### Abstract:

Phylogenetic analyses within the moss Homalothecium sericeum resolved two clades and four haplotypes lacking any molecular synapomorphy. Because they exhibit comparable levels of genetic divergence to those observed among sister species in the genus, significant morphological differences, and distinct geographic distributions, they are recognised as three distinct species. Discriminant analysis was employed to assign the types of 'forgotten' taxa previously recognized within H. sericeum s.l. to one of those three species based on their morphology. While a growing number of 'cryptic species' has been mentioned in the literature, the results suggest that thorough morpho-anatomical investigations are likely to reveal morphological discontinuities among such taxa and trigger their formal description at the appropriate taxonomic level. Homalothecium sericeum s.str., H. mandonii (Mitt.) Geh. and H. mediterraneum Hedenäs stat. et nom. nov. clearly differ in sporophytic traits but the identification of sterile specimens is challenged by the overlap in gametophytic characters. As a consequence, 8-37% of the specimens were mis-classified in discriminant analyses in an attempt to find the best combination of gametophytic traits to identify specimens that were assigned to one of the three species on the basis of their genotype. This points to the necessity of developing easy-to-use molecular identification tools in taxonomically challenging plant groups, such as bryophytes. Homalothecium mandonii is the second case of an endemic Macaronesian bryophyte species whose range encompasses the Cape Verde Islands, the Canary Islands, Madeira, and the Azores. Homalothecium
Three species for the price of one within the moss Homalothecium sericeum s.l.

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Abstract. Phylogenetic analyses within the moss Homalothecium sericeum resolved two clades and four haplotypes lacking any molecular synapomorphy. Because they exhibit comparable levels of genetic divergence to those observed among sister species in the genus, significant morphological differences, and distinct geographic distributions, they are recognised as three distinct species. Discriminant analysis was employed to assign the types
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**Key words:** Bryophytes; Cryptic species; Discriminant analysis; DNA-barcoding; Europe; Macaronesia; Mediterranean

Running head: Species within *Homalothecium sericeum* s.l.
INTRODUCTION

The acquisition of the different properties defining daughter species (when they become phenotypically diagnosable, reciprocally monophyletic, reproductively incompatible, ecologically distinct, etc.) is not simultaneous. Before the acquisition of any one of those properties, everyone will agree that there is a single species, and after the acquisition of all, everyone will agree that there are two (De Queiroz, 2007). With the increasing use of molecular characters in systematics, a growing number of studies have revealed the existence of lineages that are well-defined genetically but appear to be indistinguishable by normally used morphological features, and are hence termed ‘cryptic taxa’ (see Bickford & al., 2007, for review). Cryptic taxa have increasingly been mentioned in the recent literature (Bickford & al., 2007), but are almost never formally described, typified, and named. This reflects the reluctance of taxonomists to describe species only based on molecular characters. As Oliver & Lee (2010) claimed, taxonomists need taxa that can be separated visually, because ‘portable DNA barcoding probes are many years away, at best’. However, cryptic taxa do not necessarily share a common ancestor (e.g., Goffinet & al., 2007), and in such cases recognition of the genetically divergent but morphologically similar lineages is necessary when species are defined based on the criterion of monophyly (Hutsemékers & al., 2012). By showing that different entities exist, molecular analyses can help to detect species that would otherwise have remained hidden and call for the careful search of morphological differences among seemingly cryptic taxa, which is an essential step towards their effective recognition (e.g., Szweykowski & al., 2005; Vanderpoorten & al., 2010; Sukkharak & al., 2011; Bell & al., 2012; Medina & al., 2012).

Here, we re-analyze previously published molecular data to revisit the significance of morphological variation and taxonomy of the pleurocarpous moss *Homalothecium sericeum* (Hedw.) Schimp. Within *H. sericeum* s.l., numerous taxa were described during the second
half of the 19th and in the beginning of the 20th Century. These were later all synonymised
with *H. sericeum* (Hofmann, 1998), and this taxonomic position has been adopted in the most
recent check-lists of mosses of European and Macaronesian mosses (Hill & al., 2006; Ros &
al., 2013). Recent phylogenetic analyses (Désamoré & al., 2012) showed that accessions of
*H. sericeum* s.l. from its entire distribution range belong to three molecular groups, including
two sister clades hereafter referred to as *H. mandonii* and *H. sericeum* s.str., and four
haplotypes that did not share any synapomorphy and are hereafter referred to as *H.
mediterraneum* (Fig. 1).

In the present study, we compare the level of divergence of the molecular lineages
identified within *H. sericeum* s.l. with those observed among other species in the genus. We
then use the phylogenetic identity of a representative number of accessions to seek for
differences in morphological characters among lineages of the *H. sericeum* s.l. clade.

Because relevant type material is too old for sequencing, we compute a discriminant function
that optimizes morphological identification from the sample of molecularly analysed
specimens, and assign each type specimen to one of the molecular lineages. Finally, we make
the appropriate taxonomic changes based on the morphological and molecular data.

**MATERIAL AND METHODS**

Specimens representing each haplotype based on *rpl16* and *atpB-rbcL* sequences from all
130 accessions of *H. sericeum* s.l. included in Désamoré & al. (2012) were combined with
those generated for multiple accessions of each species of the genus by Huttunen & al.
(2008). The dataset thus created included 68 accessions in total (Appendix 1).

*Brachytheciastrum velutinum* (Hedw.) Ignatov & Huttunen was employed as outgroup.

