule opening. This pattern is common with other mosses (e.g. *Schistidium*, *Grimmia*), although in *Hymenoloma* it is more conspicuous.

TAXONOMY

Regardless the weakness of the morphological delimitation, the phylogenetic signal from molecular markers requires an exclusion of *Hymenoloma* from both Dicranales and Grimmiiales (incl. Seligeriaceae), and placement it in the Scouleriales. Despite the absence of obvious morphological synapomorphies in *Hymenoloma*, it differs both from Seligeriaceae and most of other Grimmiiales in the complex anatomy of the costa, while the peristome sculpture lacking longitudinal striolation differs it from Dicranaceae s.l. Unstable pattern of PPL cell divisions, causing unstable number of peristome ‘teeth’ could be considered as a primitive character within the clade of haplolepidous mosses, thus correlating with results of the molecular-phylogenetic reconstructions. Particularly such deviations were observed not only within Scouleriales, but in *Catoscopium* as well (Ignatov *et al.*, 2015). However, a considerable morphological and molecular distinction from both Scouleriaceae and Drummondiaceae leaves no other possibility than to describe a new family.

Hymenolomataceae Ignatov et Fedosov, fam. nov.

Type: *Hymenoloma* Dusén, Ark. Bot. 4(1). 1878.

Included genus: *Hymenoloma* Dusén

Type species: *Hymenoloma nordenskjöldii* Dusén (= *Hymenoloma antarcticum*)

Other included species: *Hymenoloma crispulum*, *H. mulahaceni*, *H. antarcticum*.

Plants glaucous-green. Stem with simple 2–4 subterminal branches. Leaves flexuose to crisped when dry, concave, from ovate base narrowed into lanceolate acumen, acuminate; margins plane or narrowly recurved, entire or minutely denticulate distally; costa percurrent, in transverse section with conspicuous guide cells, dorsal and ventral stereid bands; laminal cells quadrate to short rectangular above, elongate in basal part, moderately thick-walled, on both surfaces in distal leaf portion with irregular low cuticular papillae, on ventral surface with longitudinal cuticular ridges, occasionally smooth. Autoicous. Perichaetial leaves clasping seta base. Seta long. Capsule erect, cylindrical; annulus of 2–3 rows of large cells, persistent. Peristome single, haplolepidous, with apparent or inapparent ‘2:1 / 1:2 pattern’ on the inner surface of endostome teeth, without high trabeculae on both surfaces, occasionally with low remnants of exostome, smooth to variously papillose or with oblique-longitudinal striolation. Calyptrae papillose. Spores small to large, 12–27 µm.

CONSIDERATIONS ON ECOLOGY OF SCOULERIALES

Hymenoloma has been found in a clade that previously was known to combine two monogeneric families, Drummondiaceae and Scouleriaceae¹. When found for the first time, the position in one clade of highly specialized epiphytic *Drummondia* and aquatic *Scouleria* looked an unexplained anomaly.

Now, retrieving molecular phylogenies, one may find however a number of cases where epiphytic and aquatic mosses appear surprisingly more closely related than one might expect. The following examples are conspicuous in this respect:

(1) Fontinalaceae is the moss family most specialized to aquatic environment. In all-pleurocarp phylogeny, Fontinalaceae form a strongly supported clade with Habrodontaceae (Huttunen *et al.*, 2012); the latter family includes a single species, *H. perpusillus* (De Not.) Lindb., a small epiphytic plant of the Mediterranean.

2) Amblystegiaceae was usually circumscribed as a group with perfect peristomes (Buck & Goffinet, 2000), until molecular phylogenetic analysis placed *Anacamptodon* in this family, instead of Fabroniaceae where it was classified during the 20th century (Brotherus, 1925). The strongly specialized, or reduced, peristome in *Acamptodon* undoubtedly is the most specialized in the Amblystegiaceae. Within the latter, *Anacamptodon* forms a clade with *Hygrohypnum luridum* (Hedw.) Jenn., a subaquatic species (Vanderpoorten *et al.*, 2002).

3) Within the Brachytheciaceae, the most well-known aquatic plants were classified in the genus *Platyhypnidium*, later proved to be nested in *Rhynchostegium* (Huttunen & Ignatov, 2010) together with the genus *Eriodon*, one of the most specialized epiphytes in the Brachytheciaceae, having very long and somewhat twisted peristome.

