Molecular phylogenetics and genus delimitation in the Rhizocarpaceae (lichenized ascomycetes) with focus on the *Rhizocarpon hochstetteri*-complex

A step towards a more natural circumscription of a common and understudied family

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or

Navigating the map lichens

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Abstract The Rhizocarpaceae is a globally distributed and common family of lichenized fungi. The family consists of four genera (*Catolechia, Epilichen, Poeltinula* and *Rhizocarpon*) and c. 150 accepted species, of which all but nine belong in the genus *Rhizocarpon.* Previous molecular studies have shown that *Rhizocarpon*, as circumscribed today, may be paraphyletic with the other three genera nested. New species of *Rhizocarpon* are constantly described, suggesting there are potentially much undiscovered species diversity. My aim with this study was to provide a more natural circumscription of genera in the Rhizocarpaceae, while also investigating the *R. hochstetteri* species complex in more depth, using an integrative taxonomic approach. I have analyzed 148 Rhizocapaceae specimens phylogenetically based on three loci, both nuclear and mitochondrial, and compared the supported tree topology to morphological, anatomical, and chemical data. Obtained phylogenetic results show that there are at least four well supported clades (A—D) in the Rhizocarpaceae that do not corroborate current generic circumscriptions. Additionally, the phylogeny renders *Rhizocarpon* paraphyletic with *Poeltinula, Catolechia* and *Epilichen* nested. More specifically, *Poeltinula* is nested in the *R. hochstetteri*-complex (jointly clade A). *Epilichen scabrosus* is nested within *Catolechia* with *E. glauconigellus* as their distinct sister (jointly clade B). Representatives for the main bulk of the *Rhizocarpon* species, including the type species, form their (clades A+B) sister (clade C). *Rhizocarpon oederi* and *R. pycnocarpoides* form a sister group (clade D) to the rest of the family (clades A+B+C). I also show that the *R. hochstetteri*-complex consists of at least 11 well-separated clades, a finding that provides several insights: (1) specimen identities, (2) species limits, (3) recognition of new species, and (4) that, even though there are cases of substantial overlap, there are clear trends concerning ecology, chemistry and anatomical traits between these species.

"Oder wird Jemand zugeben wollen,

dass die Natur planlos gegen die Logik der Vernuft streite?"

- *Gustav Wilhelm Körber, 1861*

1. Introduction

The Rhizocarpaceae M. Choisy ex Hafellner (Ascomycota: Lecanoromycetes: Lecanoromycetidae: Rhizocarpales) is a family of lichenized fungi that consists of the four genera *Catolechia* Flot., *Epilichen* Clem., *Poeltinula* Hafellner and *Rhizocarpon* Ramond ex DC. The family is characterized by having lecideine apothecia (i.e. having a proper exciple without a thalline margin), richly branched, anastomosing and strongly conglutinated **paraphyses**, and **asci** with a strongly amyloid tholus with an internal, apical, deeper amyloid cap (a small structure at the apex of the ascus; Honegger 1980). The ascospores, usually 8 per ascus, are 1-septate to eumuriform, hyaline to green to dark brown and are often surrounded by a gelatinous halo. Together with the Sporastatiaceae Bendiksby & Timdal, the Rhizocarpaceae constitutes the order Rhizocarpales Miądl. & Lutzoni (Miadlikowska et al. 2014).

As of today, there is only one species in *Catolechia, C. wahlenbergii* (Ach.) Flot., which can be found growing on soil and moss in shaded crevices in north-facing rock walls. *Epilichen* consists of three small parasitic species: the type species *E. scabrosus* (Ach.) Clem., *E. glauconigellus* (Nyl.) Hafellner and *E. stellatus* Triebel. *Catolechia* and *Epilichen* lack haloes around the ascospores, but are otherwise anatomically similar to the rest of the family by having *Rhizocarpon*-type asci and brown, 1-septate ascospores. Both *Catolechia* and *E. scabrosus* contain the yellow pigment rhizocarpic acid.

Poeltinula consists of four species: the type species *P. cerebrina* (DC.) Hafellner, *P. cacuminum* (Asta, Clauzade & Cl. Roux) Clauzade & Cl. Roux, *P. cerebrinella* (Nyl.) Øvstedal and *P. interjecta* (Leight.) Hafellner. None of them makes a visible thallus, but they appear as small, black apothecia with furrows of sterile tissue, growing out from calcareous rock, and have green, 1-septate, halonate *Rhizocarpon*-type spores. The genus differs from *Rhizocarpon* mainly in the shape of the apothecia, i.e. having a deeply furrowed disc.

Of the four genera, *Rhizocarpon* is by far the most speciose and consists of c. 150 species of **crustose** lichens that are mainly living on rocks in the boreal and arctic-alpine regions, but the genus also occurs throughout the temperate, subtropical, and even tropical regions. The type species, *R. geographicum* (L.) DC. (yellow map lichen), was first described by Linnaeus (1753) as *Lichen geographicus* L. The species is both abundant and globally distributed, sometimes completely dominating alpine rock surface ecosystems. Because of its ubiquity and pioneering capabilities, it has

been widely used for dating rock surfaces too young for more precise carbon dating methods (Beschel 1973, Rosenwinkel et al. 2015).

Rhizocarpon was first described by Lamarck & De Candolle (1805) and represents one of the earliest described lichen genera. They grow mainly on siliceous rock, but some are obligate parasites on other crustose lichens, and some start their lives as parasites until they have consumed their host and then carry on autonomously (Holtan-Hartwig & Timdal 1987, Timdal & Holtan-Hartwig 1988, Poelt 1990). A typical *Rhizocarpon*-thallus is areolate (or sometimes more continuous and cracked) with a conspicuous, usually black **hypothallus** from where the areolae and apothecia originate. The genus name stems from Greek, *Rhizo-* (rhiza) meaning "root" and *-carpon* (karpón) meaning "fruit". Some species are mainly sterile and spread with **isidia** or **soredia**.

Box 1. Some lichen terms

Crustose: Growth form, tightly attached to substrate

Thallus: The "body" of the lichen. Consists of fungal tissue and algae.

Hypothallus: Pure fungal tissue growing as a mat beneath the thallus, in *Rhizocarpon* usually black.

Apothecia: Type of fruiting body of ascomycetes.

Areole: Part of a crustose thallus, has an outer cortex, an algal layer and a medulla of fungal tissue

Hymenium: The spore producing cell layer in the apothecia, consists of asci and paraphyses.

Ascus: A fertile spore-producing cell in the hymenium.

Paraphyse: A sterile supportive hypha in the hymenium.

Isidia: Vegetative lichen diaspore with a cortex, contains both algae and fungal components

Soredia: Smaller vegetative diaspore without a cortex

Historically, about 358 species names have been introduced to *Rhizocarpon*, of which 146 are found in current checklists and floras (Andreev et al. [2003; Russia], Esslinger [2018; Canada and continental USA], Fryday [2019; southern subpolar region], Galloway [2007: New Zealand], Hafellner [1995, plus updates; Lauromacaronesia], Kristinsson et al. [2010; panarctic region], McCarthy [2018; Australia], Nimis & Martellos [2019; Italy], Nimis et al. [2018; the Alps], Westberg et al. [2021; the Nordic countries], Ohmura & Kashiwadani [2018; Japan], Øvstedal & Lewis Smith [2001; Antarctica and South Georgia], Smith et al. [2009; Great Britain and Ireland]). The remaining names are either synonymized, placed in other genera or at infraspecific rank, or have simply not been treated in modern checklists or floras. New species are regularly discovered and old species concepts are redefined (e.g. Fryday & Kantvilas 2012, McCarthy & Elix 2014, McCune et al. 2016, Davydov & Yakovchenko 2017). The Nordic checklist (Westberg et al. 2021), which currently contains 73 species of *Rhizocarpon*, is mainly based on the revisional works by Timdal & Holtan-Hartwig (1988) and Ihlen (2004). About 50% of these are found in checklists from other regions.

Even though *Rhizocarpon* is both widespread and abundant, there are no comprehensive molecular phylogenetic studies focusing on genus or family level relationships. At the onset of this study, there were 118 sequences from 30 *Rhizocarpon* species available in GenBank (accessed 2019-03-28), of which 71 sequences were the internal transcribed spacer (ITS) of nuclear ribosomal DNA and 20 mitochondrial small subunit (mtSSU) ribosomal DNA; the remaining being fewer sequences of nrLSU, nrSSU, RPB1, and RPB2. Most early published *Rhizocarpon* sequences were provided for phylum and class level phylogenetics (e.g., Lutzoni et al. 2001; Buschbom & Mueller 2004; Miadlikowska et al. 2014). At the other end of the scale, McCune et al. (2016) and Davydov & Yakovchenko (2017) provided molecular phylogenies in order to support descriptions of new species.

One species group of special interest for this study is *R. hochstetteri* (Körb.) Vain. and related species. The complex is characterized by having a sharp apothecial margin, 1-septate hyaline spores and a non-amyloid medulla. Specimens with this combination of characters are henceforth referred to as the *R. hochstetteri*-complex. The group generally prefers humid habitats and can often be found growing near trickling water or streams, humid deciduous forests or in alpine or coastal habitats with frequent precipitation. Beyond that, the species complex is variable, especially regarding thallus morphology (areolate to cracked to smooth) and colour (pale gray to pale brown to dark brown), and spore size (~17–27 × 7–13 µm). Morphological, anatomical and chemical studies of the group by Fryday (2002) introduced three new taxa: *R. caesium* Fryday, *R. infernulum* f. *infernulum* (Nyl.) Lynge, and *R. infernulum* f. *sylvaticum* Fryday. Other species in the complex include the large-spored *R. hensseniae* Brodo and the poorly understood *R. expallescens* Th. Fr.

Ihlen & Ekman (2002) produced a phylogeny of the Rhizocarpaceae based on ITS and mtSSU sequences of 15 species, including three of the genera, and discussed character evolution within the family. In their study, *R. hochstetteri* is the phylogenetic sister to *P. cerebrina*. These species do not resemble each other superficially. They do share one key character, however: sharply delimited pigmented apical caps on the otherwise hyaline paraphyses, a character that is mostly lacking in the rest of *Rhizocarpon*, but is also found in *Catolechia* and *Epilichen*. Pigmented paraphyse caps are also reported in some yellow species (Runemark 1956). In Ihlen & Ekmans study, the *Poeltinula-R. hochstetteri*-clade was either sister to the rest of the family or sister to the rest of *Rhizocarpon*, with *Catolechia* as sister to the rest. In the large phylogeny of the Lecanoromycetes O.E. Erikss. & Winka by Miadlokowska et al. (2014), *Poeltinula* is not included, but the *R. hochstetteri* shows up as sister to *Catolechia*, and the metallophilic species *R. oederi* (Weber) Körb. is sister to the rest of the family.