Indels were scored using simple index coding (Simmons & Ochoterena, 2000) as
implemented in the plugin SeqState (Müller, 2004) of PhyDE v0.995 (Müller & al., 2006).
The data matrix was submitted to a MP analysis using DNApars as implemented by Seaview 4.4.2 (Gouy & al., 2010) with 10 random starts, saving a maximum of 50000 most parsimonious trees and using gaps as informative characters. Support for the branches was assessed through a non-parametric bootstrap analysis with 100 replicates.

Fourty-three out of the 130 specimens of *Homalothecium sericeum* s.l. included by Désamoré & al. (2012) were sampled to represent the morphological variation and distribution range of *H. mediterraneum* (11 accessions), *H. mandonii* (12 accessions), and *H. sericeum* s.str. (20 accessions) (see the Taxonomic treatment below for voucher information).

The types of a number of segregate taxa previously recognized within *H. sericeum* s.l., namely *Camptothecium aureolum* Kindb., *Homalothecium sericeum* var. *meridionale* M.Fleisch. & Warnst., *Hypnum mandonii* Mitt., and *Homalothecium barbelloides* Dixon & Cardot, were also examined. We did not score morphological characters of the type of *Leskea sericea* Hedw. (Hedwig, 1801). In addition to the fact that Hedwig’s type material should not be sampled unless absolutely necessary, the type of *L. sericea* exhibits the long and narrow leaves that are typical for *H. sericeum* s.str. Furthermore, Hedwig’s European type material originates mainly from the non-Mediterranean regions where only the latter occurs. As a dioecious species, *H. sericeum* s.l. is infrequently found with sporophytes and only three specimens included in Désamoré & al. (2012) indeed bore them. Therefore, a further 12 specimens with sporophytes were selected from herbarium material and assigned to one of the three lineages on the basis of their gametophytic traits: six *H. mediterraneum*, five *H. mandonii*, and one *H. sericeum* s.str. Sporophytes were only studied in one additional specimen of the latter since their character states were already largely studied in previous studies (Hedenäs, 2001, 2012). All morphologically studied specimens are cited under the Taxonomic treatment.
Seven gametophytic traits were scored: leaf length (mm); leaf width (mm); leaf length to width ratio; median leaf lamina cell length (µm); median leaf lamina cell width (µm); median leaf lamina cell length to leaf length ratio; and leaf margin denticulation (finely denticulate, denticulate, strongly denticulate). Initial measurements were made in both stem and branch leaves in three arbitrarily selected specimens of each lineage (*H. mediterraneum*: H69, H78, H86; *H. mandonii*: H28, H29, H30; *H. sericeum* s.str.: H16, H19, H91; specimens cited under Taxonomic treatment). Three stem and branch leaves that had reached their final size were measured, for median lamina cells the total size range was noted, and for all measurements the mid-point (median) values were used in the comparisons (cf. Hedenäs, 1996). These characters showed parallel patterns of variation in the two kinds of leaves, and since it was substantially easier to obtain undamaged branch leaves than stem leaves (results not shown), it was decided to use only branch leaves in order to potentially find distinguishing characters among the three lineages. Leaf lamina cell width did not distinguish the lineages based on the initial three specimens per entity due to too great overlap (branch leaf lamina cell width 4.4-6.9 µm in *H. mediterraneum*, 4.2-8.4 in *H. mandonii*, and 4.6-8.4 in *H. sericeum* s.str.). This feature was therefore not measured in the remaining material, as it seemed unlikely that it would be a useful character for taxon identification.

Shapiro Wilk’s W-test and Brown & Forsythe's test showed that the continuous gametophytic variables branch leaf length, leaf width, leaf length to width ratio, median lamina cell length, and median leaf lamina cell length to leaf length ratio did not significantly depart from a normal distribution and did not exhibit significant differences in variance, respectively. Morphological differences between *H. sericeum*, *H. mandonii*, and *H. mediterraneum* depending on the five variables were thus sought using parametric statistics, namely Analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test as implemented by STATISTICA 8.0 (StatSoft, 2008). Linear discriminant analysis (LDA)
was then employed, using the same program, to identify which is the best combination of morphological variables to identify *H. sericeum*, *H. mandonii*, and *H. Mediterraneum*. Variables were selected using backward selection with a probability to stay in the model of \( p = 0.01 \). The discriminant functions were employed to assign the types of taxa previously recognized within *H. sericeum* s.l. to one of the three species based on their morphological features. To determine what is the actual error rate when attempting at identifying specimens from morphological characters only, a cross-validation procedure, during which each specimen was successively removed from the matrix, was employed.

**RESULTS**

The datamatrix included 1273 characters, of which 94 (22 indels) were parsimony-informative. The MP analysis of *rpl16* and *atpB-rbcL* in *Homalothecium* resulted in 887 equally parsimonious trees of 121 steps, whose strict consensus is presented in Fig. 1. Within *H. sericeum* s.l., a large polytomy comprising four haplotypes labelled as *H. Mediterraneum* as well as a clade holding the accessions of reciprocally monophyletic *H. mandonii* and *H. sericeum* s. str. were recovered. Most branches did not receive bootstrap support >50%.