Summing up these examples, the ‘most aquatic’ mosses in the mentioned families are often sister to the most specialized epiphytic species, *i.e.*, having the most strongly modified peristome. Otherwise, the ‘most epiphytic’ species can link up as a sisters to the ‘aquatic’ lineages. Such rule can unlikely be proved statistically, mainly due to a low number of obviously aquatic mosses in the world. Also, there are at least some exceptions: *Fissidens fontanus* among Fissidentaceae and *Cinclidotus* spp. among Pottiaceae are not known to be associated with any epiphytic groups, as far as we know.

However, considering these cases, the affinity of aquatic *Scouleria* and epiphytic *Drummondia* may not look that anomalous. They both are escapers from the terrestrial environments where most moss species grow. Comparing with epiphytic mosses, ones growing on wet rocks look much closer to generally epilithic aquatic *Scouleria* in terms of ecology. Instead, the examples of groups,

¹ – Goffinet *et al.* (2009) included in the Scouleriaceae the genus *Tridontium* Hook f., but our preliminary data indicate in position outside this family.

which inhabit both rocks and tree trunks are so numerous, they likely do not need in additional comments.

At this point, a plasticity of *Hymenoloma* can be considered. Although the present analysis placed *Hymenoloma* in the basal position in Scouleriales-clade, a slightly different sampling in the similar analysis in Fedosov *et al.* (2016) found *Hymenoloma* to be sister to *Scouleria*+*Drummondia* (although the clade of two latter genera lacks support).

Hymenoloma crispulum is primarily an epilithic moss, but in cold regions of the world it successfully grows on soil near snow beds and on mineral soil in tundra. However, the type specimen of the type species of *Hymenoloma* has been collected on tree trunk; and in NW Russia *H. crispulum* occasionally grows on trunks of *Betula*, *Sorbus*, *Juniperus* and *Salix*. At the same time, this species also can grow on rocks along brooks and creeks, experiencing temporary flooding. It occasionally grows on rocky bottoms of temporarily inundated brooks and in pools near melting snow beds.

The above mentioned facts on ecology may provide a look of the Scouleriales as of a less unnatural aggregate.