According to the phylogenies presented by Ihlen & Ekman (2002) and Miadlikowsla et al. (2014), *Rhizocarpon*, as currently circumscribed is paraphyletic and the relationships between the genera in the

family are unclear. Additionally, in both studies, the *R. hochstetteri*-complex seems to be more closely related to one of the smaller genera than to the rest of *Rhizocarpon*. Here, I use an integrative taxonomic approach, including molecular phylogenetics, chemistry, morphology, and anatomy to investigate the diversity in the *R. hochstetteri*-complex and its relation to the rest of *Rhizocarpon*. I include specimens covering the variation of the entire family and present a dense taxon sampling of the *R. hochstetteri*-complex with the aim to discover, understand, delimit, and circumscribe the species in the complex and the genera of the Rhizocarpaceae.

2. Material and Methods

2.1 Taxon sampling

In this study, I have included 148 specimens of the Rhizocarpaceae, both freshly collected from field work in Norway during the summers of 2019 and 2020 and from various herbaria (BG, E, GZU, L, MSC, O, OSU and UPS) (Appendix 1: Table 1). Of these, 75 specimens from the *R. hochstetteri*-complex, including the holotypes for *R. caesium* and *R. infernulum* f. *sylvaticum*, were subjected to anatomical and chemical investigation. Accessions are referred to with DNA extraction numbers or GenBank IDs. I aimed to sequence three genetic markers (ITS, mtSSU, MCM7) for each accession. In addition, I included sequences from the barcoding project OLICH at NHM (Marthinsen et al. 2019) and some sequences from GenBank. However, since species determinations of the latter were often not verifiable, sequences from GenBank were kept to a minimum. Accessions without all three markers are referred to as "orphans". Specimens and sequences included in the study were selected to represent the breadth of variation of spore septation and pigmentation, chemistry, and ecology.

2.2 Molecular work

2.2.1 DNA sequence production

I extracted DNA from 1–5 apothecia per specimen, or a pencil-tip sized thallus piece if apothecia were scarce or missing, using the E.Z.N.A.® SP Plant DNA Kit (Omega Bio-Tek, Georgia, U.S.A) following a modified protocol (Bendiksby & Timdal 2013). I used Illustra™ puReTaq Ready-To-Go™ PCR Beads

(GE Healthcare, Buckinghamshire, UK) to amplify three markers (ITS, mtSSU and MCM7), following the manufacturer's protocol, (except for splitting the solution from each bead into two tubes to double the number of reactions (i.e., 9.95 ul water, 1.25 ul magnesium, 0.3 ul of each primer, 0.7 ul template DNA = 12.5 ul per reaction). The ITS and MCM7 loci were amplified as single fragments using the primer pairs ITS1F/ITS4 (Gardes & Bruns 1993; White et al. 1990) and MCM7-709for/MCM7-1348rev (Schmitt et al. 2009), respectively. The mtSSU locus was mostly amplified in two fragments using the primer pairs mtSSU1/mtSSU-RhiR (Zoller et al. 1999); this study: 5'-AAT AAC ATA CTT CAC TAC TGG T3') and mtSSU-RhiF/mtSSU3R (this study: 5'ACC AGT AGT GAA GTA TGT TAT T-3'; Zoller et al. 1999). For accessions 11041 and 11044, only half of the ITS marker was amplified using the primer pair ITS3/ITS4 (White et al. 1990).

The PCRs ran for 35 cycles with the following cycling conditions: denaturation 30 seconds at 95°C, annealing 30 seconds at 50°C for MCM7 and 58°C for ITS and mtSSU (unsuccessful PCRs were tried again at 56°C annealing temperature), and elongation for 60 seconds at 72°C. The PCR program also included an initial denaturation step at 95°C for 7 minutes and a final elongation step at 72°C for 7 minutes. Positive PCR products were cleaned using ExoStar-IT (GE Healthcare, Buckinghamshire, UK) and sequenced at Macrogen Europe (Amsterdam, The Netherlands), prepared according to the producer's protocol. Raw chromatograms were edited/assembled using de novo assembly in Geneious 6.1.8 (https://www.geneious.com), and manually corrected. In cases where mtSSU had been sequenced in two fragments, I used the map-to-reference function with a fully sequenced mtSSU from 9330 (*R. saurinum* (W.A. Weber) Bungartz), chosen because it had very high-quality chromatograms.

2.2.2 DNA sequence analysis

Sequences were aligned using MUSCLE (Edgar 2004) bundled in AliView (Larson 2014), and manually adjusted in cases where the algorithm failed to achieve homologous comparisons. Substitution models were determined using PhyML (Guindon 2010) through PartitionFinder 2.1.1 (Lanfear et al. 2012, 2016) with lowest Aikake information criterion, corrected for small sample size (AICc), and otherwise default settings. The protein coding MCM7 was partitioned according to codon positions and the same alignment was used for both the FO and the MO concatenations (position 1: TVMEF+G; position 2: HKY+I; position 3: K81UF+G). In the ITS alignment, I kept the end of 18S (the region preceding the ITS locus), due to a possibly informative insertion present in 22 accessions, and partitioned the alignment according to ITS1 and ITS2 (GTR+I+G in both FO and MO), 5.8S (K81+I+G in FO; TrNEF+I+G in MO) and the

18S insert (TrN in FO; F81+I in MO). The mtSSU locus (HKY+G in FO; TVM+I+G in MO) was not partitioned. Since RAxML only allows for one rate of heterogeneity, I used a democratic method and chose the rates most partitions got from the analysis for all partitions. Moreover, as MrBayes allows only a subset of models, I used the default substitution model settings when the best model was not available.

I inferred phylogenies using two concatenated data sets: one with 148 accessions and many orphans (MO) that include representatives from throughout the family (36 (24,2%) had all three markers, 62 (41,9%) had two markers (ITS/mtSSU=52, ITS/MCM7=7, mtSSU/MCM7=3) and 53 had only one marker (ITS=50, mtSSU=2, MCM7=1)), and a second, smaller data set with 50 accessions and few orphans (FO), still representing all major clades (32 (64%) had all three markers, 10 (20%) had two markers (ITS/mtSSU=3, ITS/MCM7=4, mtSSU/MCM7=3) and 8 (16%) had only one marker (ITS=7, MCM7=1)). In total, I include 141 ITS sequences (67 produced by myself), 91 mtSSU (46 by me) and 46 MCM7 (45 by me). In the FO phylogeny, *Sporastatia testidunea* (Ach.) A. Massal. is used as an outgroup.

For the maximum likelihood (ML) phylogenies, separate gene trees and species trees were inferred with RAxML-NG-MPI v. 1.0.2. (https://github.com/amkozlov/raxml-ng/releases/tag/1.0.2; Kozlov et al. 2019, Stamatakis 2006), with 10 random starting trees and 1000 bootstrap replicates. The same concatenated alignments used in RAxML were also analyzed with Bayesian inference using MrBayes 3.2.7a (github.com/NBISweden/MrBayes/tree/v3.2.7a; Ronquist et al. 2012). Analyses ran for 12.5 million (FO) and 25 million (MO) generations. Convergence was assessed using effective samples size (ESS) values in Tracer v 1.7.1 (Rambaut et al. 2018) and average standard deviation of split frequencies (ASDSF) in the MrBayes analyses. I assumed convergence if posterior and likelihood ESS values were above 200 and ASDSF reached values below 0.01. I used Figtree v 1.4 (Rambaut 2012) to visualize the phylogenies.

2.3 Anatomy and Chemistry

Spore measurements and hymenial characters are based on microscopic examinations of cross sections of apothecia in water. Lengths and widths were measured at the longest/widest points, excluding the halo. In cases where the spore shape was very skewed, I measured width at the septum. I aimed to measure at least 10 spores per specimen when the material allowed it. Presence/absence of crystals was examined

with polarizing filters in the microscope. Spore measurements were visualized using geom_jitter standard deviation divided by three offset to increase visibility of individual dots (Fig. 3) and geom_violin (Fig. 6) in ggplot2 (Wickham 2016).

Secondary metabolites were examined using thin-layer chromatography (TLC) in solvent system B', performed according to the methods of Culberson (1972), Menlove (1974) and Culberson & Johnson (1982).

3. Results

3.1 Phylogenetic results

Except for the placement of 218 (*R. oederi*) and 358 (*R. pycnocarpoides* Eitner) in the FO ITS tree (bootsrap support (BS)=80) (Appendix 2: Fig. 1), there were no supported incongruences between the gene trees, and the individual alignments were concatenated resulting in a 2508 bp data set (FO) and a 2547 bp data set (MO).

The RAxML and MrBayes results generated using the FO data set showed no supported topological incongruences. Four highly supported major clades appear (Fig. 1): **(A)** the *R. hochstetteri*-complex forms a clade with *P. cacuminum* and *P. cerebrina* nested within (posterior probability (PP)=0.99/BS=96) and subclades A6, A7 and A8 form a highly supported clade (PP=1/BS=92); **(B)** *Catolechia* and *Epilichen* form a highly supported clade, with *E. glauconigellus* as sister to *Catolechia* and *E. scabrosus* (PP=1/BS=99); **(C)** the group representing the rest of *Rhizocarpon* is highly supported as monophyletic (PP=1/BS=97); and **(D)** *R. oederi* and *R. pycnocarpoides* form a clade with maximum support. The relationships between the major clades are unresolved.

