

## A reappraisal of *Masonhalea* (*Parmeliaceae*, *Lecanorales*) based on molecular and morphological data

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**Abstract:** Previous studies have identified a close relationship between the monospecific *Masonhalea richardsonii* and *Tuckermannopsis inermis*. However, formal taxonomic changes were postponed until existing sequence data could be confirmed. Here we validate these data and discuss the transfer of *T. inermis* to *Masonhalea* (made by Lumbsch *et al.* in Thell & Moberg 2011), consider the morphological, anatomical and biogeographic similarities and differences between these two taxa. The two *Masonhalea* species both produce lateral apothecia, marginal pycnidia, a layer of cortical tissue beneath the pycnidial wall and bacillariform conidia.

**Key words:** cetrarioid, generic concept, lichens, molecular systematics, phylogeny, taxonomy

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### Introduction

The species *Masonhalea richardsonii* (Hook.) Kärnefelt is a unique taxon, which forms a foliose, prostrate, cartilaginous thallus, growing unattached and rolling freely, that is, vagrant, on the tundra, where it is spread primarily by wind (Fig. 1A) (Kärnefelt 1977, 1979; Thomson 1984; Kärnefelt *et al.* 1992). The unusual morphology attracted the attention of several lichenologists, resulting in its placement in several other genera, including:

*Cornicularia*, *Evernia*, *Everniopsis*, *Platysma* and *Parmelia* (Kärnefelt 1977). More recently, this taxon was segregated from *Cetraria* Ach., and the monotypic genus, *Masonhalea* Kärnefelt, was established based on several characters, including the presence of large pseudocyphellae, which form irregular patches on the lower surface of the thallus (Kärnefelt 1977). Furthermore, the lateral position of the apothecia in *M. richardsonii* differs from that of *Cetraria*, where they appear submarginally on the upper side. In addition, the short cylindrical or bacillariform conidia of *M. richardsonii* differ from the longer and fusiform, oblong citriform conidia typical of *Cetraria* s. str. (Thell 1995b). Finally, the secondary chemistry of *M. richardsonii* is unusual among cetrarioid lichens, being characterized solely by alectoronic acid (Kärnefelt 1977, 1979; Kärnefelt & Thell 1993). Early molecular studies based on ITS and  $\beta$ -tubulin sequence data, together with morphology, identified *M. richardsonii* as occupying an isolated position among the core of cetrarioid lichens (Thell *et al.* 2002a, b; Mattsson & Articus 2004). However, subsequent studies that included additional species from the cetrarioid clade (Thell *et al.* 2009; Nelsen