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LITERATURE CITED

- ANDERSON, L.E., H.A. CRUM & W.R. BUCK. 1990. List of mosses of North America north of Mexico. – *Bryologist* **93**: 448–499.
- BROTHERUS, V.F. 1924. Musci. – In: Engler, A. & K. Prantl (eds.). *Die Natürlichen Pflanzenfamilien* (ed. 2). 10. Berlin, Duncker and Humblot: 143–478.
- BROTHERUS, V.F. 1925. Musci. — In: A. Engler & K. Prantl (eds.). *Die Natürlichen Pflanzenfamilien* (ed. 2). 11. Leipzig, W. Engelmann: 1–522.
- BUCK, W.R. & B. GOFFINET. 2000. Morphology and classification of mosses. – In: Shaw, A. J. & B. Goffinet (eds.). *Bryophyte biology*. Cambridge University Press, Cambridge, UK: 71–123.
- CHURCHILL, S.P. 1981. A phylogenetic analysis, classification and synopsis of the genera of the Grimmiaceae (Musci). – In: V. A. Funk & D. R. Brooks (eds.) *Advances in cladistics*, 1. New York Botanical Garden, New York. New York Botanical Garden: 127–144.
- CHURCHILL, S. 2007. Scouleriaceae. – In: *Flora of North America Editorial Committee* (eds.). *Flora of North America North of Mexico*, Vol 27: Bryophyta: Mosses, part 1. Oxford University Press, New York, NY: 311–313.
- DUSÉN, P.K.H. 1905. Beiträge zur Bryologie der Magellansländer, von Westpatagonien und Südchile. 2. – *Arkiv för Botanik utgivet av K. Svenska Vetenskapsakademien* **4** (1): 1–45, Tafl. 1–11.
- EDWARDS, S.R. 1979. Taxonomic implications of cell patterns in haplolepidaceous moss peristomes. – In: Clarke, G.C.S. & J.G. Duckett (eds.). *Bryophyte systematics*. Academic Press, London, UK.: 317–346.
- EDWARDS, S.R. 1984. Homologies and inter-relations of moss peristomes. – In: Schuster, R.M. (ed.) *New manual of bryology*, vol. 2. Hattori Botanical Laboratory, Nichinan, Japan: 658–695.
- FEDOSOV, V.E., A.V. FEDOROVA, A.E. FEDOSOV & M.S. IGNATOV. 2016. Phylogenetic inference and peristome evolution in haplolepidaceous mosses, focusing on Pseudoditrichaceae and Ditrichaceae s. l. – *Botanical Journal of the Linnean Society* **181**: 139–155.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution* **39**: 783–791.
- FREY, W. & M. STECH. 2009. Bryophyta (Musci, mosses). – In: Frey, W. (ed.). *Syllabus of plant families A. Engler's Syllabus der Pflanzenfamilien. Part 3. Bryophytes and seedless vascular plants*. 13th ed.. Gebr. Borntraeger Verlagsbuchhandlung, Stuttgart, Germany: 116–257.
- GARDINER, A., M. IGNATOV, S. HUTTUNEN & A. TROITSKY. 2005. On resurrection of the families Pseudoleskeaceae Schimp. and Pylaisiaceae Schimp. (Musci, Hypnales). – *Taxon* **54**: 651–663.
- GOFFINET, B. & W.R. BUCK. 2004. Systematics of the Bryophyta (Mosses): from molecules to a revised classification. – In: Goffinet, B., V. Hollowell & R. Magill (Eds.) *Molecular Systematics of Bryophytes*. Missouri Botanical Garden, St. Louis: 205–239.
- GOFFINET, B., W.R. BUCK & A.J. SHAW. 2009. Morphology, anatomy, and classification of the Bryophyta. – In: Goffinet, B. & A.J. Shaw (eds.). *Bryophyte biology*, 2nd edn. Cambridge: Cambridge University Press: 55–138.
- HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucler Acids Symposium Series* **41**: 95–98.
- HEDDERSON, T.A., D.J. MURRAY, C.J. COX & T.L. NOWELL. 2004. Phylogenetic Relationships of Haplolepidaceous Mosses (Dicranidae) Inferred from rps4 Gene Sequences. – *Systematic Botany*, **29**: 29–41.
- HUELSENBECK, J. & F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. – *Bioinformatics* **17**: 754–755.
- HUTTUNEN, S., N. BELL, V.K. BOBROVA, V. BUCHBENDER, W.R. BUCK, C.J. COX, B. GOFFINET, L. HEDENAS, B.-C. HO, M.S. IGNATOV, M. KRUG, O. KUZNETSOVA, I.A. MILYUTINA, A. NEWTON, S. OLSSON, L. POKORNY, J.A. SHAW, M. STECH, A. TROITSKY, A. VANDERPOORTEN & D. QUANDT. 2012. Disentangling knots of rapid evolution: origin and diversification of the moss order Hypnales. – *Journal of Bryology* **34**: 187–211.
- HUTTUNEN, S. M. & M. S. IGNATOV. 2010. Evolution and taxonomy of aquatic species in the genus *Rhynchostegium* (Brachytheciaceae, Bryophyta). – *Taxon* **59**(3): 791–808.
- IGNATOV, M.S., O.M. AFONINA, E.A. IGNATOVA ET AL. 2006. Check-list of mosses of East Europe and North Asia. – *Arctoa* **15**: 1–130.
- IGNATOV, M.S., U.N. SPIRINA, E.A. IGNATOVA, M. KRUG & D. QUANDT. 2015. On the systematic position of the moss genus *Catoscopium* with a new approach to the peristome reduction study. – *Arctoa* **24**(2): 389–415.
- IGNATOVA, E. A., L. E. KURBATOVA, O. I. KUZNETSOVA, O. V. IVANOV, J. R. SHEVOCK, B. E. CARTER & M. S. IGNATOV. 2015. The genus *Scouleria* (Bryophyta) in Russia revisited. – *Arctoa* **24**(1): 47–66.
- INOUE, Y. & H. TSUBOTA. 2014. On the systematic position of the genus *Timmella* (Dicranidae, Bryopsida) and its allied genera, with the description of a new family Timmiellaceae. – *Phytotaxa* **181**: 151–162.
- LANFEAR, R., B. CALCOTT, S.Y.W. HO & S. GUINDON. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. – *Molecular Biology and Evolution* **29** (6): 1695–1701.

