New data on the moss genus *Hymenoloma* (Bryophyta), with special reference to *H. mulahaceni*

Olaf WERNERA, Susana RAMSB, Jan KUČERAC, Juan LARRAÍNDb, Olga M. AFONINNE, Sergio PISAA & Rosa María ROSA*

A Departamento de Biología Vegetal (Botánica), Universidad de Murcia, Campus de Espinardo, E-30100 Murcia, Spain

bEscuela Universitaria de Magisterio “La Inmaculada”, Universidad de Granada, Carretera de Murcia s/n, 18010 Granada, Spain

cUniversity of South Bohemia, Faculty of Science, Branišovská 31, CZ - 370 05 České Budějovice, Czech Republic

dUniversidad de Concepción, Departamento de Botánica, Casilla 160-C, Concepción, Chile

eKomarov Botanical Institute of Russian Academy of Sciences, Professor Popov Str. 2, St.-Petersburg 197376, Russia

Abstract – A molecular and morphological study using two chloroplast molecular markers (*rps4* and *trnL-F*) was carried out with specimens belonging to *Hymenoloma mulahaceni*, a species described at the end of the 19th century from the Sierra Nevada Mountains in southern Spain as a member of *Oreoweisia*. The comparison with Asian, European, and North American material of *Dicranoweisia intermedia* proved the conspecificity of both taxa, which was corroborated by molecular data. Therefore, the distribution area of *H. mulahaceni* is extended to U.S.A., Canada, Greenland, and several Asian countries (Armenia, Georgia, Tajikistan, and Uzbekistan). We also tested the monophyly of *Hymenoloma sensu* Ochyra et al. (2003), by including in the analysis the Holarctic taxa assigned to the genus together with Chilean material identified as *H. antarcticum* (putatively synonymous with the type of *Hymenoloma*) and *H. crispulum*. Phylogenetic analysis of basal haplolepidous taxa taking into account different genera, mainly of the Dicranales but also of Bryoxiphiales and Scouleriales, confirmed the monophyly of *Hymenoloma* and suggested a close relationship with the Scouleriaceae sensu Hedderson et al. (2004), while *Dicranoweisia* was resolved within Rhabdoweisiaceae. Molecular data helped us to show that *Hymenoloma brevipes*, morphologically closely related to *H. crispulum*, is a distinct taxon from *H. antarcticum*. This challenges the earlier records of *H. crispulum* from the Southern Hemisphere but a comprehensive revision is necessary to confirm its status in the region. A key to the genera *Dicranoweisia* and *Hymenoloma* and the European species of *Hymenoloma* is included.

Musci / Haplolepidous mosses / Dicranidae / *Hymenoloma* / *Dicranoweisia* / *Hymenoloma mulahaceni* / taxonomy / Asia / Europe / South America / North America / *rps4* and *trnL-F*
The originally monotypic Patagonian genus *Hymenoloma* Dusén has been amended substantially by Ochyra *et al.* (2003) in the course of a re-assessment of the circumscription of the morphologically heterogeneous *Dicranoweisia* S.O. Lindberg *ex* Milde. They suggested distinguishing the two morphologically distinct groups within *Dicranoweisia* at generic level, retaining only *D. cirrata* (Hedw.) Lindb. *ex* Milde and *D. africana* Dixon in *Dicranoweisia*, while moving the taxa of the “*Dicranoweisia crispula* complex” to the genus *Hymenoloma*, with which this complex was obviously morphologically closely related. Twenty-one new specific combinations were made in *Hymenoloma*. In later treatments, Ochyra *et al.* (2008a, b) summarized the current knowledge on the genus. They included it in the family Seligeriaceae and established that it consisted of about eight species worldwide, most of them restricted to the Southern Hemisphere. The austral taxa described from regions outside of the scope of the above mentioned Flora Antarctica (Ochyra *et al.*, 2008a) are however still in need of a careful taxonomic reassessment, as demonstrated e.g. in the treatment of *H. antarcticum* (Müll. Hal.) Ochyra (Ochyra *et al.*, 2008a), which was found to be synonymous with *H. nordenskjoeldii* Dusén (the type species of the genus), *H. brevipes* (Müll. Hal.) Ochyra, and *H. brevisetum* (Cardot) Ochyra and other taxa from different genera. *Hymenoloma brevipes* was understood to represent a mostly distinct phenotype, yet overlapping with the typical *H. antarcticum*.

Holarctic species of *Hymenoloma*, all of them present in Europe, include the widely accepted *H. compactum* (Schwägr.) Ochyra, *H. crispulum* (Hedw.) Ochyra (including the doubtfully distinct American *H. conterminum* (Renauld & Cardot) Ochyra), and the little known *H. mulahaceni* (Höhn.) Ochyra, first included in the genus by Ochyra *et al.* (2008b).