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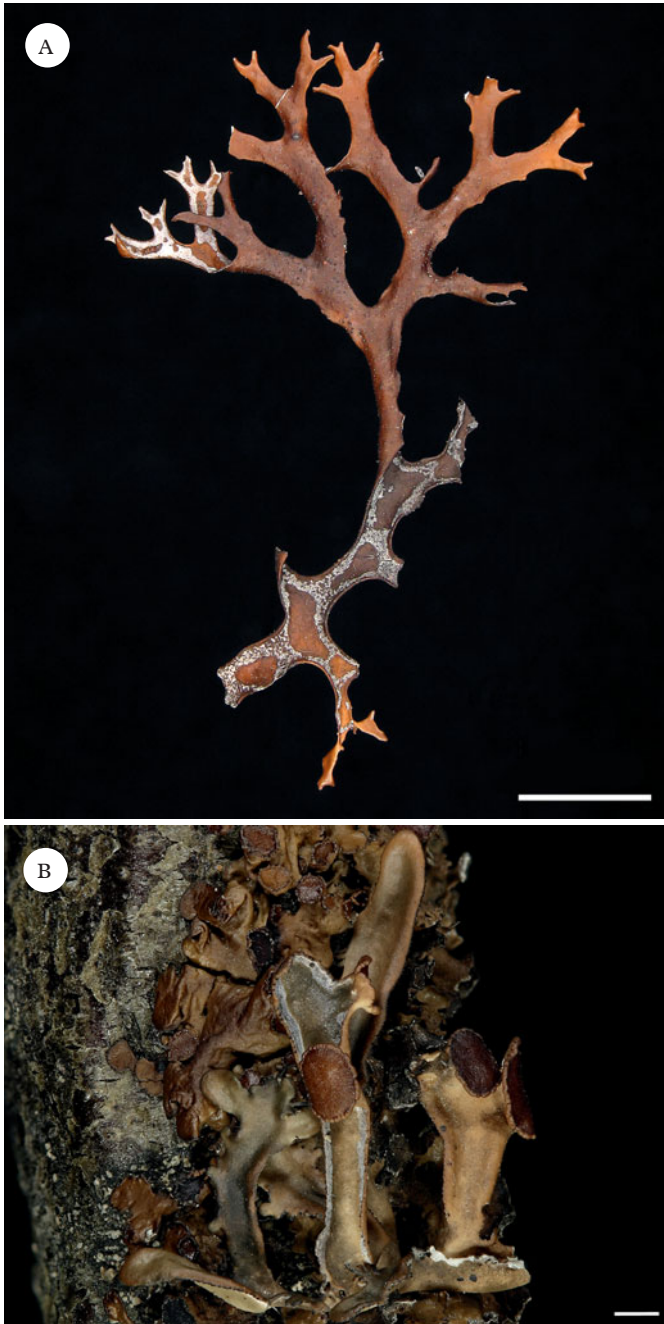


FIG. 1. A, *Masonhalea richardsonii*, Canada, Northwest Territories, Artillery Lake. *J. W. Thomson & J. A. Larsen* 92 (LD-1008288); B, *Masonhalea inermis*, USA, Alaska, Seward Peninsula, Nome area, *I. Kärnefelt* (LD-1035952). Photographs by Patrik Frödén, Lund. Scales: A = 10 mm; B = 1 mm. In colour online.

*et al.* 2011a) demonstrated a sister group relationship between *M. richardsonii* and *Tuckermannopsis inermis* (Nyl.) Kärnefelt.

Recently, the genus *Masonhalea* was expanded to include the species *Masonhalea inermis* (Nyl.) Lumbsch *et al.* (Thell & Moberg 2011). This species forms an erect, foliose to subfoliose thallus, which primarily occurs on twigs, but occasionally on soil (Krog 1973; Kärnefelt 1979; Thomson 1984; Kärnefelt *et al.* 1992; Thell & Moberg 2011). The fatty acids, protolichesterinic and lichesterinic acid, are produced in the medulla of *M. inermis*, both of which occur in a wide range of cetrarioid taxa (Kärnefelt 1979; Kärnefelt & Thell 1993). *Masonhalea inermis* (Fig. 1B) had previously been considered a form of *Cetraria crispa* (Ach.) Nyl., a synonym of *C. ericetorum* Opiz, where it was classified as *Cetraria crispa* f. *inermis* Nyl. Krog (1973) elevated it to the species level as *Cetraria inermis* (Nyl.) Krog. At this time, Krog (1973) noted the production of lateral apothecia by *C. inermis* and used this to infer a close relationship with *Cetraria subalpina* Imshaug, another species which she considered to produce lateral apothecia. However, Kärnefelt (1979) characterized the apothecia of *C. inermis* as marginal on lateral branches, a typical position for cetrarioid lichens, and stated that *C. inermis* and *C. subalpina* had few characters in common. Later, however, Kärnefelt *et al.* (1993) placed these two species together in *Tuckermannopsis*, based on the absence of a ring structure in the ascus and the production of subglobose ascospores. Subsequently, Thell *et al.* (2009) demonstrated a distant relationship between these two taxa based on molecular sequence data, and both Thell *et al.* (2009) and Nelsen *et al.* (2011a) have instead suggested a close relationship between *M. inermis* (as *T. inermis*) and *M. richardsonii*. The studies of Thell *et al.* (2009) and Nelsen *et al.* (2011a) both suggested that *M. inermis* (as *T. inermis*) was not closely related to the type species of *Tuckermannopsis*, *T. ciliaris* (Ach.) Gyelnik. For taxonomy to reflect phylogeny, it became necessary to either describe a new genus for *T. inermis* or to place it in *Masonhalea*. This taxon was then transferred to *Masonhalea* by Lumbsch *et al.* (Thell & Moberg 2011);