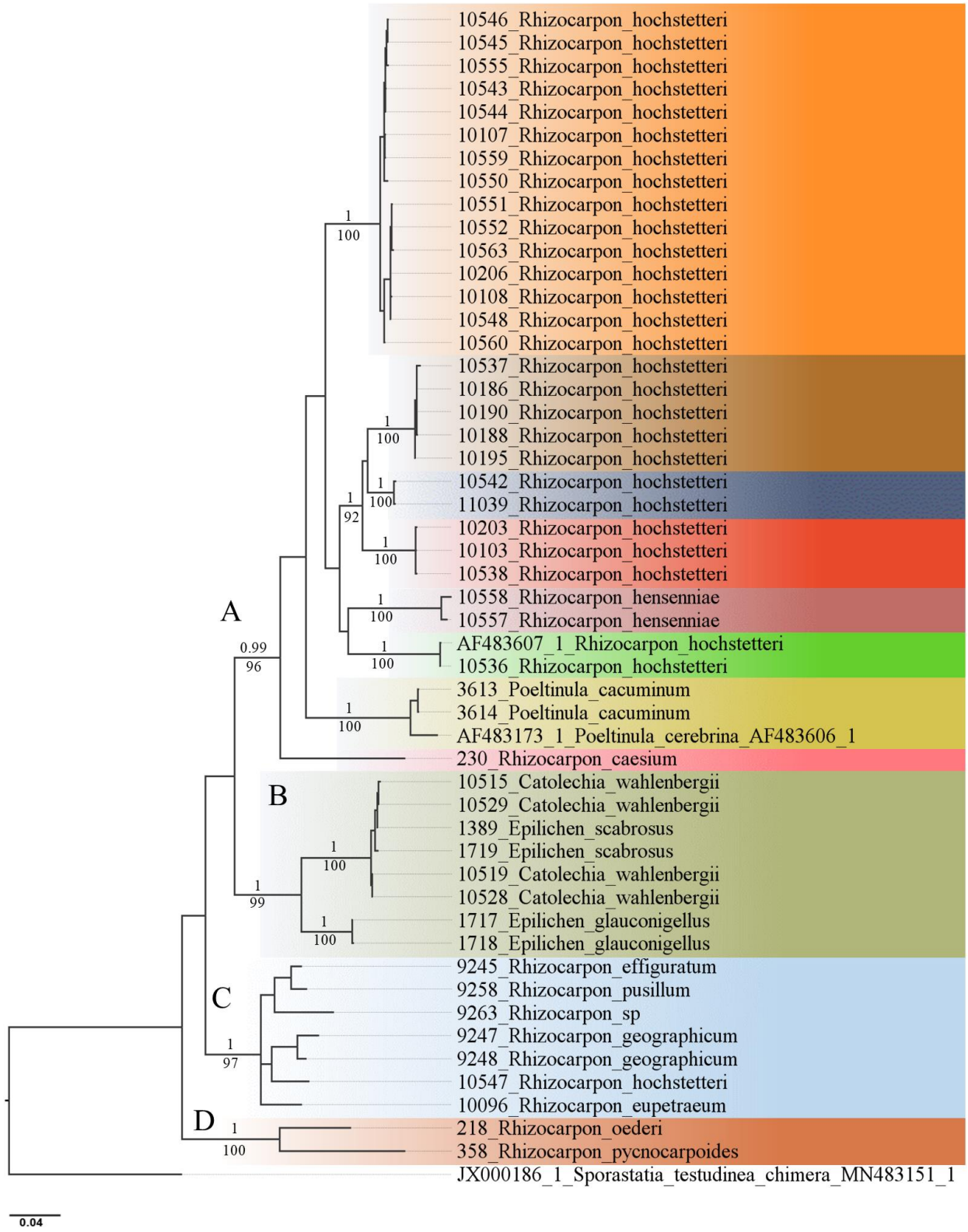

Figure 1. Phylogram (50% majority rule consensus) of three concatenated markers (ITS, mtSSU & MCM7) with few orphans (FO). Colours of subclades in clade A correspond to the colours/subclades in the MO phylogeny (Fig. 2a). The PP>0.95/BS>70 support values are indicated on branches. *Sporastatia testudinea* (MCM7+ITS) is used as an outgroup. Scale bar indicates expected changes per site.

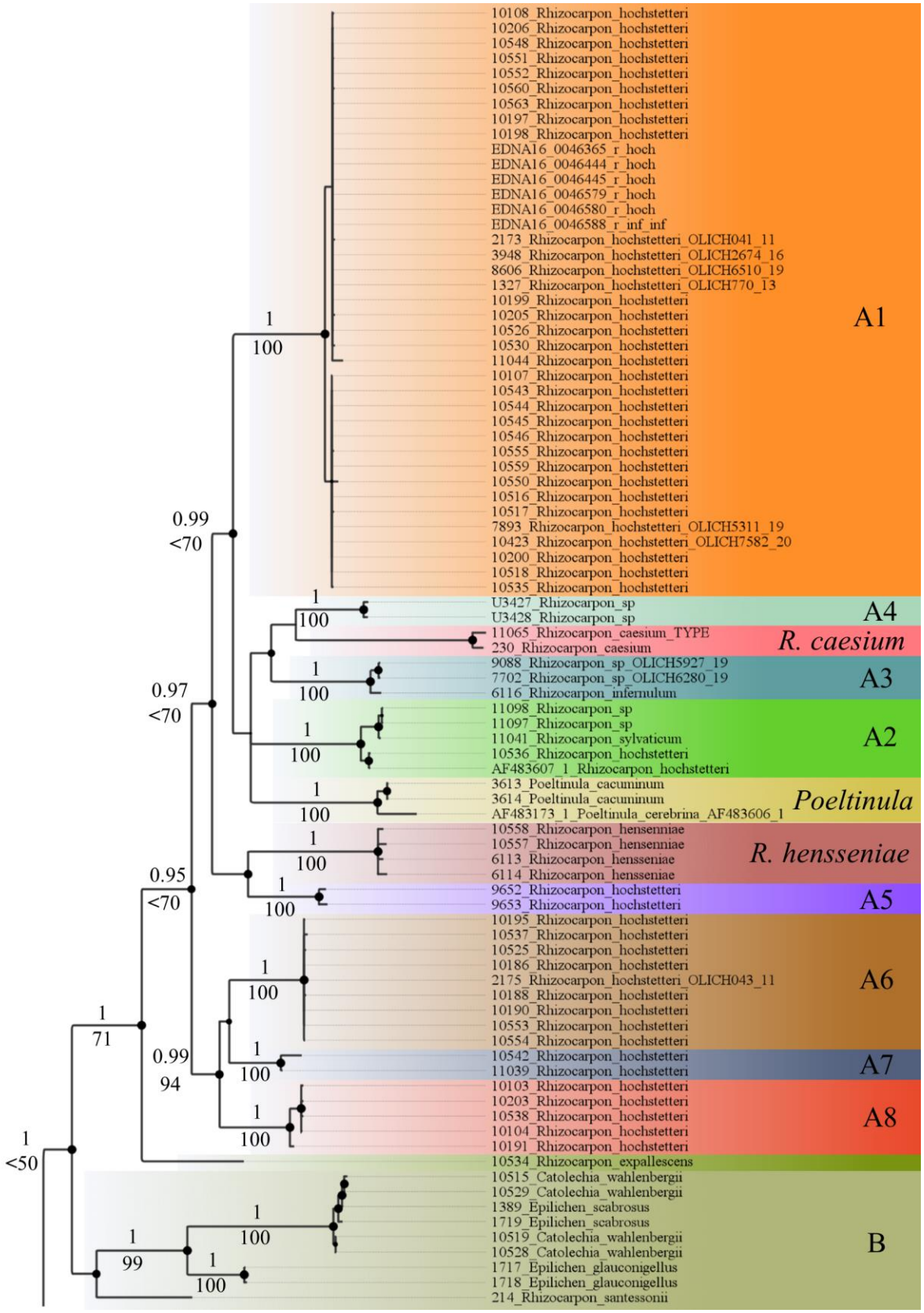

Figure 2a. Phylogram (50% majority rule consensus) of three concatenated markers (ITS, mtSSU & MCM7) with many orphans (MO). PP>0.95/BS>70 support values indicated on branches and PP support by node shape sizes. Subclades in clade A consisting of *R. hochstetteri*, *R.* sp. or otherwise uncertain names are numbered (A1-A8). Continued in 2b.

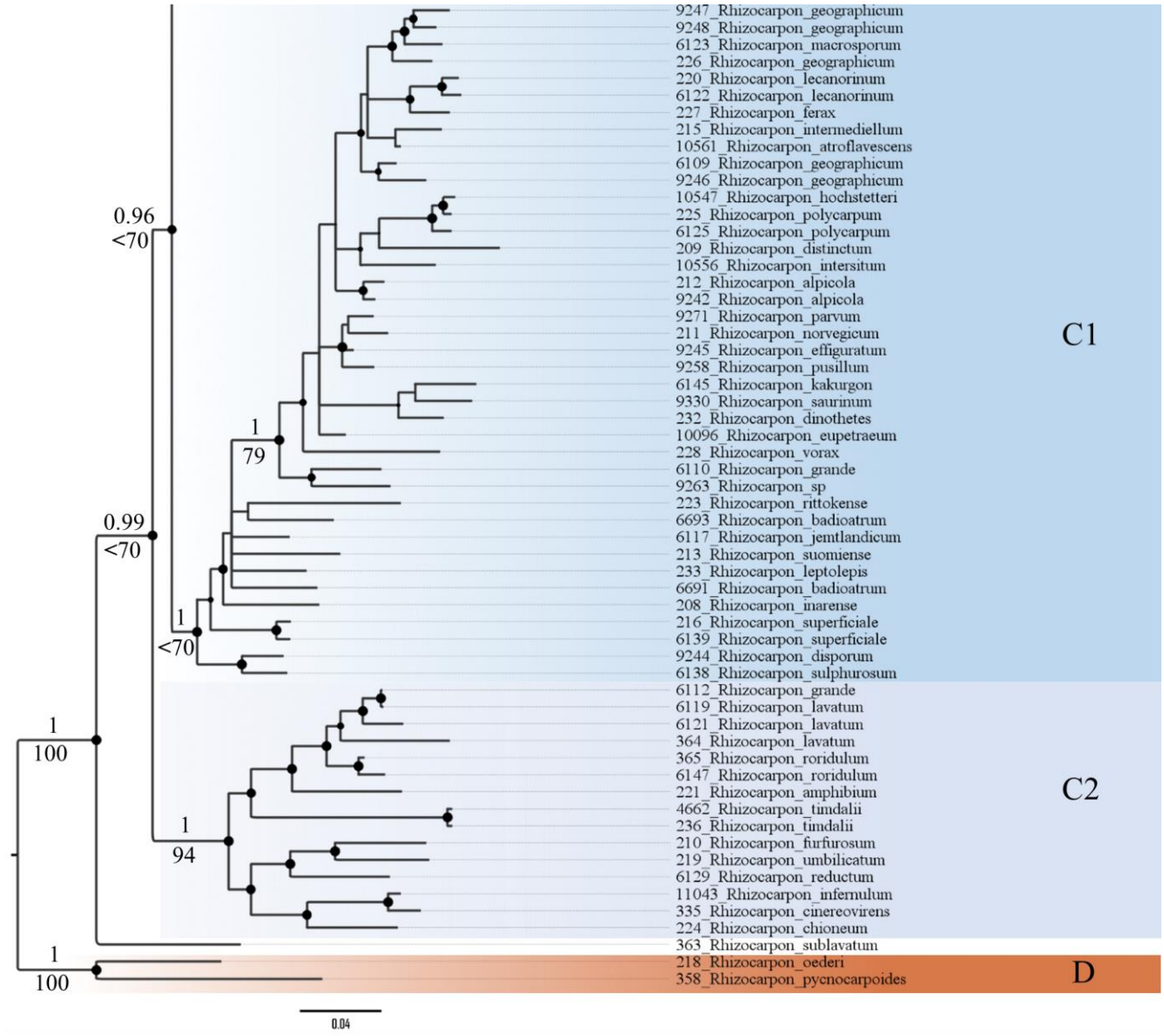

Figure 2b. Continued from 2a. Clade D is used as root. Selected support values in clades C1 & C2 are shown on branches, node shape sizes correspond to PP values. Scale bar indicates expected changes per site.

The MO data set was also congruent between phylogenetic methods, as well as with the FO data set (Fig. 2). Support was overall higher with MrBayes than with RAxML. Clade D is retained with maximum support and is here used as an outgroup. The rest of the family (clades A+B+C) now forms a maximum supported clade, but there is uncertainty concerning the placement of 363 (*R. sublavatum* Fryday). Clade C now appears as two clades: C1 (PP=1/BS=45), which represents many of the yellow species, including the type species, *R. geographicum*; and, C2 (PP=1/BS=96), which represents a group of species with hyaline and mostly muriform spores. Bootstrap support for and within C1 is quite low

compared to the other clades. Clade B is retained (PP=1/BS=99) and is sister to *R. santessonii* Timdal (PP=0.98/BS=73). Finally, clade A is retained (PP=1/BS=73). Not counting the two *Poeltinula* species, 11 highly supported subclades appear in the clade: *R. hochstetteri* clades A1 – A8, *R. hensseniae*, *R. caesium* and "*R. expallescens"* (Fig. 2a). As in the FO phylogeny, clade A6, A7 and A8 form a highly supported clade (PP=1/BS=87).