Branch leaf length (*H. Mediterraneum*, mean ± standard deviation: \( 1.74 ± 0.30 \); *H. mandonii*: \( 1.60 ± 0.19 \); *H. sericeum* s.str.: \( 1.74±0.28 \); \( p = 0.17--0.98 \), Fisher’s LSD test) and lamina cell width (see above) did not significantly differ among lineages, leaving four continuous characters (leaf width, leaf length to width ratio, median lamina cell length, and median lamina cell length to leaf length ratio; Table 1) as well as denticulation of leaf margins for consideration as potentially discriminative gametophytic characters. Specimens of *H. sericeum* s.str. exhibited significantly narrower leaves and a higher leaf length to width ratio than those of the other clade and the grade according to Fisher’s LSD test (Table 1). In *H. mandonii*, the lamina cells were significantly longer and the lamina cell length to leaf
length ratio was significantly higher than in *H. mediterraneum* and *H. sericeum* s.str. (Table 1). All of the investigated characters were, however, overlapping among the three, with an overlap of 9% in the lamina cell length to leaf length ratio to 61% in leaf width (Table 1). *Homalothecium sericeum* s.str. was further characterized by the strong denticulation or sometimes weak dentation of the leaf margin in the alar region, with at least some of the teeth distinctly bent outwards (Fig. 2).

Two variables, namely the leaf length to width ratio and the median lamina cell to leaf length ratio, were selected in the LDA. On average, 79% of the specimens were assigned to the correct taxa after cross-validation, which corresponds to a correct classification rate of 63%, 92%, and 80% in *H. mediterraneum*, *H. mandonii* and *H. sericeum* s.str., respectively. The type specimens of *H. sericeum* var. *meridionale* and *Hypnum mandonii* were assigned to *H. mediterraneum* and *H. mandonii*, respectively, while the types of *Camptothecium aureolum* and *H. barbelloides* were assigned to *H. sericeum* s.str.

Sporophytic characters, when available, further distinguished the three taxa. The seta was rough throughout its length in *H. mandonii* and *H. sericeum* s.str., but completely rough, smooth in the upper 1/4, or occasionally completely smooth, in *H. mediterraneum*. The outer exostome ornamentation was clearly cross-striolate in *H. sericeum* s.str., but smooth or only weakly cross-striolate in *H. mediterraneum* and *H. mandonii*. Finally, the exostome border in the lower portion of the teeth was broad in *H. mediterraneum* but narrow in the two clades (Fig. 3A, E).

Among the three plastid loci (*atpB-rbcL*, *rpl16*, *trnG*) investigated by Désamoré & al. (2012), *rpl16* exhibited three substitutions and one indel (Table 2) within the *H. sericeum* complex, allowing for the unambiguous identification of any specimen that is recent enough for DNA amplification. *TrnG* included one synapomorphic substitution for *H. sericeum s.l.*,
while in \textit{atpB-rbcL} one synapomorphic substitution supports \textit{H. sericeum s.str.} and \textit{H. mandonii}.

\textbf{DISCUSSION}

The analyses confirmed the monophyly of accessions assigned to \textit{H. mandonii} and \textit{H. sericeum s. str.} Levels of divergence between these two clades were similar to those observed among other species of the genus. Although these relationships were supported by the strict consensus resulting from the MP analysis of two cpDNA loci and are further fully consistent with those resolved in other species-level phylogenies of the genus (Huttunen & al., 2008) and with analyses of the \textit{H. sericeum} s.l. clade (Désamoré & al., 2012), they lacked bootstrap support in the present analyses. Although a complete set of ITS sequences was produced, we refrained from combining them with the chloroplast data as accessions of morphologically unambiguously identified species were resolved in completely unrelated clades with ITS. Such a strong incongruence among partitions could result from the amplification of paralogous ITS copies, as recently evidenced in mosses (Kosnar & al., 2012), and further studies are therefore required to explore the utility of that locus in the genus.

Although variation in \textit{rpl16} and \textit{atpB-rbcL} was sufficient to discriminate all \textit{Homalothecium} species recognized to date, the four haplotypes labelled as \textit{H. mediterraneum} formed a polytomy within \textit{H. sericeum} s.l. One possibility to accommodate those haplotypes taxonomically would be to include them within one of the closely related recognized species, but this would be at odds with their levels of divergence that are of the same order as those observed among sister species in the genus. Alternatively, each of those four haplotypes could be recognized as an individual species. However, as they share the same geographic origin and morphological identity (see below), we rather treat them here as conspecific,
although the lack of any molecular synapomorphy remains unexplained and is at odds with a
monophyletic species concept.

The discriminant analysis assigned the types of *H. sericeum* var. *meridionale* and *Hypnum mandonii* to our groups of accessions labelled as *H. mediterraneum* and *H. mandonii*, respectively, which are therefore formally recognized here as *H. mediterraneum* Hedenäs stat. et nom. nov. (see Taxonomic treatment below) and *H. mandonii* (Mitt.) Hedenäs. The new epithet *mediterraneum* was used instead of *meridionale*, since at the species level, the latter is blocked by *H. meridionale* Ravaud, a synonym of *H. sericeum* var. *robustum* Boulay. The recognition of *H. mediterraneum* and *H. mandonii* parallels previous ‘resurrections’ of ancient taxa (Rycroft & al., 2004) or de novo descriptions of moss species in the light of molecular data (Hutsemékers & al., 2012; Medina & al., 2012). The type of *Camptothecium aureolum* was unambiguously assigned to the *H. sericeum* s.str. clade, supporting the notion that the former is a synonym of the latter (Hofmann, 1998). The isotype of *H. barbelloides* has very narrow leaves, so that the specimen was assigned to *H. sericeum* by the discriminant analysis, but its lamina cells and the shape and margin denticulation of the basal leaf are similar to *H. mandonii*. The specimen appears to have grown as a pendent epiphyte, which would explain the narrow leaves, and we therefore believe that *H. barbelloides* is most likely conspecific with *H. mandonii*. However, the very uncharacteristic leaf shape of this taxon makes us refrain from making a formal reduction into synonymy. Unfortunately, only the old type material is available, and the molecular identity could therefore not be determined.