*Hymenoloma mulahaceni* was described under the name *Oreoweisia mulahaceni* Höhn, upon material collected in Sierra Nevada (South Spain). After the revision of the material kept at Höhnel’s herbarium, Schiffner (1904) confirmed the uniqueness of the species and its affinity with other *Oreoweisia* species, but later Casares Gil (1914, 1932) considered it to be only a poorly developed form of *Cynodontium bruntonii* (Sm.) Bruch & Schimp. The latter opinion prevailed and hence the species was forgotten for the following 100 years until the type of the species (at that time the only known material) was studied in the framework of the PhD. thesis of S. Rams (Rams Sánchez, 2007). It was concluded (Rams & Ros, 2006) that the taxon was conspecific with *Dicranoweisia cirrata* (Hedw.) Lindb. *ex* Milde, based on the morphological similarity, particularly the presence of pluricellular axillary gemmae characteristic of that species (Mönkemeyer, 1927; Smith, 2004), although some differences were observed between *O. mulahaceni* and *D. cirrata*, including the saxicolous habitat and the plane leaf margins. Nevertheless, the preliminary molecular analysis supported the unexpected affinity with *Hymenoloma crispulum* (Rams Sánchez, 2007). Ochyra *et al.* (2008b) mentioned without additional comments the identity of *H. mulahaceni* with *Dicranoweisia intermedia* J.J. Amann, which had been illegitimately included in *Hymenoloma* by Ochyra *et al.* (2003). Amann’s species has not been generally recognized in Europe, which might be the reason why there are, to our knowledge, no other European records of this taxon. Even in Switzerland, from where the species was described, it is regarded as a synonym of *Hymenoloma crispulum* (e.g. National inventory of Swiss Mosses, 2012). On the other hand, Russian bryologists have...
recognized the taxon (as *Dicranoweisia intermedia*) since Abramova & Abramov (1972) correctly described and emphasized the characters of the species, and this has led to a comparatively good knowledge of its distribution in northern Asia.

The molecular heterogeneity to be found in the genus *Dicranoweisia s.l.* was first supported by the data of Hedderson *et al.* (2004). Their phylogenetic reconstructions based on chloroplast *rps4* gene sequences showed that *D. cirrata* was phylogenetically closely related to *Rhabdoweisia crispata* (Dicks. ex With.) Lindb. and other taxa of Rhabdoweisiaceae Limpr. In contrast, *D. crispula* (Hedw.) Milde showed affinity to another group of species, which formed a basal clade within haplolepidous mosses, called “proto-haplolepidae”. The taxa most closely related to *D. crispula* were *Drummondia prorepens* (Hedw.) E. Britton and *Scouleria aquatica* Hook., and the authors suggested that they were both members of the Scouleriaceae, despite the obvious morphological differences among them. The molecular affinity of *Scouleria* and *Drummondia* was first revealed by Goffinet & Cox (2000) and Cox *et al.* (2000). In a later study based on a wider sampling, the *Scouleria/Drummondia* clade was consistently resolved near the base of a large lineage including the Timmiaceae, Encalyptineae and Funariineae (Goffinet *et al*., 2001), which was essentially confirmed by the large multi-gene phylogeny of mosses by Cox *et al.* (2010). Stech *et al.* (2012) resolved *Hymenoloma crispulum* as being closely related to *Drummondia* within the “protohaplolepidous” clade, based on a novel combination of non-coding chloroplast markers, although they did not include *Scouleria*. The synonymy of Scouleriaceae and Drummondiaceae, proposed by Hedderson *et al.* (2004), has been never generally accepted, as it can be observed, for instance, in the synopses of Goffinet *et al.* (2008) and Frey & Stech (2009) where both families are separately listed.

The collection of fresh material of *Oreoweisia mulahaceni* in the vicinity of the type locality 100 years after its description, allowed us to carry out a molecular study and contribute new data on the species and also on the genus *Hymenoloma*, for which molecular sequences have until now only been obtained from *H. crispulum*. Objectives of the study were (1) to assess the molecular identity and phylogenetic relationships of *H. mulahaceni*, particularly with respect to the other European species and the South American type of the genus, (2) to verify its conspecificity with *Dicranoweisia intermedia*, proposed by Ochyra *et al.* (2008b), and revise morphological characters of *H. mulahaceni* and its world distribution, and finally (3), to assess the molecular circumscription of the genus *Hymenoloma* as re-defined by Ochyra *et al.* (2003), particularly with respect to the genera *Dicranoweisia*, *Cynodontium* and *Oreoweisia*.