The gene trees were congruent with each other and with the species trees, except for clade D grouping with clade A and B in the FO ITS tree. In both the ITS trees, all putative species were retained with maximum support, but beyond species level support values are generally low to very low. In the mtSSU trees, all species are also retained, except for A6, A7 and A8, who are nested with each other in a highly supported clade (FO:BS=84/MO:BS=88). Among the gene trees, the MCM7 tree most closely resembles the species trees, and clade A (BS=100/BS100), B (BS=100/BS=100) and C (BS=95/BS=98) are all retained, as are all the putative species (BS=100 for all) and the clade consisting of A6, A7 and A8 (BS=84/BS=92).

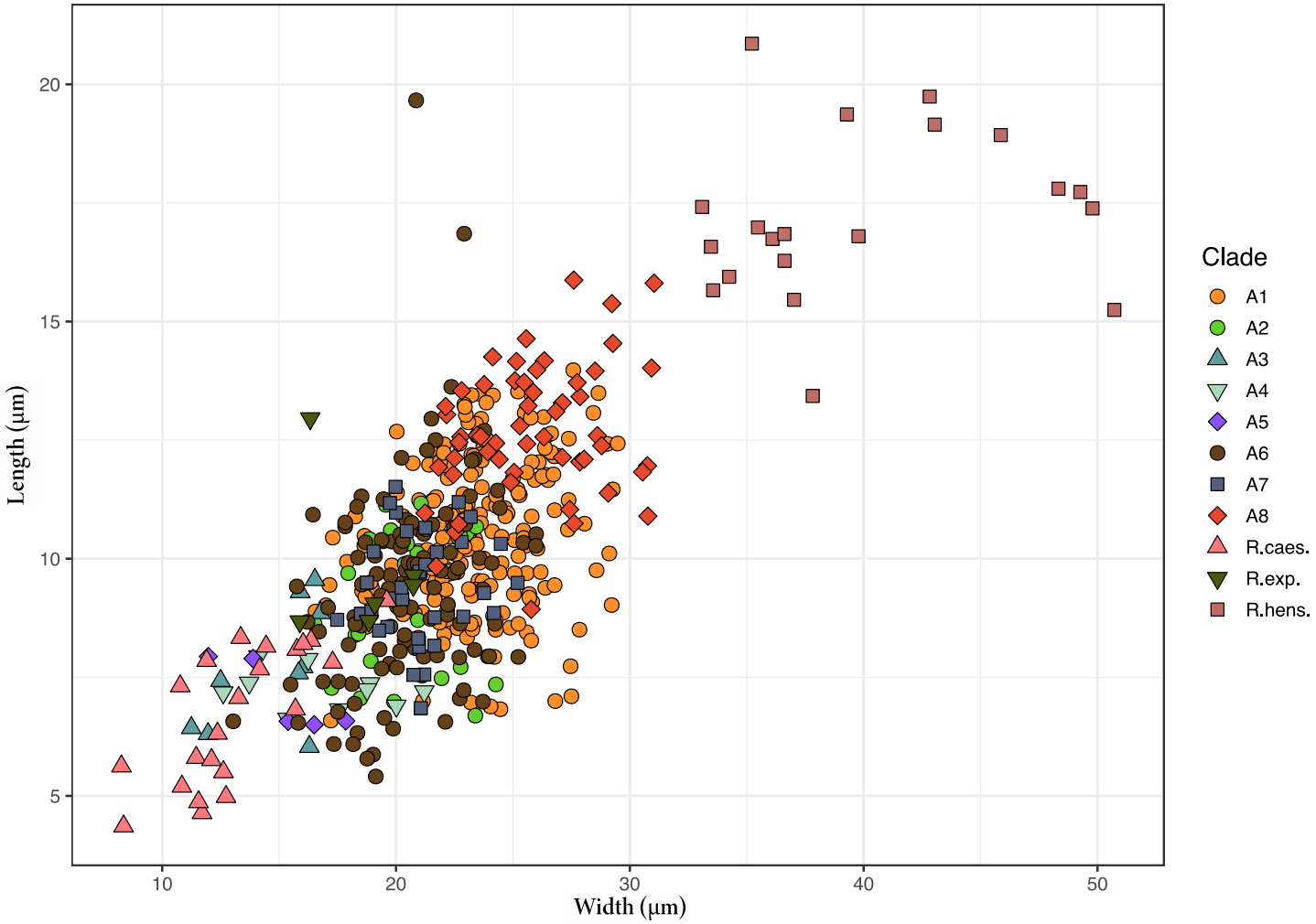

Figure 3. Scatterplot of spore lengths and widths. Clade colours and shapes correspond to colours and shapes in phylogenies (Figs. 1, 2) and maps (Figs. 10, 11).

3.2 Ascospore sizes and hymenial characters in clade A

I included 511 ascospore measurements from 52 specimens in clade A in the study (Fig. 3: 211 from 23 specimens in A1, 25 from 4 specimens in A2, 9 from two specimens in A3, 10 from one specimen in A4, 5 from two specimens in A5, 115 from 8 specimens in A6, 34 from two specimens in A7, 54 from 4 specimens in A8, 22 from two specimens in *R. caesium*, 6 from one specimen in "*R. expallescens"* and 20 from 4 specimens in *R. hensseniae*). Except for the large-spored *R. hensseniae,* there was substantial overlap in spore sizes, but trends were evident.

Epihymenial pigmentation varied from rarely bright blue and/or green (Figs. 5, 6) to more commonly dark earthy green, and sometimes almost hyaline. The degree of colouration varied within clades, the most extreme example of this being 10536 with almost half its hymenium coloured bright green (Fig. 4 D), in contrast to its close relatives AF483607 (*R. hochstetteri*), 11041 (*R. infernulum* f. *sylvaticum*), 11097 (*Rhizocarpon*) and 11098 (*Rhizocarpon*), which all had a thinner, dark earthy green layer in the epihymenium. The hypothecia were consistently light brown and I was not able to see any clear differences between neither specimens nor clades.

Presence/absence of crystals seems to be consistent in some clades and less so in others. No specimens from clades A1, A2, A3, A6, *R. caesium* or "*R. expallescens*" contained crystals. Both specimens of clade A4 and all *R. hensseniae* contained crystals. Only one of two specimens from A5 contained crystals, so did two of four examined specimens from clade A8.

3.3 Chemistry in clade A

Of the 75 specimens from clade A, 56 were subjected to TLC. Some clades were consistent in their chemistry: clades A2, A3, A5, A6 and *R. caesium* never had lichen substances and clade A8 and *R. hensseniae* always had stictic acid. The two specimens constituting clade A4 both had norstictic acid, albeit very low concentrations judging from the weak spots they produced. One of the two specimens (11039) in clade A7 also had norstictic acid, the other (10542) had stictic acid. Norstictic acid has not been recorded as a main compound in the group previously.

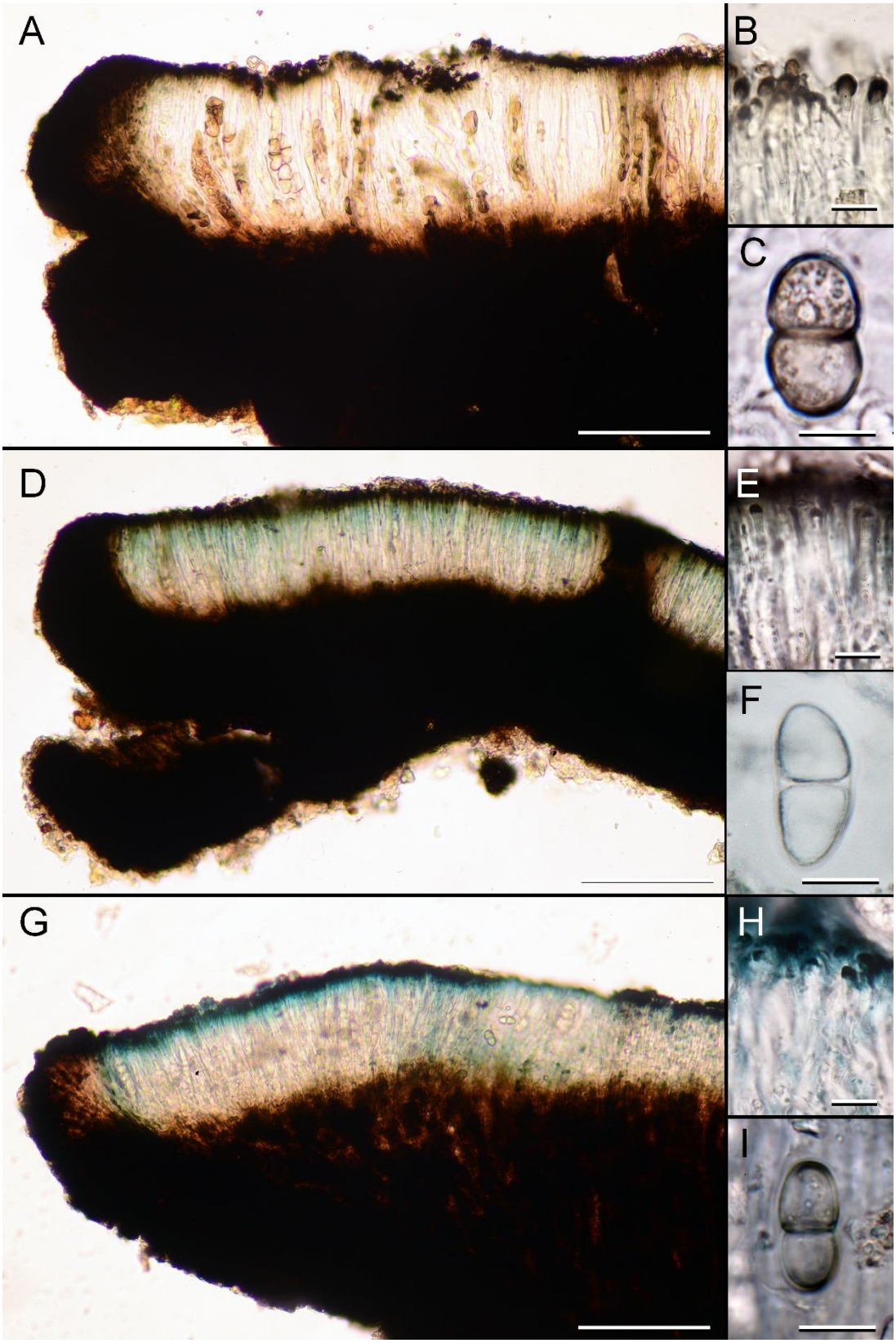

Figure 4. Cross-sections of apothecia (A, D, G, scale bar = 100 µm), with close up on the sharply delimited paraphyse caps (B, E, H, scale bar = 10 µm) and ascospores (C, F, I, scale bar = 10 µm). A-C: 10526 (O-L-227846), clade A1; D-F: 10536 (O-L-228034), clade A2; G-I: 230 (O-L-160773), *R. caesium*