- LIMPRICHT, K.G. 1894. Die Laubmoose Deutschlands, Oesterreichs und der Schweiz. 1 Abt. – *Eduard Kummer, Leipzig*.
- NYHOLM, E. 1987. Illustrated flora of Nordic mosses. Fasc. 1. Fissidentaceae–Seligeriaceae. – *Copenhagen & Lund, Nordic Bryological Society: 1–74*.
- OCHYRA, R. 1993. Antipodal mosses: I. A revision of the genus *Holodontium* (Seligeriaceae). – *Fragmenta Floristica et Geobotanica* **38**: 75–98.
- OCHYRA, R. 1998. The moss flora of King George Island, Antarctica. – *Kraków, Institute of Botany, Polish Academy of Sciences, 278 pp*.
- OCHYRA, R., J. ŻARNOWIEC & H. BEDNAREK-OCHYRA. 2003. Census Catalogue of Polish Mosses. – *Biodiversity of Poland 3. Kraków, Institute of Botany, Polish Academy of Sciences, 372 pp*.
- OCHYRA, R., R.I. LEWIS SMITH & H. BEDNAREK-OCHYRA. 2008a. The illustrated moss flora of Antarctica. – *Cambridge, Cambridge University Press*.
- OCHYRA, R., A. STEBEL & H. BEDNAREK-OCHYRA. 2008b. The genus *Hymenoloma* (Bryophyta, Seligeriaceae) in the Polish Carpathians. – In: *Stebel A. & R. Ochyra (eds). Bryophytes of the Polish Carpathians. Poznań, Sorus: 211–225*.
- RAMBAUT, A. & A.J. DRUMMOND. 2007. Tracer. – *Computer program and documentation distributed by the author, website http://beast.bio.ed.ac.uk/Tracer*.
- REIMERS, H. 1936. Über die Laubmoosgattungen *Bryopteris* Thér., *Camptodontium* Dus., *Hymenoloma* Dus., *Verrucidens* Card. und ihre systematische Stellung. – *Notizblatt des Königlichen botanischen Gartens und Museums zu Berlin* **13**(116): 36–52.
- ROS, R.M., V. MAZIMPAKA, U. ABOU-SALAMA, M. ALEFFI, T.L. BLOCQUEL, M. BRUGUÉS, R.M. CROS, M. G. DIA, G.M. DIRKSE, I. DRAPER, W. EL-SAADAWI, A. ERDAĐ, A. GANEVA, R. GABRIEL, J.M. GONZÁLEZ-MANCEBO, C. GRANGER, I. HERRNSTADT, V. HUGONNOT, K. KHALIL, H. KÜRSCHNER, A. LOSADA-LIMA, L. LUÍS, S. MIFSUD, M. PRIVITERA, M. PUGLISI, M. SABOVLJEVIĆ, C. SÉRGIO, H.M. SHABBARA, M. SIM-SIM, A. SOTIAUX, R. TACCHI, A. VANDERPOORTEN & O. WERNER. 2013. Mosses of the Mediterranean, an annotated checklist. – *Cryptogamie, Bryologie* **34**(2): 99–283.
- SCHOFIELD, W.B. 2007. *Dicranoweisia*. – In: *Flora of North America Editorial Committee (eds.). Flora of North America North of Mexico, Vol 27: Bryophyta: Mosses, part 1. Oxford University Press, New York, NY: 395–396*.
- SMITH, A.J.E. 2004. The moss flora of Britain and Ireland. 2 ed. – *Cambridge, Cambridge University Press, 1012 pp*.
- STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. – *Bioinformatics* **22**: 2688–2690.
- STECH, M., S.F. MCDANIEL, R. HERNANDEZ-MAQUEDA, R.M. ROS, O. WERNER, J. MUÑOZ & D. QUANDT. 2012. Phylogeny of haplolepidous mosses – challenges and perspectives. – *Journal of Bryology* **34** (3): 173–186.
- TSUBOTA, H., Y. AGENO, B. ESTÉBANEZ, T. YAMAGUCHI & H. DEGUCHI. 2003. Molecular phylogeny of the Grimmiaceae (Musci) based on chloroplast *rbcL* sequences. – *Hikobia* **14**: 55–70.
- TSUBOTA, H., E. DE LUNA, D. GONZÁLEZ, M.S. IGNATOV & H. DEGUCHI. 2004. Molecular phylogenetic and ordinal relationships based on analyses of a large-scale data set of 600 *rbcL* sequences of mosses. – *Hikobia* **14**: 149–170.
- WERNER, O., S. RAMS, J. KUČERA, J. LARRAÍN, O.M. AFONINA, S. PISA & R.M. ROS. 2013. New data on the moss genus *Hymenoloma* (Bryophyta), with special reference to *H. mulahaceni*. – *Cryptogamie, Bryologie* **34**(1): 13–30.
- VANDERPOORTEN, A., L. HEDENÄS, C. J. COX & A. J. SHAW. 2002. Phylogeny and morphological evolution of the Amblystegiaceae (Bryopsida). – *Molecular Phylogenetics and Evolution* **23**: 1–21.