**MATERIALS AND METHODS**

**Plant Material**

For DNA extraction, we used eight samples of *Hymenoloma mulahaceni*, two from Spain (Sierra Nevada), one from USA (Alaska), and five from Russia (Altai, Chukotka, Kamchatka), the last six originally identified as *Dicranoweisia intermedia*; four European samples of *H. crispulum*, one of *H. compactum*, and four samples of *Hymenoloma* from the extreme south of Chile, one of them corresponding according to Ochyra *et al.* (2008a) to *H. antarcticum* (typical
phenotype), and three of them corresponding to *H. antarcticum* (*brevipes* phenotype), from now on named *H. antarcticum* and *H. brevipes* respectively (all the data related to the origin of these specimens and their original identifications are indicated in the Appendix).

To assess the broader phylogenetic context, and to test the earlier hypotheses on the relations of *Hymenoloma mulahaceni* with *Cynodontium* or *Oreoweisia*, we also included two specimens of *C. bruntonii* (Sm.) Bruch et Schimp., and one of *O. torquescens* (Hornsch. ex Brid.) Wijk et Margad., and completed the selection with sequences obtained from GenBank among those species belonging to Dicranales, Bryoxiphiales and Scouleriales from which *rps4* and *trnL-F* sequences were available. *Orthotrichum jettae* B.H. Allen was used to root the trees. In an initial phase we tested additional outgroup species, but in the final version these were not included, because of the problematic alignment of the most variable parts of the *trnL* intron and the poorer resolution within the *Hymenoloma* clade due to large indels in this region. The low support for many of the basal clades of the haplolepidous mosses has also been observed by Stech et al. (2012). GenBank accession numbers and specimen details are summarized in the Appendix, together with other specimens of *Hymenoloma mulahaceni* used for the morphological study.

**DNA isolation and amplification of chloroplast regions**

Total DNA was extracted from dry herbarium material using the NaOH extraction method as explained in Werner et al. (2002). The partial *rps4* gene was amplified using the primers *rps5* (occupying the first positions of the *rps4* gene, Nadot et al., 1995) and *trnas* (Buck et al., 2000) at a final concentration of 400 nM. For the amplification of the *trnL-F* region the primers C and F of Taberlet et al. (1991) were used, adding 4 μl of stock DNA as template, 200 μM of each dNTP, 2 mM MgCl₂, 2.3 units Taq polymerase (OncorAppligene), 1 μl BLOTTO (10% skimmed milk powder and 0.2% NaN₃ in water) and the buffer provided by the enzyme supplier were added. BLOTTO attenuates PCR inhibition caused by plant compounds (De Boer et al., 1995). The amplification conditions were as follows: 3 min at 94°C, 35 cycles with 30 sec at 94°C, 30 sec at 50°C and 1 min at 72°C, and a final 7 min extension step at 72°C. Amplification products were controlled on 1% agarose gels and successful reactions were cleaned with the help of the GenElute PCR Clean-Up Kit (Sigma-Aldrich). Cycle sequencing was performed with the amplification primers, using a standard protocol at the facilities of Secugen (Madrid).

**Data analysis**

The sequences were edited using Bioedit 5.0.9 (Hall, 1999) and aligned manually. As discussed in Quandt et al. (2003) and Quandt & Stech (2005), the known hairpin associated inversion residing in the *trnL-F* intergenic spacer was reverse-complemented for the phylogenetic reconstructions.

The genetic distances between the sequences of the alignment were calculated with the help of MEGA5 (Tamura et al., 2011). In the case of the *trnL-F* region, indels were coded with the help of SeqState 1.25 software (Müller, 2005) using the simple coding option (Simmons & Ochoterena, 2000). The aligned sequences together with the coded indels were analysed using Maximum Parsimony
New data on the moss genus *Hymenoloma* (Bryophyta) (MP; Fitch, 1971) and Neighbor Joining (NJ) methods. The MP and NJ analyses were run with PAUP*4b10 (Swofford, 2002), using the following settings for MP: RANDOM additions (100 replicates), TBR branch-swapping, MULTREES = yes, steepest descent = no, COLLAPSE = yes. The number of maxtrees (2000) was not reached. All characters were equally weighted. A bootstrap analysis (Felsenstein, 1985) with 1000 replicates was performed with the settings as mentioned. Neighbor joining analyses were run with uncorrected pairwise distances. Branching confidence for MP and NJ was assessed using 1000 bootstrap replicates. A Bayesian analysis was carried out with MrBayes 3.2 (Ronquist et al., 2012). The best models for nucleotide substitution were determined for each region with jModeltest (Posada, 2008), a program that intensively uses PhyML (Guindon & Gascuel, 2003). Following the indications of this program the applied settings were nst = 6 and rates = equal for the *rps4* gene and nst = 6 and rates = gamma for the *trnL-F* region. Indels were treated as standard data. Three runs were conducted with 2 000 000 generations. Trees were sampled every 100th generation and the first 2 000 trees were discarded (burn-in) in order to exclude the trees before the chain reached the stationary phase. Tracer 1.5.0 (Rambaut & Drummond, 2007) was used to inspect the results of the MCMC chains and the effective sample size of >680 for all parameters indicated that sufficient generations were sampled. Trees were edited with the help of TreeView (MP, Bayes; Page, 1996) and TreeGraph 2 (Stöver & Müller, 2010). A ML analysis was carried out using MEGA5 without indel-coding. The best model for the combined *rps4-trnL-F* dataset (T92 + G) was identified with MEGA5. Tree Inference Options were set to Nearest Neighbor Interchange. Gaps/missing data were treated as partial deletion with site coverage cutoff = 95%. A bootstrap analysis with 1000 replicates was carried out.