however, co-authors for the new combination were not included in that publication.

Here we highlight anatomical, morphological, chemical and ecological similarities between these two taxa and draw comparisons with variation present in other cetrarioid genera. In addition, we have obtained new DNA sequences from both *M. richardsonii* and *M. inermis* and find that the newly obtained sequences validate the originals, thereby supporting the monophyly of *M. inermis* and *M. richardsonii*.

## Materials and Methods

### Characterization of phenotype and habitat

A thorough summary of the morphological, anatomical, chemical, ecological, and biogeographical characteristics of these taxa, and other cetrarioid genera, was compiled. These data were drawn from numerous publications cited throughout the text and were used to compare and contrast the two *Masonhalea* species, as well as to discuss the variability of these characters in other cetrarioid genera.

### Molecular phylogeny

We also sought to verify the apparent sister relationship between *M. inermis* and *M. richardsonii*. New ITS sequences were generated for two species: *Masonhalea inermis*, USA, Alaska, Copter Peak, Plot 4, *C. Hampton-Miller* 912 (LD), GenBank Acc. No. JQ361046; *Masonhalea richardsonii*, USA, Alaska, Steese Highway, *S. A. Harris* 4597 (F), GenBank Acc. No. JQ361047. GenBank accession numbers for other taxa are included in Nelsen *et al.* (2011a). All DNA extraction, PCR reaction, purification, cycle-sequencing, precipitation and sequencing protocols follow Leavitt *et al.* (2011) and Nelsen *et al.* (2007, 2011b). New ITS sequences were added to the full alignment (ITS, nuLSU, mtSSU, RPB1) of Nelsen *et al.* (2011a); sequences were manually aligned, and ambiguous regions removed. A maximum likelihood analysis (ML) and Bayesian analysis were conducted in RAxML 7.2.8 (Stamatakis 2006) and MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), respectively. Substitution models and other settings for the analyses followed Nelsen *et al.* (2011a) and were performed using the CIPRES Science Gateway 3.1 (Miller *et al.* 2010).

## Results

We briefly highlight the similarities and differences between the *Masonhalea* species, and note the variability of these characters in other cetrarioid genera.

### Growth form

One of the most noticeable differences between *M. inermis* and *M. richardsonii* is their growth form. Whereas both have foliose thalli with weakly channelled lobes, *M. richardsonii* produces prostrate, unattached thalli with irregularly to weakly dichotomously branching lobes, and *M. inermis* is characterized by an erect, foliose thallus that is attached to its substratum and produces unbranched to weakly branched lobes (Krog 1973; Kärnefelt 1977, 1979; Thomson 1984; Kärnefelt *et al.* 1992). Macroscopic growth form is known to be quite variable within the cetrarioid core. In some genera, such as *Cetraria* s. str. (Kärnefelt 1979) and *Nephromopsis* (Randlane *et al.* 1995; Randlane & Saag 1998), growth form is conserved; in other genera, such as *Cetrariella* (Kärnefelt *et al.* 1992) and *Kaernefeltia* (Thell & Goward 1996) species vary greatly in their vegetative morphology. Furthermore, the species *Cetraria aculeata* varies from fruticose to foliose, depending on its habit (attached vs. vagrant) (Pérez-Ortega *et al.* 2012). Consequently, macroscopic differences in thallus morphology are known within other genera, and even species within the cetrarioid core.