Appendix 1. Species studied with GenBank accession numbers for *nad5/rps4/trnL*. Newly obtained sequences include specimen voucher information. *Blindia acuta* AY908928/ JQ890483/ AF023721; *Bryoxiphium norvegicum* **AY908957/ AF231267/ KX446938** **Iceland, B. Jóhannsson 2-805 (ICEL)**; *Bryum pseudotriquetrum* DQ640127/ JF277327/ AY150357; *Catoscopium nigrum* AY908927/ AF307001/ AF497128; *Ceratodon purpureus* AY908862/ AB848717/ AF435310; *Dicranoweisia cirrata* **KX452407** / AF231274/ AF478333 **Krasnodar Territory, Anapa Distr., Malyj Utrish, Ignatov & Ignatova 05-348 (MW)**; *Dicranum scoparium* AY908884/ AF234158/ KF424001; *Diphyscium foliosum* AY312874/ AF223034/ AF229891; *Distichium capillaceum* AY908786/ AB853082/ AF435326; *Distichium inclinatum* KR026969/ AB914715/ AF435327; *Drummondia obtusifolia* AY908926/ AF223038/ AF229895; *Drummondia prorepens* –/ AF306977/ JQ690728; *Drummondia sinensis* **KX369286 / KX369281**/ FJ572458 **Russia, Primorsky Territory, Ignatov et al. 13-1792 (MW)**; *Encalypta streptocarpa* AJ622818/ AF478282/ EU186541; *Flexitrichum flexicaule* KR026965/ AJ554007/ AF231247; *Funaria hygrometrica* Z98959/ JN088980/ JN088948; *Grimmia plagiopodia* AY908919/ AY908144/ AJ879761; *Hedwigia ciliata* AY908380/ AF478289/ LN714327; *Hookeria lucens* AY908489/ AY306930/ AY306764; *Hymenoloma crispulum 1* AY908925/ AY908164/ JX123832; *Hymenoloma crispulum 2* **KX369287/ KX369279**/ JX123830 **Yakutia, Chersky Mts, Ignatov & Ignatova 15-1232 (MW)**; *Hymenoloma mulahaceni 1* **KX369289/ KX369280**/ JX123836 **Altai Mts, 31.VII.1993, Ignatov s.n.**; *Hymenoloma mulahaceni 2* **KX369288**/ JX123862/ JX123835 **Kazakhstan, Alma-Ata, 9.VII.1991, Allen 10540A (MHA)**; *Oreas martiana* AY908892/ AY908084/ AF435342; *Paraleucobryum enerve* AY908883/ AY908106/ AF231184; *Pleurochaete squarrosa* AY908854/ AY950373/ GU953730; *Ptychomitrium gardneri* AY908951/ AF231290/ AF023719; *Rhabdoweisia crispata* AY908966 / AF222899/ AF231259; *Schistidium apocarpum* AY908920/ JQ040708/ GQ428079; *Scouleria aquatica* AY312887/ AF023780/ AF023723; *Scouleria pulcherrima* **KX369284/ KX369282/ KX446936** **Russia, Irkutsk Province, Vitim, Mamontov 321/I (LE)**; *Scouleria rschewinii* **KX369285/ KX369283/ KX446937** **Russia, Irkutsk Province, Vitim, Mamontov 464/I (LE)**; *Seligeria pusilla* **KR026971/ KR026960/ KX387262** **Russia, Dagestan, Ignatov & Abakarova 11-31 (MW)**; *Seligeria recurvata* **KX086711/ KX086707/ KX387259** **Russia, Dagestan, Gunib, Ignatov & Ignatova 09-604 (MW)**; *Timmia anomala* AY908958/ AB914721/ **KX446934** **Cyprus, Seregin s.n. (MW)**; *Trematodon longicollis* AY908865/ AY908087/ AF435352; *Trichodon cylindricus* AY908863/ AY908125/ **KX446935** **Russia, Moscow Province, Fedosov 13-1-3 (MW)**.