The sequences alignment and all trees are available at TreeBase (submission ID 12712).

The computer program TCS (Clement *et al.*, 2000) was used to estimate the gene genealogy of the combined *rps4-trnL-trnF* data of the *Hymenoloma*-clade with the inclusion of *Drummondia obtusifolia*. Gaps were treated as missing data.

**RESULTS**

**Molecular analysis**

We obtained 20 new sequences for the *trnL-F* region and the *rps4* gene (Appendix). The partial sequence of the *rps4* gene had a length of 601 bp. The adjoining *rps4-trnS* spacer was not used because of incomplete data and alignment problems. The *trnL-F* region had a length of 468-470 bp for the ingroup *Hymenoloma* species (including the *trnL* intron, *trnL* exon 2, *trnL-F* spacer, and bp of the *trnF* gene). Both samples of *Hymenoloma mulahaceni* from Spain had identical sequences in both studied regions. For the *rps4* gene the number of pairwise differences between the two Spanish *H. mulahaceni* and the samples identified as *Dicranoweisia intermedia* from Russia and Alaska were in the range of 0–2, with *H. crispulum* 2–5, with Chilean *Hymenoloma* there were 3-7 mutations and with *H. compactum* 9. All other previously discussed possible close relatives like *Oreoaweisia* and *Cynodontium* differed by more than 20 mutations. In case of the *trnL-F*-region, the number of pairwise differences was quite similar with
0-2 differences between Spanish *H. mulahaceni* and the samples identified as *D. intermedia*, 3 between Spanish *H. mulahaceni* and *H. compactum*, 3-5 between Spanish *H. mulahaceni* and Chilean *Hymenoloma*, and 3-4 between Spanish *H. mulahaceni* and *H. crispulum*. As in the case of the *rps4* gene, all other species that were mentioned before as putative close relatives are clearly separated by more than 20 mutational steps.

The TCS network of the combined *rps4+trnL-trnF* dataset illustrates the genetic distances between the *Hymenoloma* specimens. *Hymenoloma antarcticum* and *H. brevipes* were the first species to separate from the branch, starting with *Drummondia obtusifolia* (Fig. 1). The distance between them was seven mutational steps. The *H. crispulum* samples formed a loop because gene genealogy was not clearly resolved. *Hymenoloma mulahaceni* from Spain and the Russian Altai (samples 6-8) were three mutational steps away from the Russian Far East specimens (samples 2-5) and one more step from the Alaska specimen (sample 1). The inclusion of gaps would have added one more step between the first and the last two.

The complete *rps4+trnL-F* alignment had a final length of 1226 positions (including codified gap information). Of the resulting final alignment, 829 positions

![Fig 1. Parsimony network created by TCS (Clement et al., 2000). The graph represents the gene genealogy among the *Hymenoloma* samples. *Drummondia obtusifolia* was added since the phylogenetic analyses suggest that it is the closest relative of *Hymenoloma*. Small dots represent hypothetical intermediate steps that were not actually found in this study.](image-url)
New data on the moss genus *Hymenoloma* (Bryophyta) were constant and 210 characters were parsimony-informative: 77 of them corresponded to the *rps4* gene, 102 to the *trnL-F* sequence and 31 to the coded indel information of the *trnL-F* region. The 48 most parsimonious trees had a length of 815 steps (RI = 0.804, CI = 0.656).