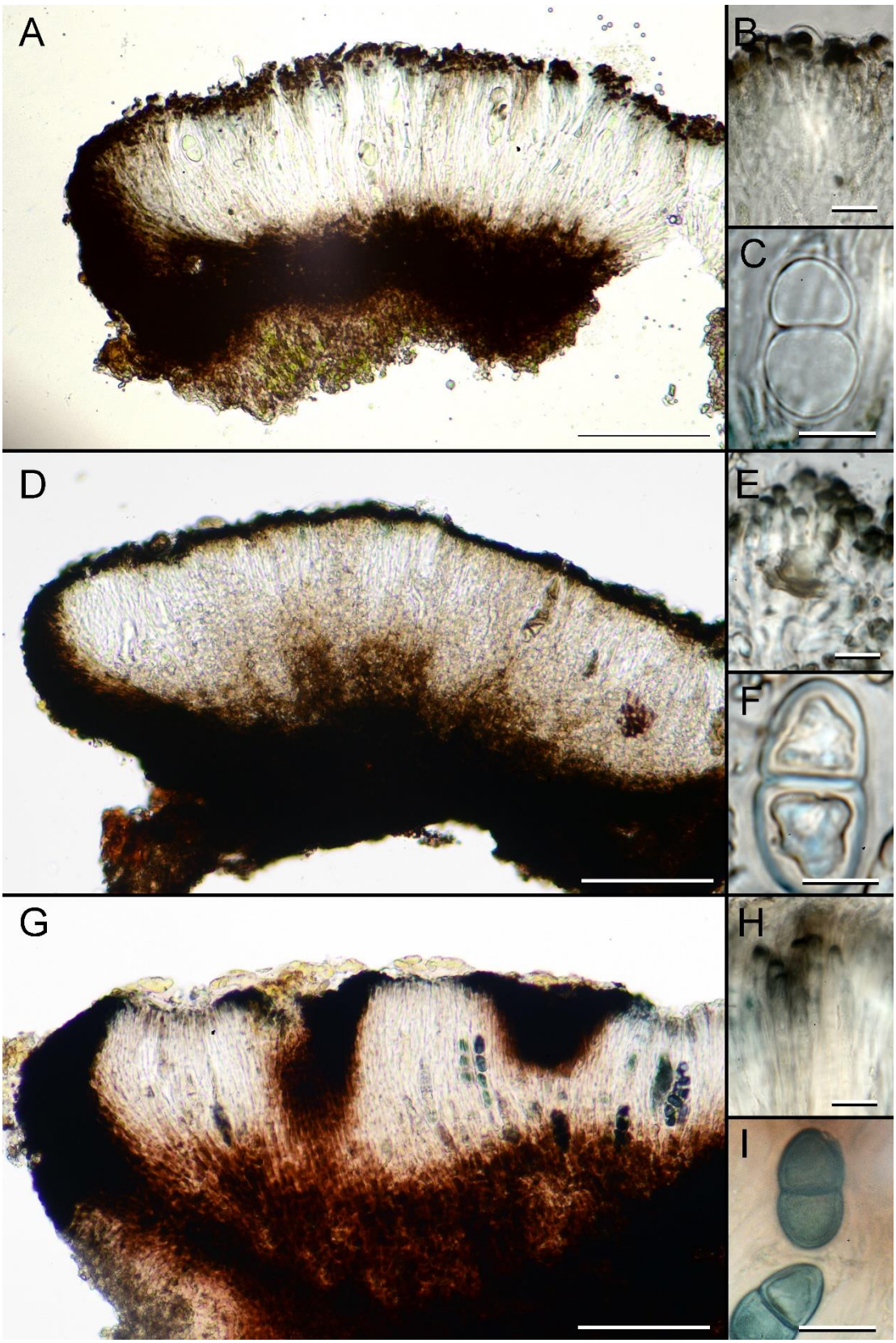

Figure 5. Cross-sections of apothecia (A, D, G, scale bar = 100 µm), with close up on the sharply delimited paraphyse caps (B, E, H, scale bar = 10 µm) and ascospores (C, F, I, scale bar = 10 µm). A-C: 10186 (O-L-165731), clade A6; D-F: 10203 (O-L-183846), clade A8; G-I: 3613 (Hafellner 63791), *Poeltinula cacuminum.* The sterile furrows are visible and seem to be growing down from the epihymenium.

Clade A1 had variable chemistry: of 27 examined, 4 had stictic acid, 14 had no lichen substances, one had three unknown lichen substances. Furthermore, 8 specimens had an unknown substance that fluoresced in UV (366nm) before the sulfuric acid dousing step. Unpublished notes in specimens in O show that this substance was also observed in a *R. hochstetteri* by Holtan-Hartwig in 1985.

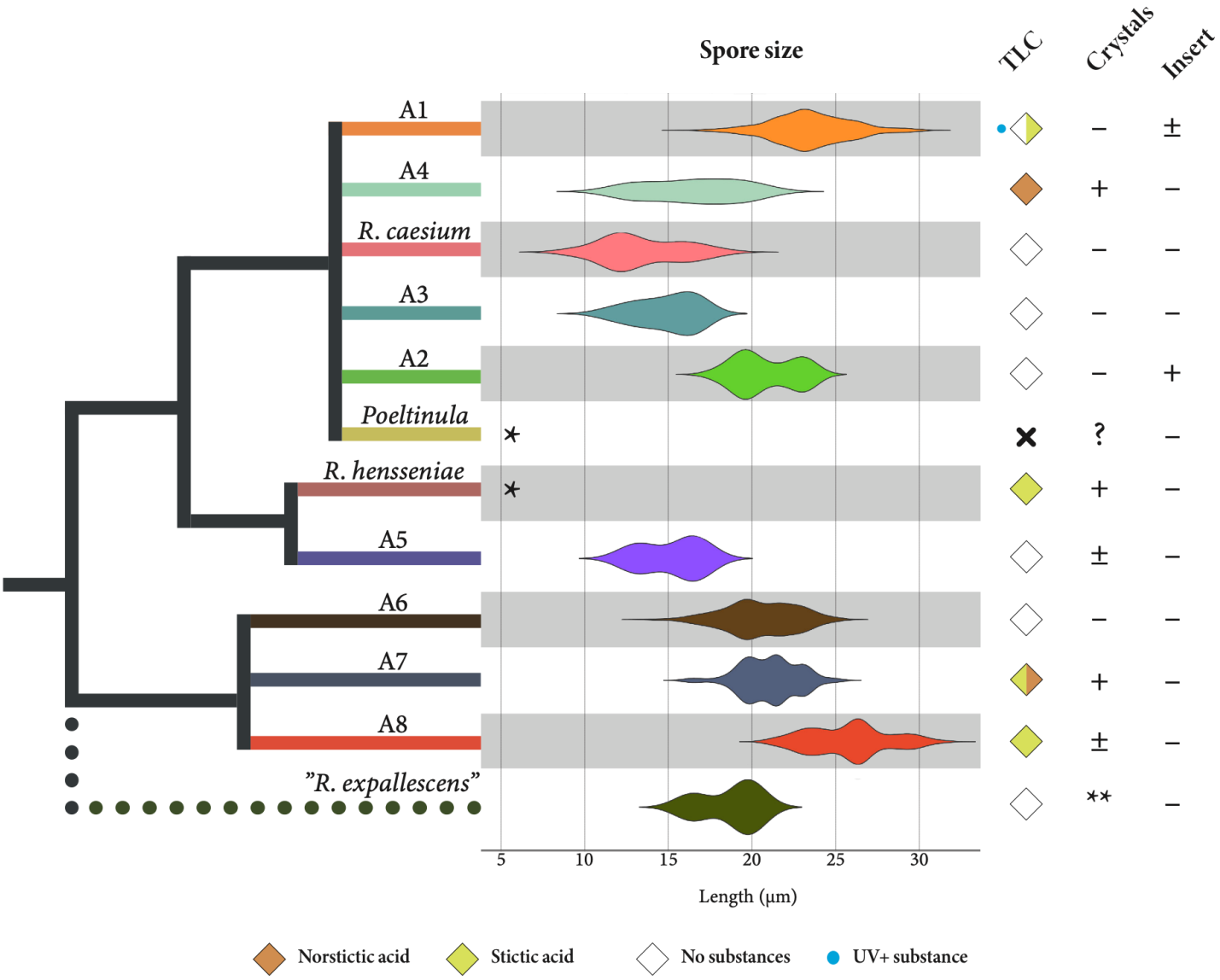

Figure 6. Illustration cladogram of clade A (from Fig. 2a). Branches with PP<0.95 are collapsed and the placement of "*R. expallescens*" is uncertain. Spore lengths and presence/absence of secondary metabolites, hymenial crystals and ITS1 insert plotted.

***R. hensseniae* (>50um spores) is excluded from the spore length plot to increase visibility of the species that are more difficult to separate and the two *Poeltinula*-species were excluded from both microscopic examinations and TLC.

****The single specimen called "*R. expallescens*" seemed to have crystals among only some paraphyses.

4. Discussion

I have aimed to circumscribe and delimit natural genera in the Rhizocarpaceae and investigate the species diversity in the *R. hochstetteri*-complex. My results, from molecular and anatomical evidence combined, suggest that the family consists of at least four major lineages (Fig. 3: A, B, C and D), which do not reflect current generic circumscriptions. My three-locus phylogeny renders *Rhizocarpon* paraphyletic with *Poeltinula, Catolechia* and *Epilichen* nested. More specifically, *Poeltinula* is nested in the *R. hochstetteri*-complex as part of clade A, *E. scabrosus* is nested within *Catolechia* with *E. glauconigellus* as their distinct sister (clade B). *Rhizocarpon oederi* and *R. pycnocarpoides* form a sister group (clade D) to the rest of the family. The rest of the species form either one clade (clade C in the FO phylogeny) or two clades (clades C1 and C2 in the MO phylogeny).

The results also show 11 putative species (excluding the two *Poeltinula* species) in clade A, which exceeds the number of currently accepted names in the group (*R. hochstetteri, R. caesium, R. infernulum* f. *infernulum, R. infernulum* f. *sylvaticum* and *R. hensseniae*), and that there are ecological, morphological and anatomical trends, albeit with substantial overlap in e.g. spore sizes and thallus morphology.

4.1 Phylogenetic results

4.1.1 Genus delimitation in the Rhizocarpaceae

The phylogenetic results in this study support the findings of Ihlen & Ekman (2002) and Miadlikowska et al. (2014): *Rhizocarpon*, as currently circumscribed, is non-monophyletic. Four major clades appear in both the FO and MO phylogenies, not corroborating every aspect of current taxonomy. In clade A, *Poeltinula* is nested in the *R. hochstetteri*-complex and must be included in *Rhizocarpon* unless *R. hochstetteri* is excluded. This is highly supported in the FO phylogeny and moderately to highly supported in the MO phylogenies (Figs. 1, 2). In addition to molecular evidence, clade A is characterized by the sharply delimited paraphysis caps and the 1-septate, mostly hyaline ascospores (Figs. 5, 6). Similar ascospores also occur in the *R. polycarpum* (Hepp) Th. Fr. group, though, but that species has a strongly

amyloid medulla. However, the large genetic distance, together with these anatomical features, suggest that it is more natural to include the *R. hochstetteri*-complex in *Poeltinula*, than to lump *Poeltinula* with *Rhizocarpon*.