### Upper cortex

The two *Masonhalea* species also possess different types of upper cortex. *Masonhalea inermis* produces a single-layered upper cortex composed of paraplectenchyma (Kärnefelt *et al.* 1992, 1993), whereas *M. richardsonii* has a bi-layered upper cortex, composed of a layer of pachydermatous paraplectenchyma produced above a layer of prosoplectenchyma (Kärnefelt 1977; Kärnefelt *et al.* 1992). The production of a bi-layered upper cortex is not restricted to *M. richardsonii* as several other cetrarioid species are known to produce a bi-layered upper cortex (Kärnefelt *et al.* 1992, 1993). We speculate that the thickened, and dense, bi-layered arrangement of cortical hyphae in *M. richardsonii* may be related to its vagrant lifestyle. For instance, a thickened cortex in *Cetraria aculeata* has been viewed as a response to its vagrant life-

style (Pérez-Ortega *et al.* 2012). Although the number of cortical layers is conserved in some cetrarioid genera, such as *Allocetraria* (Thell *et al.* 1995a), *Tuckermannopsis* (Kärnefelt & Thell 2001) and *Vulpicida* (Mattsson 1993), it is known to be variable in others. For example, the genus *Cetraria* s. str. is composed of species with a uni-, bi- or even tri-layered cortex, and the number of cortical layers is even known to be variable within the species *C. aculeata* (Kärnefelt *et al.* 1992; Pérez-Ortega *et al.* 2012).

### Pseudocyphellae

Both *Masonhalea* species bear conspicuous, confluent pseudocyphellae on the lower surface, albeit of different shapes and in different locations. In *M. richardsonii* they form irregular patches or areas, whereas in *M. inermis* they appear as a continuous line on a flat border along the margin (Fig. 1A). Some variation in the presence or absence of pseudocyphellae, as well as location, is known among other cetrarioid genera. The genus *Cetrellopsis*, which appears embedded in a paraphyletic *Nephromopsis* (supplementary Fig. 1; Thell *et al.* 2009; Nelsen *et al.* 2011a), forms pseudocyphellae on both surfaces, while *Nephromopsis* has pseudocyphellae solely on the lower cortex (Randlane *et al.* 1995; Randlane & Saag 1998). The genus *Allocetraria* also exhibits variability with *A. flavonigrescens* lacking pseudocyphellae, and *A. ambigua* and *A. sinensis* developing them marginally (Thell *et al.* 1995a). Finally, in the genus *Cetraria*, pseudocyphellae are situated on the lower cortex, but *C. islandica* is known to also produce marginal pseudocyphellae.

### Ascomata

Both *Masonhalea* species bear apothecia in a lateral position. This is quite rare among cetrarioid lichens which usually have sub-marginal apothecia, most commonly laminal on the upper side along the margins, or marginally on the lower side but later turning upwards in nephromoid apothecia (*Nephromopsis*) (Kärnefelt *et al.* 1992), suggesting that the lateral position of the apothecia is a

synapomorphy of *Masonhalea*. The two *Masonhalea* species differ in the number of exciple layers present in their apothecia (Kärnefelt *et al.* 1992). Differences in this character are also known to occur within, for example, the genera *Cetraria* s. str. and *Nephromopsis* (Kärnefelt *et al.* 1992; Randle *et al.* 1995; Randle & Saag 1998).