The first impression based on the distances is confirmed by all four methods applied for the phylogenetic analyses (Fig. 2). The genus *Hymenoloma* sensu *Ochyra et al.* (2003) received a strong support (1.0 pp, 97-99%), with *H. antarcticum* being clearly nested within this clade. Within *Hymenoloma*, not all species were resolved as monophyletic in all analyses. This is, for example, the case of *H. crispulum*, which is only supported by the Bayesian and the ML analyses. *Hymenoloma brevipes* and *H. antarcticum* were only resolved as belonging to one clade by MrBayes and NJ with low support values. Both species share two indels that separate them from the remaining *Hymenoloma* specimens and two more indels that they share with *H. crispulum* and *H. compactum*, but not with *H. mulahaceni*. This explains why the Bayesian inference and NJ (including coded gaps) suggest a closer relationship of *H. antarcticum* with *H. brevipes* (with low support values) although MP (including gaps) favours a basal position of *H. antarcticum* within the genus *Hymenoloma* (data not shown). The two phenotypes of *H. antarcticum* seem to be sufficiently different to warrant their specific status, as corroborated by the

---

**Fig 2.** Cladogram based on the Bayesian analysis of the combined *trnL-F* and *rps4* sequence data set. Clade credibility values and bootstrap support obtained by ML, MP, and NJ are indicated at the branches. Specimens of *Hymenoloma mulahaceni* originally identified as *Dicranoweisia intermedia* are indicated with an asterisk (*).
morphological data discussed below. The *H. mulahaceni* clade is strongly supported (1.0 pp, 96-100%) in all analyses but clearly consists of two clades, one containing the European samples and the one from Altai (western clade), the other containing samples from Russian Far East and Alaska (eastern clade). The morphological study of samples of both subclades showed subtle quantitative differences. For example, the plants of the eastern clade have a more pronounced thickening of the leaf margins and a slightly longer seta (6.0-6.5 mm vs. max 5.0 mm in the western clade). These differences do not allow drawing any taxonomic conclusion due to the low number of specimens studied.

There was no evidence for a close relationship of *H. mulahaceni* with *Oreoweisia*, *Cynodontium* or *Dicranoweisia cirrata*.

**Morphological analysis**


**Plants** about 1 cm high, blackish, dark green, to deep green tufts. **Stems** erect, to 160 μm thick, cross-section with well-delimited cortex formed by 2-3 layers of small, thick walled, brown-yellowish cells, central cylinder with 2-3 layers of big, thin-walled, yellowish cells and central strand weakly to well-developed. **Leaves** incurved to twisted when dry, erect to erect-patent when moist, lanceolate, gradually tapering to the apex, upper leaves 2.25-2.60 × 0.30-0.45 mm, basal leaves shorter; lamina unistratose in the basal third, irregularly bistratose, sometimes in patches and in longitudinal rows, in the upper part, base sheathing; apex acuminate; margins plane, entire, bistratose in the upper half, sometimes slightly undulate. **Costa** ending below or in the apex, 70-110 μm wide at the base, yellowish; in cross-section with 2-4 guide cells, dorsal and ventral stereids in 0-1 layers, sometimes only some dorsal substereids present, almost homogeneous in the upper part of leaf, differentiated ventral and dorsal epidermis present. **Cells** in the upper part of lamina isodiametric, bulging on both surfaces, papillose by means of longitudinal cuticular ridges densely covering both surfaces and making them appear rugged, 8-14 μm wide and 6-10 μm long, with slightly thickened brown-reddish cell walls; basal cells hyaline, shortly rectangular, thin walled, alar cells not differentiated. Pluricellular, green-brown or brownish, short to long ellipsoidal, 80-130 μm long **gemmae**, developing at the apex of branched axillary filaments, rarely present (to-date only known from the Spanish material). Monoicous. **Perichaetia** apical, inner perichaetial leaves widely sheathing, obtuse, markedly differentiated from vegetative leaves, the external longer, hardly differentiated from the vegetative
leaves. **Perigonia** below perichaetia on short lateral branches, consisting of 2-3 small inner perigonal leaves, widely ovate to rounded, membranaceous, smooth and with a weak costa, enclosed by 2-3 outer sheathing perigonal leaves. **Seta** 5-6 mm long, dextrorse below, sinistrorse above. **Capsule** erect, long cylindrical to ellipsoidal, brown-yellowish, $1.5-1.75 \times 0.5-0.6$ mm, striate when dry, narrowed at mouth, neck hardly swollen; exothecial cells shortly rectangular, thin-walled, with 5-8 rows of small, oblate or isodiametric, thick-walled cells at mouth, becoming thicker, brown-reddish in the middle, with large stomata at base; columella ending below the capsule mouth. **Annulus** absent. **Peristome** of 16 lanceolate teeth, deeply inserted, 110-120 $\mu$m long, unequally developed, sometimes reduced or shorter, acute, with 10-12 segments, papillose to smooth, hyaline at margins. **Lid** rostrate. **Spores** smooth, rounded, 12-18 $\mu$m in diameter. **Calypttra** cucullate.