As for the two genera *Catolechia* and *Epilichen*, they are so genetically similar that that I propose to lump them together, even though the two genera are morphologically and ecologically quite different (Figs. 1, 2). *Catolechia* grows almost exclusively in crevices in shady, more or less north facing, rocky walls, while all *Epilichen* species are obligate parasites on other lichens, often *Baeomyces* Pers., on the ground. However, in addition to genetic similarity and presence of rhizocarpic acid, *Catolechia* and *Epilichen* both have 1-septate, brown ascospores without a halo. Lacking a halo is unique in the family. Within clade B, the yellow *Catolechia* and *E. scabrosus* are nested with each other on a long branch, with *E. glauconigellus* as sister. Unfortunately, none of the more conserved markers were produced for any of the *Epilichen* species, and the nesting is a result of the two species being 97.08 – 98.62% similar on the ITS marker.

There is also the question about the placement of the parasitic *R. santessonii*. In the MO phylogeny it is moderately supported as sister to *Catolechia+Epilichen* (PP=0.97/BS=69). It is also yellow, but more faintly so, and unlike that of *Catolechia* and *Epilichen* (rhizocarpic acid), the pigment is yet to be identified (Timdal 1986). Instead of lacking a halo, *R. santessonii* has a very thick halo surrounding its ascospores (Timdal 1986, Fig. 1B). The species also has a swollen, pigmented paraphysis cap. I consider this sufficient evidence for taking it out of *Rhizocarpon*, but there is still not enough molecular support to confidently place it in either clade A or B. Acquiring MCM7 or LSU for the species may solve this.

In my study, clade C is highly supported as monophyletic in the FO phylogeny, but is split in two clades, C1 (PP=1/BS=47) and C2 (PP=1/BS=94), and appears paraphyletic in the MO phylogeny. Ihlen & Ekman (2002) include representatives from both C1 (*R. geographicum, R. polycarpum, R. distinctum* Th. Fr., *R. norvegicum* Räsänen, and *R. suomiense* Räsänen) and C2 (*R. amphibium* (Fr.) Körb., *R. lavatum* (Fr.) Körb., *R. petraeum* (Wulfen) A. Massal and *R. reductum* Th. Fr.). In their study, their version of clade C2 is also highly supported. In fact, the only phylogenetic incongruence between this study and Ihlen & Ekman's (2002) is the placement of *R. oederi*. In their study, the species is either sister to their version of clade C or their version of clade C1, albeit weakly supported. Judging from my results, there is little question that clade D, consisting of two metallophilic species (*R. oederi* and *R. pycnocarpoides*), is monophyletic. It was placed at the base of the family by Miadlikowska et al. (2014), and it was sister to the rest with maximum support in all my analyses, except for appearing in a clade with clades A and B in the FO ITS gene tree (Appendix 2: Fig. 1).

In my opinion, the most natural delimitation of the genera in the family is to raise all major clades (Fig. 1, 2: clades A, B, C and D) to generic rank. Some question marks remain, like the placement of 10534 ("*R. expallescens")*, 214 (*R. santessonii*)) and 363 (*R. sublavatum*) (Fig. 2). Acquiring additional conserved markers for these accessions, in addition to a denser sampling from clade C will probably help to resolve the deep topology in the family.

There are some inconsistencies regarding support values between RAxML and MrBayes phylogenies that must be addressed, most notably in the MO phylogeny. For example, the branch leading to clade A has in the MO phylogeny maximum PP support with MrBayes, but only moderate BS support (MO: PP=1/BS=71). In the FO phylogeny, however, both the PP and BS values are high (FO: PP=0.99/BS=96). Even more extreme is the branch leading to clade A+B (FO: not supported, MO: PP=1/BS=48). Studies have shown that Bayesian PP and ML-BS can provide conflicting support values at short internodes (Alfaro et al. 2003). Determining which of the nodes in my phylogenies have an artificially high PP and which have an artificially low BS is difficult to assess from the current results. A first step in investigating this would be to remove uncertainly placed singletons from the analysis (e.g. 10534 ("*R. expallescens"*) and 214 (*R. santessonii*)), but obviously the best long-term solution is to get the missing markers for these accessions. Lacking that, one is left with anatomical and morphological evidence to interpret the trees. In light of this, I still do not doubt that clade A is monophyletic, even though BS support for it plummets when adding many orphans to the analysis. In contrast, I cannot confidently say that clade A and B are sisters just because it has maximum PP support in one of the analyses. The message here is that caution must be taken when there is conflict between support values and that we cannot blindly trust the numbers that the software returns. It is always safest to explore different phylogenetic methods and data subsets.

Overall, this means that there is high support for raising clades A, B and D to generic rank, but more data is needed to resolve the relationship between them, and to confidently delimit and circumscribe clade C. Combined, the MCM7 and mtSSU loci provided moderate to good support for testing monophyly of the genera, but the relationship between them remains unresolved. Preliminary results of the LSU locus were consistent with the results of this study, but a series of unfortunate events in the NHM DNA lab put a stop to further investigation, and the marker was left out of this study. For future studies, I suggest acquiring MCM7 and LSU sequences for the under-sampled and unresolved parts of the tree.

4.1.2 Species delimitation in the R. hochstetteri*-complex / Clade A*

One of the more surprising results of this study was the discovery of 11 putative species in the *R. hochstetteri*-complex. The most recent taxonomic paper dealing with the *R. hochstetteri*-complex (Fryday 2002) concluded that the group consisted of at least four taxa (*R. hochstetteri*; *R. caesium*; *R. infernulum* f. *infernulum* and *R infernulum* f. *sylvaticum*) and that these four were distinguishable by thallus and apothecium morphology and by spore size. Here, I discovered 11 well-separated and highly supported clades apparently deserving taxonomical recognition. There is substantial overlap in spore size between many of them, but there seem to be trends in all characters that Fryday recognized.

After examining the holotype specimen, Fryday (2002) concludes that the true *R. hochstetteri* must be one of the large spored species. Clade A1 fits the description very well, with its gray to brown cracked to areolate thallus (Fig. 7 A-D). It seems to be the most common clade and collections are from Norway, Scotland and New Hampshire, suggesting a northern Atlantic distribution. There is very little genetic variation within the clade (Fig. 2a). Even though the description given in the paper could fit more than one clade in this study, I consider clade A1 the most logical candidate for the name.

Less of a problem is *R. caesium*. It is reported to have very small ascospores (Figs. 3, 6), a thin hymenium with a brightly blue-green coloured epihymenium (Fig. 4) and large apothecia compared to the rest of the group; hence it seems to be quite easily distinguishable (Fryday 2002). I have provided an ITS sequence from the holotype which forms a highly supported clade with the only other accession determined as *R. caesium* in my data set. The holotype is from western Scotland and the other specimen is from Iceland.

Like *R. caesium*, *R. hensseniae* also has large apothecia, but very large and slightly darker spores compared to the rest of the group, make it easily distinguishable. The species also sometimes has nitrogen-fixating cyanobacteria organized in structures called cephalodia, a unique character within the family. The Japanese specimens (6113 & 6114) do not have cephalodia, but both the Alaskan specimens (10557 & 10558) do. Acquiring cephalodia has been hypothesized to allow enlargement of thallus and apothecia in lichens (Schneider et al. 2016). Interestingly, the species has relatively small paraphysis caps. The specimens included in this study are from Japan and southern Alaska (Fig. 11) and all records in GBIF (https://www.gbif.org/species/3425426) are from western North America, which suggests a northern Pacific distribution.

Rhizocarpon infernulum f. *infernulum* is harder to take a stance on. The species was described by Nylander (1885) and the type material has not been attempted sequenced. Specimens originally determined to this taxon appear in three clades in the FO phylogeny: EDNA16_0046588 in A1, 6116 in

clade A3, and 11043 as sister to 335 (*R. cinereovirens* (Müll. Arg.) Vain.) in clade C2. *Rhizocarpon cinereovirens* is a species with similar spores and hymenial pigmentation to the *R. hochstetteri*-complex, but it lacks the swollen paraphysis caps, has more bulbous areolae and is genetically far separated from the *R. hochstetteri*-complex (Fig. 2b). The ones in clade A1 and sister to *R. cinereovirens* are without a doubt wrongly determined. However, specimens in clade A3 fit well with Fryday's (2002) description of the species regarding spore size, morphology and ecology. He notes that the species might be adapted to stress and can be found at high altitudes and sometimes at old, abandoned mines. The two Norwegian specimens in the clade are collected at metal rich localities: 9088 was collected from a spoil heap at an old copper mine and 7702 was collected from a very metal rich rock wall. Another argument for A3 being *R. infernulum* is that the third specimen in the clade (6116) was collected in western Alaska, not far from the Bering Sea, i.e. from the type locality (Fryday 2002). The two specimens of clade A5 were also collected in Alaska and are similar in spore size to A3. However, they have a much smoother, thinner thallus and smaller, more scattered apothecia (Fig. 8 C, D). I find it most likely that clade A3 is what Fryday (2002) meant by *R. infernulum* f. *infernulum*, but more accessions of both clades are needed, to determine whether these characters are representative for the species, or if there is more variation within them.

As for *R. infernulum* f. *sylvaticum*, Fryday was in doubt whether this taxon should have species or infraspecific rank. He first regarded it as a distinct species, '*R. oceanicum'* (ined.) (Fryday 1996), but changed his mind due to the many overlaps in mainly hymenial characters between it and *R. infernulum* and chose to regard them both as forms of the latter. Forma *sylvaticum* was supposed to have slightly larger spores and a smooth, cracked thallus compared to the more areolate thallus of f. *infernulum*. Specimens from clade A2 fit this description well (fig. 7 D-E), and form a highly supported clade together with a specimen (11041) Fryday himself determined as f. *sylvaticum.* This is supported by my own observations of the type specimen. Regrettably, the sequencing plate containing a sample from the type was destroyed in the mail on its way to Macrogen Europe, so as of now it is not possible to be certain about its identity. However, spore measurements and hymenial pigmentation, chemistry and ecology all fit Fryday's (2002) description so well that I regard clade A2 as f. *sylvaticum.* The clade is well supported and separated, and I propose to raise it to species rank with the epithet *sylvaticum*, since '*R. oceanicum'* was never formally described.

Rhizocarpon expallescens is a poorly understood and scarcely collected species with no reference sequence, and the name should be treated with caution. The specimen here named "*R. expallescens"* is only tentatively determined by myself. There was no sequence that matched it, and it appears as a singleton in the tree. The specimen was growing half-submerged in an alpine stream, had a white to pale

gray thallus with scattered patches of tiny areolae, and really does not look like any other lichen I have seen. I do not believe this is the true *R. expallescens*, but I kept the name in the analyses since no other clade made claims to the name and because records of erroneous determinations can be useful information in future studies.