Gametophytic traits significantly differ among *H. mandonii*, *H. mediterraneum* and *H. sericeum* s.str., but exhibit substantial overlap (Table 1). In mosses, the gametophyte is the dominant phase and gametophytic traits are largely employed for species identification. During its lifetime the gametophyte is permanently exposed to environmental variation, and is hence prone to plasticity and sometimes convergence (Vanderpoorten & al., 2002; Olsson
Variation in gametophytic traits for taxonomy and species identification might therefore be misleading (Zander & Vitt, 1979; Olsson & al., 2011, 2012; Bell & Hyvönen, 2012; Câmara & Carvalho-Silva, 2013). In the *H. sericeum* complex and other moss genera where species differentiation mostly relies on continuous characters (e.g., *Leucobryum*, Vanderpoorten & al., 2003; *Rhynchostegium*, Hutsemékers & al., 2012), specimen identification is challenged by the overlap in characters among species. In the *H. sericeum* complex, this overlap results in a misidentification rate of 8-37% when only gametophytic characters are used. Sporophytic traits substantially assist species identification but, as in many of the about 60% of moss species that are dioecious (e.g., Wyatt, 1982; Hedenäs & Bisang, 2011), sporophytes are mostly lacking in the *H. sericeum* complex. Although a combination of gametophytic and sporophytic characters allows distinguishing the three species of the *H. sericeum* complex, as summarised in the identification key given below, the present study points to the necessity of developing easy-to-use molecular identification tools. Such molecular identification tools (DNA barcoding markers) will improve biodiversity assessments and ecological research in taxonomically challenging bryophyte groups (e.g., Stech & al., 2013; Lang & Stech, in press). Of the three plastid markers (*atpB-rbcL*, *rpl16*, *trnG*) used in the phylogeographic study of Désamoré & al. (2012), *rpl16* was the only locus that displayed sufficient levels of variation to on its own allow for an unambiguous differentiation between the three species (Table 2). Although *atpB-rbcL* and *trnG* were also partly informative, *rpl16* can best serve as an easy molecular tool for identifying poorly developed and/or sterile *Homalothecium sericeum* s.l. specimens. The locus was similarly shown to exhibit appropriate levels of inter-specific variation in other genera, such as *Plagiomnium* (Wyatt & Odrzykoski, 2012), *Forssstroemia* (Olsson & al., 2012), *Leptodon* (Sotiaux & al., 2009), and *Cratoneuron* (Hedenäs, 2011). *Rpl16* is not among the regions that have been explored as DNA barcoding marker in mosses so far (see
Stech & al., 2013; Lang & Stech, in press; and references therein), but can be considered a potential candidate for distinguishing closely related bryophyte species, although its applicability to a wider range of mosses remains to be tested. Contrary to the original idea of species identification based on a single short, standardized DNA region, recent DNA barcoding attempts of mosses indicate that different markers (or different combinations of markers) may work best in different moss lineages, including standard markers such as trnL-F and ITS (but see above), but also newly considered regions such as atpF-atpH (Hassel & al., 2013) or rps19-rpl12 (Lang & Stech, in press). The present results concerning rpl16 are in line with these observations.

The three Homalothecium species exhibit distinct, albeit slightly overlapping geographic ranges. Homalothecium mandonii is a strict Macaronesian endemic. It is distributed across the four Macaronesian archipelagos, a pattern that is otherwise found in bryophytes only in Exsertotheca intermedia (Brid.) S. Olsson, Enroth & D. Quandt, raising the question of why apparently so vagile organisms failed to reach the North African and South-Western European coasts. Homalothecium mediterraneum is a Mediterraneo-Macaronesian endemic that is widespread across the Mediterranean but was found in one locality in Lanzarote in the Canary Islands. Finally, H. sericeum is a temperate species distributed across central Europe and the South of Scandinavia, with a few scattered localities in the Mediterranean and eastern North America. The restricted distributions of the segregate species within H. sericeum s.l. reinforce the notion that many disjunctions typically observed in moss distribution ranges are due to taxonomic shortcomings (Hutsemékers & al., 2012; Medina & al., 2012) and call for the necessity of substantial taxonomic revisions of previously broadly defined bryophyte species.

**Taxonomic treatment**
**Homalothecium sericeum** (Hedw.) Schimp., Bryol. Eur. 5: 93. 456 (fasc. 46–47 Mon. 3. 1).

1851.


Synonym: *Camptothecium aureolum* Kindb., Rev. Bryol. 22: 85. 1895. Lectotype:


Plants medium-sized, sometimes small, irregularly or pinnately branched, branches and upper shoot ± strongly curved upwards-inwards when dry. Stem with central strand, a cortex (including epidermis) of 2-3(-4) layers of small and incrassate cells, without hyalodermis; rhizoids inserted at or just below costa insertion, red-brown, slightly branched, smooth; axillary hairs 1-2 per axil, strictly axillary, with 1-3 short, hyaline upper cells, 6.0-12.0 µm wide, basal cells 1-2, quadrate, hyaline or brownish; pseudoparaphyllia foliose; paraphyllia absent. Stem leaves when moist erect to erecto-patent, when dry erect, straight or slightly homomallous, from ovate-triangular triangular or narrowly triangular base gradually narrowed to longly acuminate apex, not or slightly narrowed towards insertion, slightly concave, plicate; costa single, ending 50-65% way up leaf, 29.5-80.0 µm wide near base, cells on both ad- and abaxial sides linear and similar to adjoining lamina cells, smooth or often ending in a spine on back, in transverse section near base plano-convex, 3-4-stratose, cells homogeneous; margin plane or on one or both sides shortly recurved or reflexed, without border, above finely denticulate or denticulate, sometimes partly entire, around upper alar region mostly distinctly denticulate to dentate, denticles or teeth often spreading or recurved; median leaf lamina cells 36.0-130.0 x 4.0-8.5 µm, linear, with moderately to longly tapering ends, slightly incrassate, scattered cells sometimes dorsally and distally prorate; basal lamina cells slightly wider and much shorter than median cells, strongly incrassate,
more or less porose; *alar cells* triangular, transversely rectangular, quadrate or shortly rectangular, in basal part rectangular and widened, irregular, incrassate, eporose, forming a large and well differentiated, ± isodiametric or approximately triangular group, extending from leaf margin 25-35% of distance to leaf middle at insertion, decurrent 50-80% way down to leaf below. *Branch leaves* smaller and more shortly acuminate than stem leaves, widest 0-20% way up, costa ending in a spine, upper margin more strongly denticulate than in stem leaves, median leaf lamina cells 19.0-119.5 x 4.5-8.5 µm, many lamina cells distally and dorsally prorate; median values (three leaves) for leaf width 0.28-0.54 µm, length to width ratio 3.94-5.88, mid-leaf lamina cell length 49.35-82.95 µm, lamina cell length (µm) to leaf length (mm) ratio 29.70-45.64. *Sexual condition* dioicous, with normal-sized or dwarf male plants. *Perigonia* lateral on stem, in dwarf males lateral or apical, paraphyses present, in dwarf males absent. *Perichaetia* lateral on stem; inner perichaetial leaves straight and erect, from ovate or triangular-ovate base suddenly or gradually narrowed to flexuose acumen, longly acuminate, plicate; costa single, weak; margin in acumen denticulate or partly strongly so; paraphyses 6-13 cells long, slightly incrassate. *Calyptra* cucullate, 3-5-stratose, smooth, naked or with a few basal paraphyses. *Seta* 9-17 mm long, orange or red, rough throughout, when dry untwisted or dextrorse. *Capsule* longly cylindrical to longly elongate-ovoid, straight, or slightly curved, not furrowed, not constricted at mouth when moist or dry, orthotropous or slightly homotropous; exothecial cells 27.5-65.0 x 15.5-27.5 µm, quadrate to elongate-rectangular, evenly incrassate, smooth, below mouth 2-4 rows of isodiametric or transversely rectangular cells; stomata round-pored; annulus separating, of 2(-3) rows of relatively small cells; operculum longly conical or short-rostrate, basal cells radial, slightly incrassate. *Exostome* reduced, teeth narrow, red or pale reddish, lower outside cross-striolate, not furrowed, upper outside papillose or strongly so, margin entire, border in lower portion of teeth narrow, gradually narrowed at transition zone, absent above, primary peristomial layer
reduced or strongly reduced. *Endostome* basal membrane low, with short and imperfect processes, hyaline or brownish, papillose, cilia 1-2, short or rudimentary. *Spores*

14.5-23.0(-29.0) μm, papillose, mature in winter half-year.


Homalothecium mediterraneum Hedenäs stat. et nom. nov. Fig. 3A-D


Plants medium-sized, sometimes small, irregularly or pinnately branched, branches and upper shoot ± strongly curved upwards-inwards when dry. Stem with central strand, a cortex (including epidermis) of 2-4 layers of small and incrassate cells, without hyalodermis; rhizoids inserted at or just below costa insertion, red-brown, hardly to moderately strongly branched, smooth; axillary hairs 1-2 per axil, strictly axillary, with 1-4 short, hyaline upper cells, 8.5-10.5 µm wide, basal cells 1-2, transversely rectangular to shortly rectangular, brownish; pseudoparaphyllia foliose; paraphyllia absent. Stem leaves when moist erect to patent, when dry erect, straight or slightly homomallous, from cordate-triangular or rounded-triangular base gradually narrowed to longly acuminate apex, markedly narrowed towards insertion, slightly concave, plicate; costa single, ending 55-75% way up leaf, 38.0-73.5 µm wide near base, cells on both ad- and abaxial sides linear and similar to adjoining lamina cells, smooth or often ending in a spine on back, in transverse section near base plano-
convex, 3-4-stratose, cells homogeneous; *margin* plane or on one or both sides entirely or partly broadly recurved, without border, entire or finely denticulate, denticles around upper alar region weak or absent, rarely bent slightly outwards; *median leaf lamina cells* 29.5-92.5 x 4.0-7.5 µm, linear, with shortly to moderately tapering ends, slightly incrassate, scattered cells sometimes dorsally and distally prorate; *basal lamina cells* wider and much shorter than median cells, incrassate, eporose; *alar cells* quadrate, transversely rectangular, rhomboidal, especially towards insertion rectangular, in distal portion of group often irregular, slightly incrassate, eporose, forming a large and well differentiated, ± isodiametric or along margin slightly elongate group, extending from leaf margin 25-35% of distance to leaf middle at insertion, decurrent 20-50% way down to leaf below. *Branch leaves* smaller and more ovate than stem leaves, widest 15-30% way up, costa ending in a spine, upper margin more strongly denticulate than in stem leaves, *median leaf lamina cells* 25.0-115.5 x 4.5-7.0 µm, many lamina cells distally and dorsally prorate; median values (three leaves) for leaf width 0.34-0.56 µm, length to width ratio 3.03-4.26, *mid-leaf lamina cell length* 46.20-81.90 µm, *lamina cell length* (µm) to leaf length (mm) ratio 24.75-48.21. *Sexual condition* dioicous, with normal-sized or dwarf male plants. *Perigonia* lateral on stem, in dwarf males lateral or apical, paraphyses present, in dwarf males few. *Perichaetia* lateral on stem; inner perichaetial leaves straight and erect, from ovate or ovate-oblong base suddenly or gradually narrowed to flexuose acumen, acuminate, smooth or weakly plicate; costa single, weak; margin in acumen denticulate or finely so, at shoulder strongly so or partly dentate; paraphyses 8-19 cells long, slightly incrassate. *Calytra* cucullate, 3-5-stratose, smooth, naked. *Seta* 8-15 mm long, red, rough almost throughout or above weakly so or smooth, occasionally smooth almost throughout, when dry dextrorse. *Capsule* cylindrical, shortly or gradually narrowed towards mouth, straight, or mouth slightly oblique, not furrowed, often constricted at mouth when moist but not more so when dry, orthotropous; exothecial cells 21.0-65.0 x 12.5-40.0 µm,
quadrate or shortly to longly rectangular, evenly incrassate or slightly collenchymatous with
superficial thickenings, smooth, below mouth 1-4 rows of isodiametric, shortly transversely
rectangular, or rectangular cells; stomata round-pored, occasionally ovate-pored; annulus
separating, of 1-3 rows of relatively small cells; operculum conical or rostrate, basal cells
radial, incrassate. Exostome reduced, teeth short and narrow, yellow-brown or pale yellow-
brown, lower outside indistinctly cross-striolate to smooth, not furrowed, upper outside
strongly papillose, margin entire or irregular, border in lower portion of teeth broad,
gradually narrowed at transition zone, absent above, primary peristomial layer strongly
reduced. Endostome basal membrane low, with short and imperfect or vestigial processes,
hyaline, vestigial processes sometimes brownish, papillose above, sometimes more finely so
below, cilia 1-3, vestigial. Spores 13.0-21.0 µm, finely or strongly papillose, mature in winter
half-year.