**Ecology:** In Sierra Nevada, the species was found growing on acidic substrate, in rock crevices with a thin layer of soil, from 3079 to 3482 m a.s.l. The type of *Dicranoweisia intermedia* was found on rotten *Larix decidua* wood. Central and North Asian samples were collected on various types of stones and rocks, including base-rich carbonates, but commonly also on the bark of various trees including *Juniperus*, *Betula* and *Populus*. Hence it seems that there is no clear affinity to some specific site conditions, although the ecological information on the labels was not precise enough to draw firm conclusions.

**Distribution:** The presently known distribution of *Hymenoloma mulahaceni* comprises large parts of the Holarctic, including Europe: Spanish Sierra Nevada (Höhnel, 1895, our data), Swiss western Bernese Alps (Amann, 1918), and Iceland (Podpěra, 1954); Asia: Armenia (our data), Georgia (our data), Uzbekistan (our data), Kazakhstan (Abramova & Abramov, 1972, our data), Tajikistan (our data), Kyrgyzstan (Abramova & Abramov, 1972, our data), China: Qilian Mts (Abramova & Abramov, 1985) and Altai Mts (our data), Mongolia: Khovd, Mongolian Altai (Tsegmed, 2010), Russia: Altai, Northern Siberia and Russian Far East (Ignatov *et al.*, 2006, our data) and Russian Caucasus (our data); northernmost North America (U.S.A. (Alaska) and Canada, our data), and Greenland (our data). The distribution is summarized in Fig. 14.

**Nomenclature:** Höhnel (1895) and later authors deliberately used the spelling "mulahacenii", although the epithet refers to the name of a mountain (Mulhacén) rather than to a name of a person and the protologue also does not infer the possibility of intentional latinization of the geographical name. The origin of this mistake can be explained since the name of this mountain is dedicated to a person (Muley Hacen, King of Granada: 1464-1482). Therefore the epithet’s termination needs to be corrected according to Art. 60.11 of the Vienna ICBN.

Ochyra *et al.* (2003) newly combined *Dicranoweisia intermedia* J.J. Amann to *Hymenoloma*. The combination *Hymenoloma intermedium* (J.J. Amann) Ochyra was however illegitimate, since it was already occupied by *Hymenoloma intermedium* (Dixon) Broth., *Nat. Pflanzenfam*. (ed. 2) 10: 172. 1924. The latter species is based on *Verrucidens intermedius* Dixon, which has been considered synonymous with *Kiaeria pumila* (Mitt.) Ochyra (Ochyra, 1999). *Oreoweisia mulahaceni* (1895) is nevertheless an older name than *Dicranoweisia intermedia* (1918), and hence *Hymenoloma mulahaceni* is the correct name for this species in *Hymenoloma*. 
Fig. 14. Known distribution of *Hymenoloma multilacteum*. Full diamonds, sequenced specimens; full circles, specimens seen; empty circles, literature reports.
DISCUSSION

Phylogenetic circumscription

The molecular data presented here clearly suggest that the morphological concept of *Hymenoloma* of Ochyra et al. (2003) agrees with the phylogenetic delimitation, as reconstructed by the analysis of chloroplast *rps4* and *trnL-F* regions. The molecular segregation of *Hymenoloma* from *Dicranoweisia* is also supported by morphological characteristics (see key for the generic differentiation below). Nevertheless, it must be taken into account that Ochyra’s concept of *Hymenoloma* is significantly dependent on his taxonomic identification of the type of *Hymenoloma nordenskjoeldii* Dusén with the earlier described *H. antarcticum*. However, the protologue data (Dusén, 1905) illustrate completely smooth lamina cells and capsules that are sulcate when dry, characteristics that are not normally present in *Hymenoloma* sensu Ochyra et al. (2003 and following). Should *H. nordenskjoeldii* prove to be different from the above described concept, it would be necessary to suspend all earlier amendments of *Hymenoloma*, and move its members to the later described and very probably synonymous *Verrucidens* Cardot. The synonymy of *Verrucidens* with *Hymenoloma* has already been proposed by Cardot & Brotherus (1923) and their conclusion was accepted by Ochyra et al. (2008a).

Our data support the earlier suggested (Hedderson et al., 2004) close relationship between *Hymenoloma* and *Drummondia* and *Scouleria* (see the well-supported clade marked in black in Fig. 2), which does not seem to be supported by any morphological synapomorphy (Hedderson et al., 2004). Care should also be taken with the genus *Tridontium* Hook. f., formerly considered to belong to Scouleriaceae by Goffinet et al. (2008), but recently shown to belong to Pottiaceae (Cox et al., 2010). The proposal of Ochyra et al. (2003, 2008a, b) to include *Hymenoloma* in the family Seligeriaceae has not received any support from molecular data, as shown by Hedderson et al. (2004) and Stech et al. (2012).