### Hamathecium, asci and ascospores

The two *Masonhalea* species form short, straight and nearly unbranched paraphyses, which are thickened towards the tips (Thell *et al.* 1995b). This type of paraphysis is shared with a wide range of other cetrarioid taxa including *Alloctraria* and *Tuckermannopsis* (Thell *et al.* 1995b). Both *Masonhalea* species produce asci of the *Lecanora*-type (Thell *et al.* 1995b); this ascus type is widespread among cetrarioid lichens, with numerous species also producing the *Cetraria*-type of ascus, characterized by a large tholus with an amyloid ring (Thell *et al.* 1995b). *Masonhalea inermis* possesses the *Tuckermannopsis*-form of the *Lecanora*-type ascus, which is characterized by globose ascospores arranged uniseriately in a cylindrical ascus with a small tholus, broad axial body and a broad ocular chamber (Kärnefelt *et al.* 1992; Thell *et al.* 1995b). In contrast, asci of *M. richardsonii* are categorized as the *Nephromopsis*-form of the *Lecanora*-type, containing oblong-obovate ascospores (not arranged uniseriately), which are produced in narrowly clavate asci with a smaller axial body and a conical ocular chamber with a narrow beak (Kärnefelt *et al.* 1992; Thell *et al.* 1995b). Ascospore shape also generally appears conserved among species within individual genera in the cetrarioid core (Mattsson 1993). However, differences in ascospore shape among closely related cetrarioid species are known to occur in *Nephromopsis*, where most species have oblong-obovate ascospores, while a small number of taxa, such as *N. komarovii* and *N. nephromoides*, have ellipsoid ascospores (Randle *et al.* 1995; Randle & Saag 1998). Furthermore, the genus *Cetrellopsis*, which is closely related to or part of *Nephromopsis* (supplementary Fig. 1; Thell *et*

*al.* 2009; Nelsen *et al.* 2011a), is characterized by ellipsoid ascospores (Randle *et al.* 1995; Randle & Saag 1998). Kärnefelt *et al.* (1992) have suggested that spore arrangement, size of the axial body and ascospore shape are often correlated, though this has not been tested in a quantitative framework. If the evolution of these three characters is indeed correlated, it may not be surprising that *M. inermis* and *M. richardsonii* differ in these three characters.

### Pycnidia and conidia

Both *Masonhalea* species are characterized by 1-layered, raised, marginal pycnidia, with a layer of cortical tissue beneath the pycnidial wall (Kärnefelt *et al.* 1992). Several other cetrarioid genera, such as *Cetraria*, *Nephromopsis* and *Tuckermannopsis*, include species with similar pycnidia (Kärnefelt *et al.* 1992). However, both *Masonhalea* species form bacillariform conidia (Kärnefelt *et al.* 1993; Thell 1995b), which are quite rare in the cetrarioid core (Thell 1995b). Together, this combination of pycnidia and conidia appears unique in the cetrarioid core.

### Secondary chemistry

*Masonhalea richardsonii* and *M. inermis* differ in their medullary secondary chemistry, with *M. richardsonii* producing the depsidone alectoronic acid, and *M. inermis* developing the fatty acids protolichesterinic and lichesterinic acids (Kärnefelt *et al.* 1992). The production of protolichesterinic and lichesterinic acids is quite widespread in the cetrarioid core (Kärnefelt & Thell 1993). Though less common, alectoronic acid is also known from a range of cetrarioid genera (Kärnefelt & Thell 1993), including *Cetrariella* (Kärnefelt *et al.* 1992; Thell 1995a; Kärnefelt & Thell 2000; Rico *et al.* 2005), *Nephromopsis* (Randle *et al.* 1995) and *Tuckermannopsis* (Kärnefelt & Thell 2001). Interestingly, the species *Cetrariella commixta* is known to be variable with respect to the presence or absence of alectoronic acid (Kärnefelt *et al.* 1992; Thell 1995a); consequently, the production of alectoronic acid is not constant

in the genus *Cetrariella* (Kärnefelt & Thell 2000), or even the species *C. commixta* (Rico *et al.* 2005). Somewhat similarly, the species *Nephromopsis pallescens* is known to develop either alectoronic acid or protolichesterinic and lichesterinic acids in the medulla, or occasionally, both sets of substances (Randlane *et al.* 1995). Finally, within the genus *Tuckermannopsis*, *T. americana* produces alectoronic acid (Thell 1998; Kärnefelt & Thell 2001), while the closely related *T. orbata* is characterized by protolichesterinic acid (Kärnefelt & Thell 2001).