According to Hofmann (1998), H. sericeum var. meridionale (H. mediterraneum) and H.
sericeum var. tunetanum differ from H. sericeum s. str. only in their smooth seta. Because the
often occurring smooth or partly smooth seta is one feature that distinguishes H.
mediterraneum from the other two species recognized here (Table 2), Homalothecium
sericeum var. tunetanum is considered as a synonym of H. mediterraneum.

Known geographical distribution: Homalothecium mediterraneum is circum-
Mediterranean and is known from one locality in Lanzarote in the Canary Islands (Désamoré
& al., 2012).

Specimens studied (except types; ‘D’ with number = specimens included in Désamoré &
al. (2012): Cyprus. Troodos Mts, at Pano Platres village, 18 September 2001, B.Papp (D
H44), BP: 177881; Troodos Gebirge, Pano Platres, Frahm 200691 (D H31), Herb. J.-
P.Frahm BONN. Greece. Crete, Chania, Frahm K-158 (D H34), Herb. J.-P.Frahm BONN;
Crete, Irakleion region, 18 April 2001, B.Papp (D H86), BP: 170680; Crete, environs de
Homalothecium mandonii (Mitt.) Geh., Flora 69: 348. 1886. Fig. 3E-H


Homalothecium sericeum var. meridionale Schimp. in Geh., Flora 69: 349. 1886, nom. nud. (Geheeb, 1886)

Plants medium-sized, sometimes small, irregularly pinnately branched, branches sometimes turning to new stems, branching angle relatively narrow, branches straight or curved upwards-inwards when dry. Stem with central strand, a cortex (including epidermis) of 1-2(-3) layers of small and incrassate cells, without hyalodermis; rhizoids inserted at or just below costa insertion, red-brown, not or slightly branched, smooth; axillary hairs 1-3 per axil, strictly axillary, with 1-2 short, hyaline upper cells, 10.0-12.5 µm wide, basal cells 1-2,
transversely rectangular to shortly rectangular, brown; *pseudoparaphyllia* foliose;

*paraphyllia* absent. *Stem leaves* when moist erect to patent, when dry erect, straight or slightly homomallous, triangular or ovate-triangular, from shortly above insertion gradually narrowed to acuminate apex, distinctly constricted at insertion, concave, strongly plicate;