*Hymenoloma* and *Oreoweisia* are not closely related. The latter is represented here by its newly sequenced generitype *O. torquescens*, and is confirmed as belonging to the Rhabdoweisiaceae, along with the genera *Amphidium*, *Arctica*, *Cynodontium*, *Dicranoweisia*, *Kiara*, *Oncophorus*, and *Rhabdoweisia*.

The species originally described as *Oreoweisia mulahaceni* clearly belongs to *Hymenoloma*, and it is separated by many mutational steps from *Dicranoweisia cirrata*, *Cynodontium bruntonii* and *Oreoweisia torquescens*. The data also suggest that *H. mulahaceni* and *D. intermedia* are indeed one species despite the two well-supported clades of *H. mulahaceni*, as they do not correspond to the former identification of the specimens and no clear morphological characteristics could be assigned to each clade. The number of mutations separating the two clades is lower than the number of mutations separating the other sequenced species of *Hymenoloma* (Fig. 1). A future analysis including a significantly higher number of specimens could resolve the question as to whether there are one or two species and clarify their morphological delimitation.

The broad concept of some *Hymenoloma* species, e.g. in the Flora of North America (McIntosh, 2007) and Flora of Antarctica (Ochyra et al., 2008a), seems to be in need of revision. McIntosh’s concept of *H. crispulum* obviously encompasses *H. mulahaceni*, as he admits distally bistratose lamina, typical of the latter taxon. Similarly, the two phenotypes of *H. antarcticum* described by Ochyra et al. (2008a).
which we were able to study in several specimens from southern Chile proved to belong to distinct species, based on both morphological and molecular data. *Hymenoloma brevipes* is morphologically extremely close to *H. crispulum* except for the larger spores, which match the size of *H. antarcticum*, and somewhat differently shaped alar cells; the differentiation of non-sporulating plants of *H. crispulum* and *H. brevipes* proved nevertheless difficult, and this seems to be the reason for the frequent misidentifications occurring in herbarium material. The presence of *Hymenoloma crispulum* in the Southern Hemisphere could not be confirmed in this study.

**Differentiation of the Holarctic species of Hymenoloma, including the generic differentiation from Dicranoweisia**

A key is given for the distinction of *Dicranoweisia s.str.* and *Hymenoloma* that is based in Ochyra *et al.* (2003) but modified in the view of the specimens of *Hymenoloma mulahaceni*. It is important to highlight that the presence of axillary pluricellular gemmae is not exclusive of *Dicranoweisia s.str.*, but they can be also present in the genus *Hymenoloma* and therefore should not be considered as a distinguishing character. The diagnostic characters for the *Hymenoloma* species are considered to be the colour of the plants, the leaf posture when dry, the differentiation of the alar cells, stratification in the upper lamina and margins, the colour and length of seta, and the papillosity of the peristome.

1. Lamina cells smooth or occasionally papillose, in this case lacking longitudinal cuticular ridges; leaf margins strongly and broadly recurved, bistratose; perichaetial leaves undifferentiated; annulus compound, deciduous............. *Dicranoweisia*

1. Lamina cells papillosely rugged due to the presence of longitudinal cuticular ridges densely covering both surfaces of the leaf; leaf margins plane to distally incurved, occasionally bistratose; perichaetial leaves markedly differentiated; annulus absent or occasionally indistinct and persistent............. 2. *Hymenoloma*

2. Leaves straight, slightly curved to twisted when dry, blackish, dark to deep green; pluricellular irregularly ellipsoid gemmae at the apex of axillary filaments sometimes present; alar cells usually not or weakly differentiated; leaf margins and sometimes the upper lamina bistratose ............ *H. mulahaceni*

2. Leaves crispate when dry, deep green; gemmae absent; alar cells usually well differentiated; leaf margins and upper lamina unistratose ............. 3

3. Seta yellow, becoming brownish with age, slender, 5-12 mm long; peristome teeth vertically striate in the proximal part and irregularly papillose with low papillae distally on the outer surface; spores yellow, smooth to rough, (11)14-20 μm in diameter ............................................ *H. crispulum*

3. Seta reddish-brown, stout, 3-5 mm long; peristome teeth prominently and roughly papillose with high papillae covering all the outer surface; spores reddish-brown, roughly papillose, (17)20-27 μm in diameter ............ *H. compactum*

**Acknowledgements.** We are grateful to Dr. R. Ochyra for his advice during the preparation of the PhD. thesis of S. Rams. This work was supported by the Spanish Ministry of Science and Innovation (CGL2008-00275/BOS project and CGL2012-22936/BOS), cofunded with FEDER program from the EU. Jan Kučera acknowledges funding of his part of the project by the grant from the Ministry of Education of the Czech Republic, No. MSM 6007665801.
REFERENCES


APPENDIX

Appendix: GenBank accession numbers for rps4 and trnL-F sequences used in the study with voucher information for the newly obtained accessions. Finally, voucher information of other Hymenoloma mulahaceni specimens used for the morphological study is included.