### Ecology and distribution

Both *Masonhalea* species share a similar northern Beringian distribution, although the distribution range of *M. inermis* is smaller, except for an isolated occurrence in northern Norway (Krog 1973; Kärnefelt 1977, 1979; Tønsberg & Elvebakk 1993). Despite this similarity, the two species differ ecologically, with *M. richardsonii* occurring vagrant on soil, and *M. inermis* instead occurring mostly on twigs, but occasionally also on soil (Krog 1973; Kärnefelt 1977, 1979; Thomson 1984; Thell & Moberg 2011). A number of other cetrarioid genera, such as *Allocetraria* (Thell *et al.* 1995a), *Arctocetraria* (Kärnefelt 1979; Kärnefelt *et al.* 1993) and *Vulpicida* (Mattsson 1993; Mattsson & Lai 1993), also include both corticolous and terricolous species or individual species that occur in both habitats.

A summary of the morphological, anatomical, chemical and ecological characteristics of the two *Masonhalea* species is provided in Table 1.

### Phylogeny

The final concatenated alignment consisted of 2628 unambiguously aligned characters, and the topology recovered (Appendix Fig. A1) is consistent with that in fig. 2 of Nelsen *et al.* (2011a). The newly generated ITS sequences are strongly supported as sister to the earlier *M. inermis* and *M. richardsonii* sequences, thereby confirming the original sequences. Subsequent sequencing of the

mtSSU, nuLSU and RPB1 loci (S. D Leavitt & H. T. Lumbsch, unpublished data) of the recently collected *M. inermis* specimen produced sequences nearly identical to those used in Thell *et al.* (2009) and Nelsen *et al.* (2011a), thereby confirming their identity. As in Thell *et al.* (2009) and Nelsen *et al.* (2011a), *M. inermis* is strongly supported as being sister to *M. richardsonii* (Appendix Fig. A1).

### Taxonomy

We previously considered transferring *T. inermis* to *Masonhalea*, and indeed proposed this combination in the original Nelsen *et al.* (2011a) submission. Ultimately, we refrained from making this change until our results could be validated with new DNA sequences (Nelsen *et al.* 2011a). However, this change was included and printed in Thell & Moberg (2011). Thus, the publication of the name *Masonhalea inermis* (Nyl.) Lumbsch *et al.* in Thell & Moberg (2011) is the valid combination according to article 33:7 of the International Botanical Code (McNeill *et al.* 2006), not Nelsen *et al.* (2011a) as printed in Thell & Moberg (2011). We correct the authors of this combination below, following a brief summary of the revised circumscription of the genus *Masonhalea*, which largely adheres to that of Thell & Moberg (2011).

### *Masonhalea* Kärnefelt

*Bot. Notiser* 130: 101–102 (1977);—type: *Masonhalea richardsonii* (Hook.) Kärnefelt.

*Thallus* foliose to subfoliose, upper surface brown, lower surface whitish to brown, sometimes prostrate, unbranched to weakly dichotomously branching. *Pseudocyphellae* present on the lower surface; occurring in patches, or as a continuous line along the margin.

*Ascomata* zeorine, apothecia formed laterally on margins, asci cylindrical to narrowly clavate with an axial body. *Ascospores* hyaline, globose to oblong-ovate, 3.5–6.5 × 3–6 µm. *Photobiont* trebouxoid.

*Pycnidia* marginal. *Conidia* bacillariform.

TABLE 1. Summary of the numerous morphological, anatomical, chemical and ecological characteristics of the two *Masonhalea* species. Data are derived from literature sources cited throughout the manuscript.