Other names that have been used for species in the complex include *Lecidea colludens* Nyl., *R. applanatum* (Fr.) Th Fr., *R. crenulatum* H. Magn. and *R. decinarescens* (Nyl.) Zahlbr. Fryday (2002) regards the latter as a synonym to *R. infernulum* and the other three as synonyms to *R. hochstetteri*. Fryday's circumscription of those two taxa differs from the one presented here, so the application of the synonyms are ambiguous. Future type studies may show that some of them are available for the unnamed clades in my phylogeny. I consider clades A4, A5, A6, A7 and A8 as distinct species, but without type studies it is impossible to know if any of the mentioned names belong to any of them.

Of these, clade A4 is perhaps the least problematic. The clade consists of two specimens collected by U. Arup in the nature reserve Kullahammar in Skåne, Sweden (Fig. 10). It differs from the rest by having a very small thallus and apothecia (fig. 7 B), small ascospores (c. 13–18 (–20) × 7–10 µm), containing norstictic acid and having hymenial crystals. The only other clade with norstictic acid (A7) also has crystals in the hymenium, but it has larger spores. However, there are few accessions of both clade A4 and A7, and there may be more variation within both species.

Then there is the group consisting of A6, A7 and A8. They form a highly supported clade in both species trees as well as in the mtSSU and MCM7 gene trees. Clade A6 has medium sized spores (c. 18–22 × 8–10 µm), no lichen substances, and no crystals in the hymenium. The thallus morphology varies from scattered areolate to more continuous and cracked, and gray to pale brown. Morphologically, many of them look like intermediates of other species. If anything stands out, it is that the mature specimens all have numerous apothecia, almost forming a scattered net-pattern across the thallus (Fig. 8 E, F). The species seems to have a continental distribution, at least judging from the Norwegian collections (Fig. 10). Interestingly, the specimen from Siberia (Fig. 11) is genetically very similar (99.8% on ITS) to its Norwegian conspecifics. It is morphologically similar to clade A7, which also has medium sized spores (c. 20 × 10 µm), but of the two specimens in clade A7, one has stictic acid and the other norstictic acid, and they both have hymenial crystals. Like A4, they are both quite small (c. 1 cm thallus), and one is collected on a small rock and the other is growing on some pebbles (Fig. 9 A, B). Conversely, clade A8 is easy to separate, with its large spores (c. 25–30 × 10–15 um) and thick grayish thallus. Some specimens in the clade are collected on metal rich rocks or slag heaps, and two out of four examined specimens had hymenial crystals.

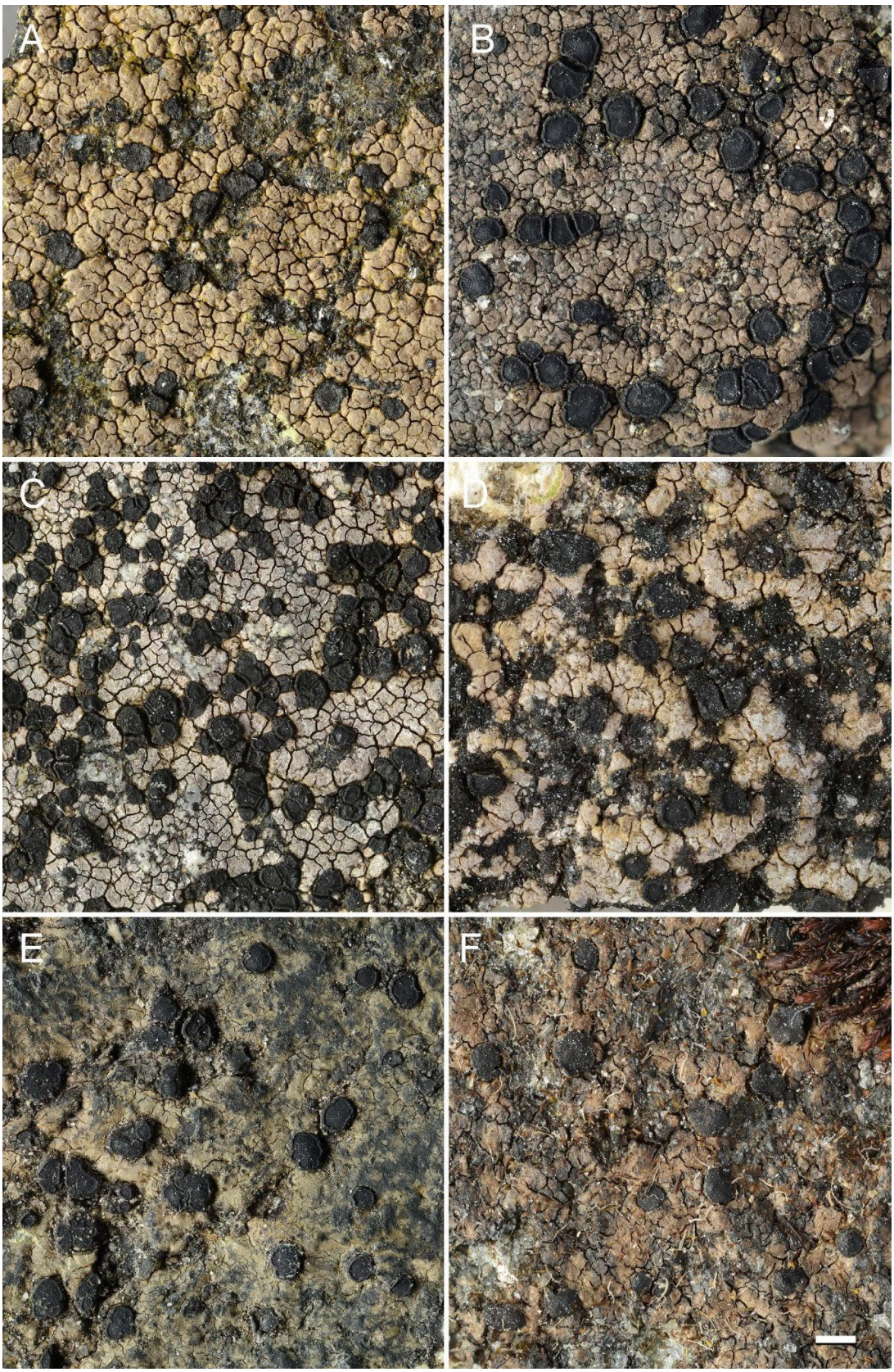

Figure 7. Photos of specimens of clade **A1** (A-D) and **A2** (E, F) A: 10206 (O-L-222653), B: 10526 (O-L-227846), C: 10205 (O-L-174055), D: 10200 (O-L-165824), E: 10536 (O-L-228034), F: (AF483607) O-L-119602. Scale bar = 1 mm for all images.

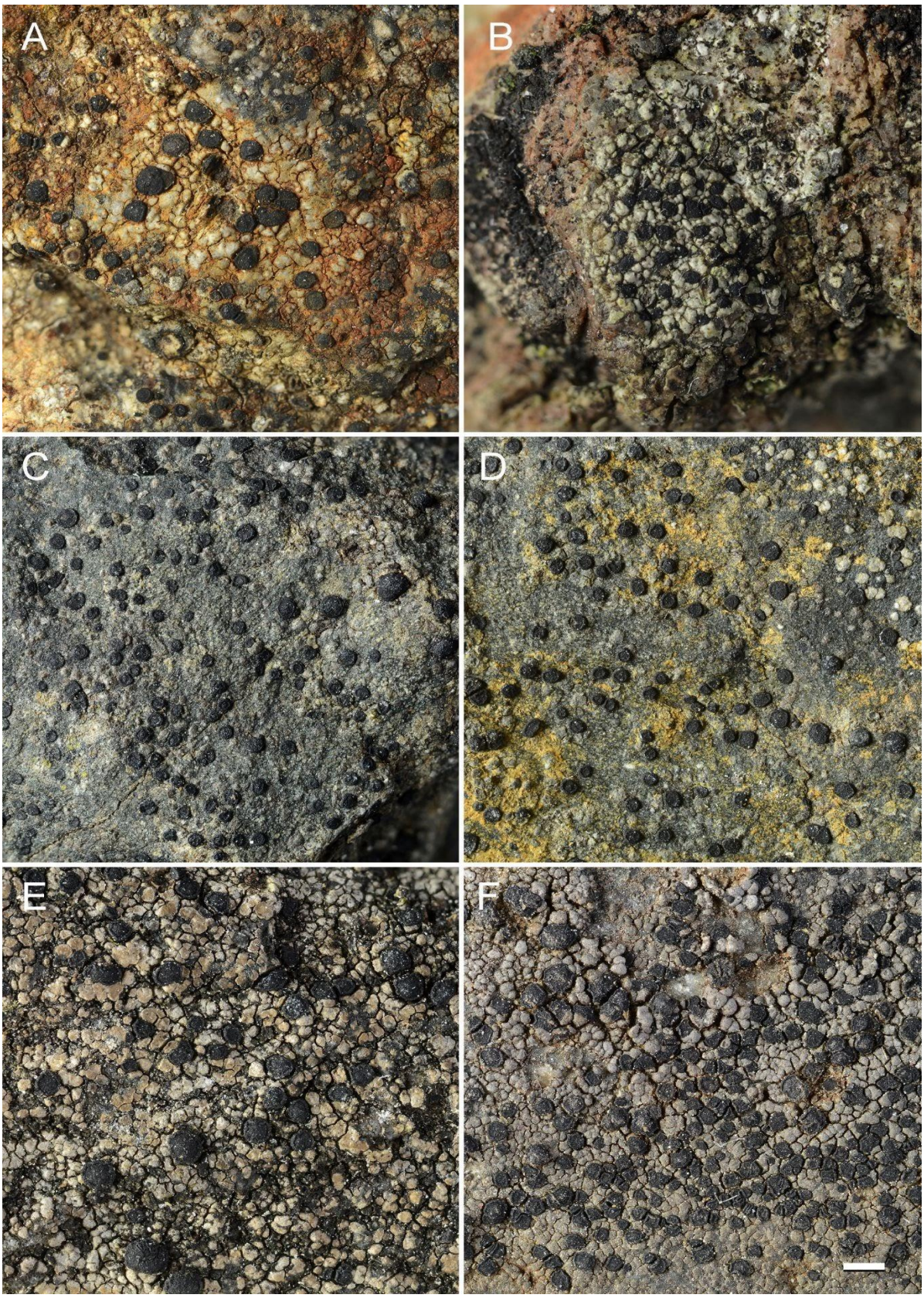

Figure 8. Photos of specimens of clade **A3** (A), **A4** (B), **A5** (C, D) and **A6** (E, F). A: 7702 (O-L-163727), B: U3428 (L20096), C: 9653 (Wheeler 4314), D: 9652 (Wheeler 4284), E: 10186 (O-L-165731), F: 10188 (O-L-179453). Scale bar = 1 mm for all images.