costa single, ending 50-75% way up leaf, 31.5-63.0 µm wide near base, cells on both ad- and abaxial sides linear and similar to adjoining lamina cells, smooth, in transverse section near base plano-convex, 4(-5)-stratose, cells homogeneous; *margin* plane or shortly to longly weakly reflexed, without border, finely denticulate throughout, a few denticles around upper alar region sometimes stronger, rarely bent slightly outwards; *median leaf lamina cells* 46.0-178.5 x 4.0-8.0 µm, linear, with moderately to longly tapering ends, slightly incrassate or incrassate, smooth or slightly distally and dorsally prorate; *basal lamina cells* wider and much shorter than median cells, strongly incrassate, porose; *alar cells* in upper portion of group transversely rectangular, quadrate, or rectangular, often rounded, below rectangular to longly rectangular, incrassate, slightly porose, forming a rounded, shortly oblong, or triangular group, extending from margin 35-40% of distance to leaf middle at insertion, decurrent or shortly so. *Branch leaves* smaller than stem leaves, widest 15-25% way up, costa occasionally ending in small, obtuse spine, upper margin denticulate, median leaf lamina cells 33.5-147.0 x 4.0-8.5 µm, occasional lamina cells distally and dorsally prorate; median values (three leaves) for leaf width 0.37-0.61 µm, length to width ratio 2.96-3.60, mid-leaf lamina cell length 65.10-103.95 µm, lamina cell length (µm) to leaf length (mm) ratio 44.93-59.64. *Sexual condition* dioicous, with normal-sized or dwarf male plants. *Perigonia* lateral on stem, in dwarf males lateral or apical, paraphyses present, in dwarf males few. *Perichaetia* lateral on stem and branch bases; inner perichaetal leaves straight and erect, narrowly ovate or triangular ovate, above shortly narrowed to acumen, apex narrowly acuminate, smooth or plicate; costa single, weak, indistinct; margin in acumen entire or
weakly denticulate, at shoulder denticulate, strongly so, or with single teeth, not or weakly
bordered; paraphyses 6-19 cells long, incrassate. Calyptra cucullate, 3-5-stratose, smooth or
sometimes with one low ridge, naked. Seta 11-22 mm long, red, rough throughout, when dry
dextrorse. Capsule ovoid to cylindrical, not furrowed, not or when dry sometimes weakly
constricted below mouth, orthotropous or almost so; exothecial cells 21.0-50.5 x 12.5-31.5
μm, quadrate or rectangular, slightly evenly incrassate or longitudinal walls incrassate, not
collenchymatous, smooth, below mouth 1-4 rows of small, rectangular, transversely
rectangular, or quadrate cells; stomata round-pored; annulus separating, of 1-3 rows of
relatively small cells; operculum shortly rostrate, basal cells radial, incrassate. Exostome
reduced or strongly so, teeth narrow and sometimes short, light orange-brown, lower outside
weakly cross-striolate to smooth, not furrowed, upper outside papillose to almost smooth,
margin entire, border absent, or present, narrow, and gradually narrowed upwards, primary
peristomial layer strongly reduced. Endostome basal membrane low, with short and imperfect
or vestigial processes, yellowish or brownish, papillose or finely so, cilia 0-2(-3), absent or
vestigial. Spores 14.5-22.0 μm, rather strongly papillose, mature in winter half-year.

In the original description of Hypnum mandonii Mitt. (Mitten, 1870), this taxon was said
to have more closely imbricate leaves than H. sericeum, and acute rather than more narrowly
pointed leaves. The latter is probably to some degree reflected in the narrower leaves that
widen from closer to the leaf insertion in H. sericeum than in H. mandonii.

Known geographical distribution: Homalothecium mandonii is a Macaronesian endemic
distributed across Cape Verde, all of the Canary Islands, Madeira, and the Azorean islands
São Miguel and Santa Maria

2013).

\textit{Taxon with uncertain position}


\textit{Key to the European and Macaronesian species of Homalothecium sericeum s.l.}

1. Branch leaves 3.9-5.9 times as long as wide, mostly widest 0-20% way up leaf; margin denticulation at alar region well developed, with at least some teeth distinctly bent outwards (Fig. 2). Exostome distinctly cross-striolate on lower outside.

\textit{H. sericeum} s.str.
1. Branch leaves 3.0-4.3 times as long as wide, mostly widest 15-30% way up leaf; margin
denticulation at alar region weak or absent, teeth rarely and only slightly bent outwards
(Fig. 3C, G). Exostome smooth or weakly cross-striolate on lower outside.

2. Ratio between branch leaf lamina median cell length (µm)/median leaf length (mm)

24.7-48.2. Seta frequently partly or entirely smooth; exostome border broad (Fig. 3A).

\[ H. \text{ mediterraneum} \]

2. Ratio between branch leaf lamina median cell length (µm)/median leaf length (mm)

44.9-59.6. Seta rough throughout; exostome border narrow (Fig. 3E).

\[ H. \text{ mandonii} \]

ACKNOWLEDGEMENTS

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reviewers significantly improved the manuscript. This research was funded through an
Integrating Research Grant (IRG) of the European Distributed Institute of Taxonomy (EDIT).

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Table 1  Branch leaf and sporophyte characters that differentiate the three *Homalothecium sericeum* s.l. species. 1--4: Average and standard deviation of the median (mid-point) values of measured characters in *Homalothecium mediterraneum* (n = 11), *H. mandonii* (n = 12), and *H. sericeum* s. str. (n = 20) and median values of the measures from type specimens of *Camptothecium aureolum, Homalothecium sericeum* var. *meridionale, Hypnum mandonii* and *Homalothecium barbelloides*. Minimum and maximum values for each variable are shown in parentheses. Measurements highlighted in bold in one species indicate a significant difference (p < 0.05) from those observed in the two other species according to Fisher’s LSD test. 5--9. Other branch leaf and sporophyte characters that differentiate the three *Homalothecium* species.