	<i>M. inermis</i>	<i>M. richardsonii</i>
Thallus	Foliose to subfoliose, upper surface brown, with pale lower surface, unbranched to weakly branched lobes	Foliose, upper surface brown, with whitish or brown lower surface, prostrate, irregularly to weakly dichotomously branching
Substratum	Shrubs, attached	Soil, unattached
Upper Cortex	Uni-layered, paraplectenchymatous	Bi-layered, layer of pachydermatous paraplectenchyma over a prosoplectenchymatous layer
Pseudocyphellae	Present, formed marginally on lower surface in a linear fashion	Present, formed on lower surface in irregular patches
Ascomata	Regularly produced, formed laterally on margins; exciple 1–2-layered	Irregularly produced, formed laterally on margins; exciple 3-layered
Paraphyses	Short, straight, nearly unbranched, thickened near tips	Short, straight, nearly unbranched, thickened near tips
Asci	<i>Lecanora</i> -type, with amyloid plug and lacking amyloid structures in tholus. <i>Tuckermannopsis</i> -form with cylindrical ascus with small tholus, broad axial body and ocular chamber, and ascospores arranged uniseriately	<i>Lecanora</i> -type, with amyloid plug and lacking amyloid structures in tholus. <i>Nephromopsis</i> -form with narrowly clavate ascus with smaller axial body, conical ocular chamber with a narrow beak, and ascospores not arranged uniseriately
Ascospores	Globose, 3.5–5.0 × 3.5–6.0 μm	Oblong-ovate, 6.0–6.5 × 3–6 μm
Pycnidia	1-layered, raised, marginal pycnidia, with a layer of cortical tissue beneath the pycnidial wall	1-layered, raised, marginal pycnidia, with a layer of cortical tissue beneath the pycnidial wall
Conidia	Bacillariform	Bacillariform
Secondary chemistry	Protolichesterinic and lichesterinic acids	Alectoronic acid
Distribution	Beringian, and N. Norway	Beringian

**Chemistry.** Alectoronic or lichesterinic and protolichesterinic acids in the medulla.

**Substratum.** Growing attached on shrubs or unattached on soil.

**Distribution.** Beringia and northern Norway.

### **Masonhalea inermis (Nyl.) Lumbsch, M. Nelsen & A. Thell**

In Thell & Moberg (eds), *Nordic Lichen Flora* 4: 67 (2011) as *Masonhalea inermis* (Nyl.) Lumbsch et al.

*Cetraria crispa* f. *inermis* Nyl., *Bull. Soc. Linn. Normandie* 4, 1: 214 (1887);—*Cetraria tenuifolia* var. *inermis* (Nyl.) Räsänen, *Kuopion Luonnon Ystävään Yhdistyksen Julkaisuja, ser. B*, 2(6): 23 (1952);—*Cetraria inermis* (Nyl.) Krog, *Bryologist* 76: 299 (1973);—*Tuckermannopsis inermis* (Nyl.) Kärnefelt, *Bryologist* 96: 403 (1993).

### **Summary**

Macroscopically, *M. inermis* and *M. richardsonii* appear quite distinct from one another. However, upon closer examination, several morphological and anatomical similarities can be seen. Furthermore, many of the differences between *M. inermis* and *M. richardsonii* appear minor when considered in the context of the vagrant habit of *M. richardsonii* and when compared with differences included in other genera. Most of the differences observed between these two species exist among species in other cetrarioid core genera. These two species are not found on short branches, suggesting they have been separated for some time, and/or undergone a substantial amount

of molecular evolution. Consequently, these two species should be expected to have accumulated a number of differences. They appear to share a unique suite of morphological and anatomical character states among the cetrarioid core, which include the production of lateral apothecia, and bacillariform conidia formed in marginal pycnidia with a layer of cortical tissue beneath the pycnidial wall (Kärnefelt *et al.* 1992).

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## Appendix

Fig. A1. Maximum likelihood phylogram for the cetrarioid lichens with new ITS sequences for *Masonhalea inermis* and *M. richardsonii* added to the full alignment in Nelson et al. 2011a. ML bootstrap values  $\geq 70$  and Bayesian posterior probabilities  $\geq 0.95$  are listed either above or below line or left and right of slashes, respectively.