- **Amphidium lapponicum** (Hedw.) Schimp. AJ554011, AF231233; **Arctoa falcata** (Hedw.) Loeske AF231265, AF231234; **Arctoa fulvella** (Dicks.) Bruch & Schimp. AF231266, AF231235; **Bryoxiphium norvegicum** (Brid.) Mitt. MUB 25067, JX123848, JX123828; **Cynodontium bruntonii** (Sm.) Bruch & Schimp. - 1 Spain, Sierra Nevada, Rams et al. s.n., MUB 25027, JX123849, JX123829; **Dicranoweisia cirrata** (Hedw.) Lindb. - 1 AF478279, AF231243; **Distichium capillaceum** (Hedw.) Bruch & Schimp. AF435326; **Ditrichum flexicaule** (Schwägr.) Hampe AJ845204, AF487854; **Ditrichum pallidum** (Hedw.) Hampe AF306979, AF231248; **Drummondia obtusifolia** Müll.Hal.AF223038, AF229895; **Eucamptodontopsis brittoniae** (E.B. Bartram) B.H. Allen AF435285, AF435328; **Holomitrium calycinum** (Hedw.) mitt. MUB 25067, JX123848, JX123828; **Hymenoloma antarcticum** (Müll. Hal.) Ochyra - 1 Chile, Comuna Cabo de Hornos, Isla Navarino, W.R. Buck 41093, CONC, NY, JX123866, JX123846; **Hymenoloma brevipes** (Müll. Hal.) Ochyra (sub H. crispulum) - 1 Chile, provincia de Tierra del Fuego, J. Larraín 31010, CONC, JX123865, JX123845; **Hymenoloma brevipes** (sub H. antarcticum) - 3 Chile, provincia de Tierra del Fuego, J. Larraín 31010, CONC, JX123865, JX123845; **Hymenoloma crispulum** (Hedw.) Ochyra - 1 Austria, Kučera 7058, CBFS, JX123852, JX123832; **Hymenoloma crispulum** (Hedw.) Ochyra - 2 Austria, Kučera 7133, CBFS, JX123852, JX123832; **Hymenoloma mulahaceni** (Höhn.) Ochyra (sub Dicranoweisia intermedia) - 1 USA, Alaska, Southern Seward Peninsula Coast, O.M. Afonina s.n., 2001, LE, JX123855, JX123835; **Hymenoloma mulahaceni** (sub D. intermedia) - 2 Russia, Arctica, Chukotka, O.M. Afonina s.n., 1983, LE, JX123857, JX123837; **Hymenoloma mulahaceni** (sub D. intermedia) - 3 Russia, Arctica, Chukotka, O.M. Afonina s.n., 1975, LE, JX123858, JX123838; **Hymenoloma mulahaceni** (sub D. intermedia) - 4 Russia, Far East, Kamchatka Peninsula, I.V. Czernyadjeva s.n., 2003, LE, JX123859, JX123838; **Hymenoloma mulahaceni** (sub D. intermedia) - 5 Russia, Far East, Kamchatka Peninsula, I.V. Czernyadjeva s.n., 2001, LE, JX123860, JX123840; **Hymenoloma
mulahaceni (sub D. intermedia) - 6 Rusia, Altai, Kalbakaya Creek, M. Ignatov 36/316, 1993, MHA, JX123856, JX123836; *Hymenoloma mulahaceni* - 7 Spain, Sierra Nevada, Rams s.n., MUB 21884, JX123861, JX123841; *Hymenoloma mulahaceni* - 8 Spain, Sierra Nevada, Rams s.n., MUB 21885, JX123862, JX123842; *Kiaeria blyttii* (Bruch & Schimp.) Broth. AF231283, AF231252; *Kiaeria starkei* (F. Weber & D. Mohr) I. Hagen AF435289, AF435334; *Oncophorus wahlenbergii* Brid. AY908083, AF231256; *Oreoweisia mulahaceni* specimens used for the morphological study (all of them sub Dicranoweisia intermedia, except the type of *Oreoweisia mulahaceni*):