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<td><strong>H. mediterraneum</strong></td>
<td>(0.34)</td>
<td>(3.03)</td>
<td>(46.20)</td>
<td>(24.75)</td>
<td>15-30%</td>
<td>Weak or absent, rarely above</td>
<td>Rough, smooth in upper ¼, or weakly</td>
<td>Broad</td>
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<td>0.48±0.02</td>
<td>3.60±0.11</td>
<td>61.09±3.44</td>
<td>35.92±2.32</td>
<td>leaf base</td>
<td>bent slightly</td>
<td>occasionally cross-</td>
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<td>(0.56)</td>
<td>(4.26)</td>
<td>(81.90)</td>
<td>(48.21)</td>
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<tr>
<td>Species</td>
<td>Width</td>
<td>Length</td>
<td>Thickness</td>
<td>Teeth/Leaf Base</td>
<td>Teeth/Bend</td>
<td>Bend Outwards</td>
<td>Smooth/Striolate</td>
<td>Additional Details</td>
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<td>H. mandonii</td>
<td>0.37</td>
<td>2.96</td>
<td>65.10</td>
<td>44.93</td>
<td>15-25%</td>
<td>Weak</td>
<td>Smooth</td>
<td>Rough, sometimes a few stronger teeth, rarely bent outwards Smooth or Narrow (Fig. 3E)</td>
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<td>0.49±0.01</td>
<td>3.29±0.06</td>
<td>88.26±3.64</td>
<td>55.05±1.27</td>
<td>above</td>
<td>sometimes a</td>
<td>weakly</td>
<td>(Fig. 3E)</td>
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<td></td>
<td>0.61</td>
<td>3.60</td>
<td>103.95</td>
<td>59.64</td>
<td>leaf base</td>
<td>few stronger</td>
<td>cross-striolate</td>
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<tr>
<td>H. sericeum</td>
<td>0.28</td>
<td>3.94</td>
<td>49.35</td>
<td>29.70</td>
<td>0-20%</td>
<td>Mostly</td>
<td>Rough</td>
<td>Cross-striolate</td>
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<td>0.38±0.01</td>
<td>4.61±0.10</td>
<td>65.31±2.12</td>
<td>37.92±1.02</td>
<td>above</td>
<td>strong,</td>
<td>throughout</td>
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<td></td>
<td>0.54</td>
<td>5.88</td>
<td>82.95</td>
<td>45.64</td>
<td>leaf base</td>
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<td>C. aureolum</td>
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<td>4.62</td>
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<tr>
<td>H. sericeum var. meridionale</td>
<td>0.50</td>
<td>3.23</td>
<td>71.40</td>
<td>43.87</td>
<td>-</td>
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<tr>
<td>H. mandonii</td>
<td>0.48</td>
<td>3.06</td>
<td>95.55</td>
<td>64.54</td>
<td>-</td>
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<tr>
<td>H. barbelloides</td>
<td>0.20</td>
<td>5.63</td>
<td>79.80</td>
<td>69.09</td>
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Table 2. Species-specific polymorphisms in the rpl16 gene among *Homalothecium mandonii*, *H. sericeum*, and *H. mediterraneum*.

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<th>Position</th>
<th>594</th>
<th>703</th>
<th>619</th>
<th>832</th>
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<tr>
<td><em>H. mandonii</em></td>
<td>C</td>
<td>Poly-A (8 repeats)</td>
<td>T</td>
<td>A</td>
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<tr>
<td><em>H. sericeum</em></td>
<td>T</td>
<td>Poly-A (9 repeats)</td>
<td>C</td>
<td>G</td>
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<tr>
<td><em>H. mediterraneum</em></td>
<td>T</td>
<td>Poly-A (9 repeats)</td>
<td>T</td>
<td>A</td>
</tr>
</tbody>
</table>
Appendix 1. Voucher information and Genbank accession numbers of the specimens of Homalothecium included in the present phylogenetic analysis. Hap and SH numbers are used in Fig. 1, and refer to haplotypes included in Désamoré & al. (2012) and specimens in Huttunen & al. (2008), respectively. The GenBank accession numbers for SH specimens correspond with rpl16 and atpB-rbcL.


SH391: EF531079, EF530992. *Homalothecium philippeanum* (Spruce) Schimp. - SH121:
EF531069, EF530994. SH310: EF531074, EF531000. SH315: EF531071, EF530996. SH316:
EF531073, EF530999. SH317: EF531070, EF530995. SH323: EF531072, EF530997. SH389:

*Homalothecium sericeum* (Hedw.) Schimp. s.str. - Hap1, Hap2, Hap3, Hap4, Hap5, Hap6,
Hap7, Hap9, Hap10, Hap17, Hap18, Hap20: Désamoré & al. (2012). SH35: EF531061,
EF531007. SH319: EF531066, EF531012. SH324: EF531067, EF531013. SH359: EF531060,
EF531006. SH360: EF531062, EF531008. SH393: EF531059, EF531005. SH394: EF531058,
EF531004. **OUTGROUP: Brachytheciastrum velutinum** (Hedw.) Huttunen& Ignatov –
SH78: EF531033, EF530965.
Figure captions:

**Figure 1.** Strict consensus of 887 equally parsimonious trees resulting from the MP analysis of *rpl16* and *atpB-rbcL* in the moss genus *Homalothecium*. Thick branches indicate bootstrap support above 50.

**Figure 2.** Variation in leaf margin denticulation in the alar region of branch leaves in *Homalothecium sericeum* s.str. (Sweden. Södermanland, Utö, 15 May 2010, *L.*Hedenäs, S: B175290). Scale: 50 µm.

**Figure 3.** A-D: *Homalothecium mediterraneum* (Greece. Crete, Chania, *Frahm K-158*, Herb. J.-P.Frahm BONN); E-H: *H. mandonii* (Canary Islands. El Hierro, Riscos de Sabinosa, March 1906, *C.*J.Pitard, S: B185186). A, E: lower exostome seen from the outside – note the exostome borders, visible as semi-translucent marginal portions of each tooth; B, F: branch leaves; C, G: leaf margin in alar region; D, H: median leaf lamina cells. Scales: a: 50 µm (A, E); b: 1 mm (B, F); c: 50 µm (C, D, G, H).
Figure 2
Click here to download Figure: Homalotheicum se