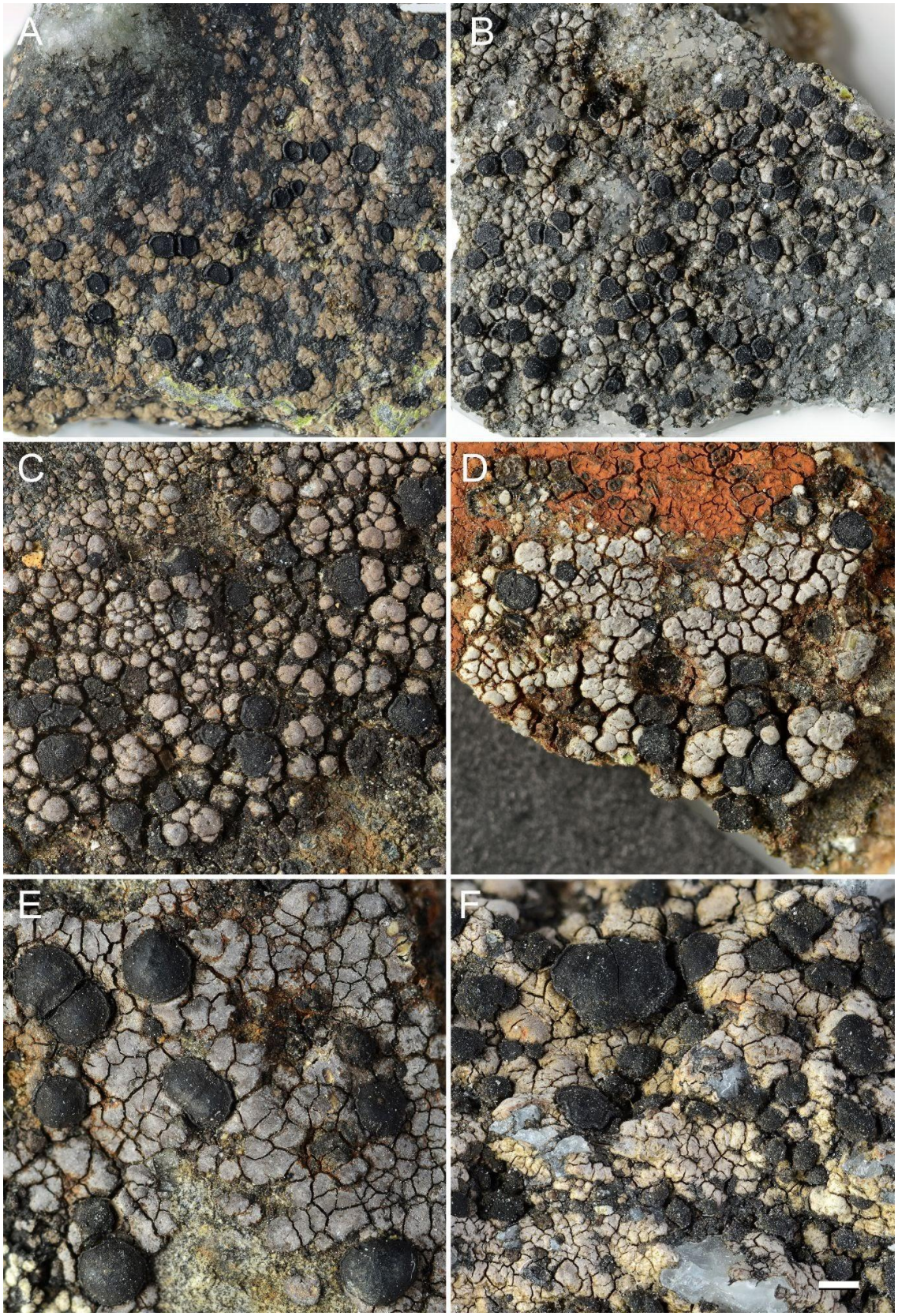

Figure 9. Photos of specimens of clade **A7** (A,B), **A8** (C, D) and ***R. hensseniae*** (E, F) A: 11039 (O-L-228008), B: 10542 (O-L-227917), C: 10203 (O-L-183846), D: 10103 (O-L-225964), E: 6113 (McCune 35464), F: 6114 (McCune 36273). Scale bar = 1 mm for all images.

4.2 Thoughts on ascospore and hymenial character evolution

Due to the interspecific diversity of ascospore morphology in the genus, spores have long been an important character used to describe and determine species in *Rhizocarpon* (Runemark 1956, Hafellner 1984). Species often differ in spore size, pigmentation, septation and number of spores in the asci, and closely related species are often similar in this respect. One example of this is the *R. reductum*-group (highly supported clade C2 in this study), where most of the species have hyaline muriform spores (some have hyaline 1-septate spores). Another is the sister species pair of *R. geminatum* Körb. (2 spores / ascus) and *R. disporum* (Nägeli ex Hepp) Müll. Arg. (1 spore / ascus), which both have a reduced number of enlarged spores. Another still is the *R. geographicum*-group with pigmented muriform spores.

It is easy to imagine the importance of ensuring spore survival, both during spore maturation and after release, and that different groups have ended up with different strategies to tackle this problem. Although speculative, it is not unthinkable that the pigmented paraphysis caps that are found in clade A and B work as protection against UV radiation. If this is true, one would expect reduction of this trait if a species were to adopt other strategies to protect the spores, like spore pigmentation or enlargement. This might be what we see in the large-spored *R. hensseniae*, and there are more examples: *P. cacuminum*, with its green spores in an almost perithecial shaped apothecia protected by thick furrows of sterile tissue, has small paraphyses, with only remnants the pigmented cap remaining (Fig. 5 H); *Catolechia* has brown spores, and the dark caps are barely visible.

Epihymenial colouration has also been widely used as a character in lecideoid lichens and *Rhizocarpon* is no exception (e.g. Runemark 1956, Hafellner 1984). Mayer & Printzen (2000) proposed to standardize the nomenclature and methodology of hymenial colours and pigments in lichens. The need for such a standard became apparent in this study, as the colours seemed to depend somewhat on the instruments used (e.g. light source in the microscope), the thickness of the cross-section, and possibly that the colours can be mixtures of two or more pigments. There is also some evidence in this study that the intensity of the colour can be ecologically induced. For example, the vividly green 10536 (*R. hochstetteri*) was collected on an exposed coastal rock wall, while all its less coloured conspecifics were collected on shaded boulders in deciduous forests.

Likewise, some studies suggest that there is variation of spore sizes within species that can be ecologically induced (Masson & Magain 2020), and it does make sense that species with wide distributions are phenotypically more plastic in some respects. In some clades, epihymenial pigmentation and spore measurements were more consistent, but this might be false since there are many clades I only

have a few accessions from (A3, A4, A5, A7, *R. caesium* and *"R. expallescens"*). The clade with the most accessions, A1, is the most variable on most characters (e.g. spore size, hymenial thickness and pigmentation, chemistry, thallus morphology and colour), while the ones with few accessions are more homogenous. This is very possibly a result of sampling bias, and it would not be surprising if a denser sampling of the clades with few accessions proves these patterns to be false. Especially since many of the clades with only two accessions are collected close to each other or in similar ecologies.

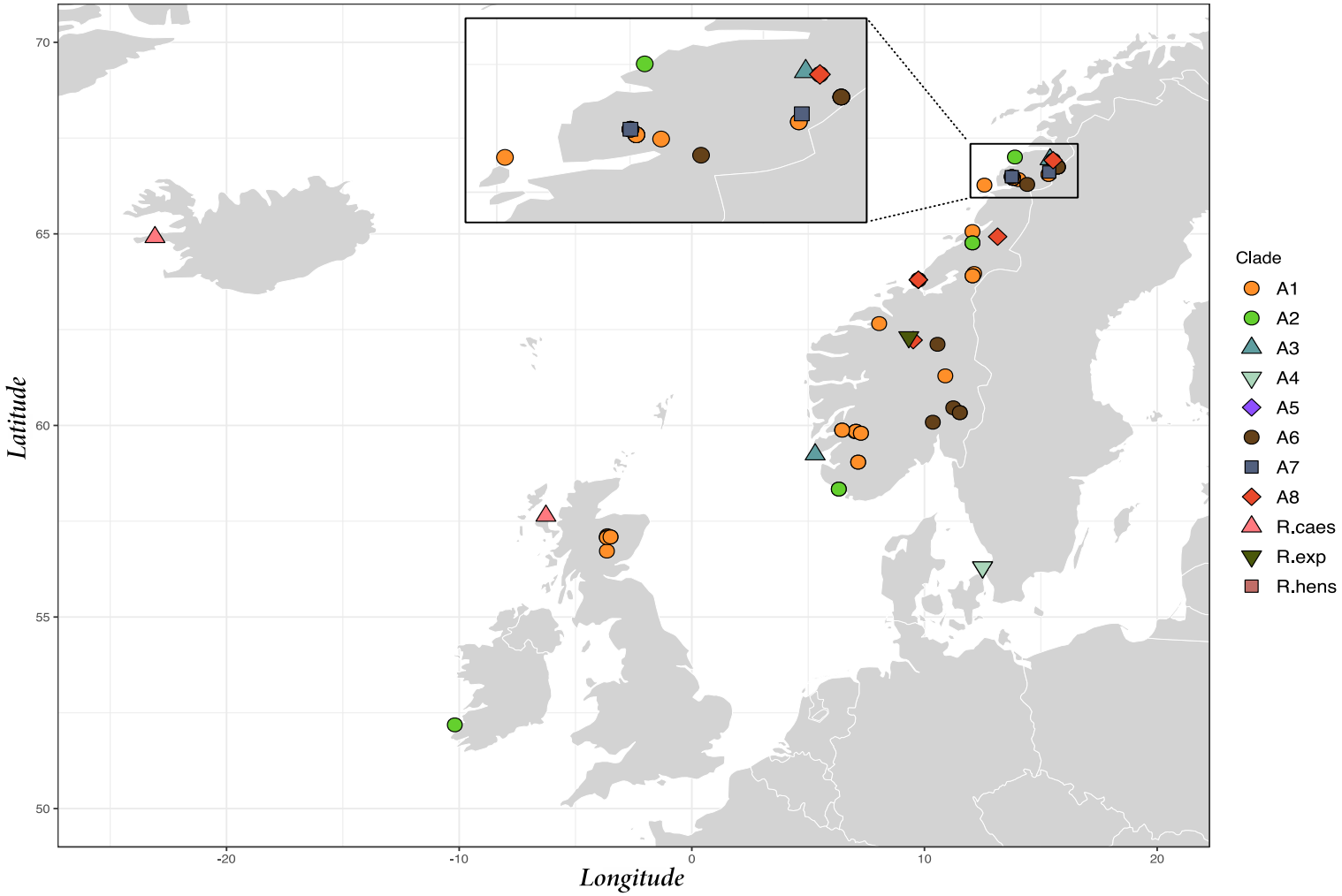

Figure 10. Map of European examined collections. A region in North Norway containing six of the taxa in the *R. hochstetteri*-complex is highlighted. Colours correspond to clades in phylogenies (Figs. 1, 2).

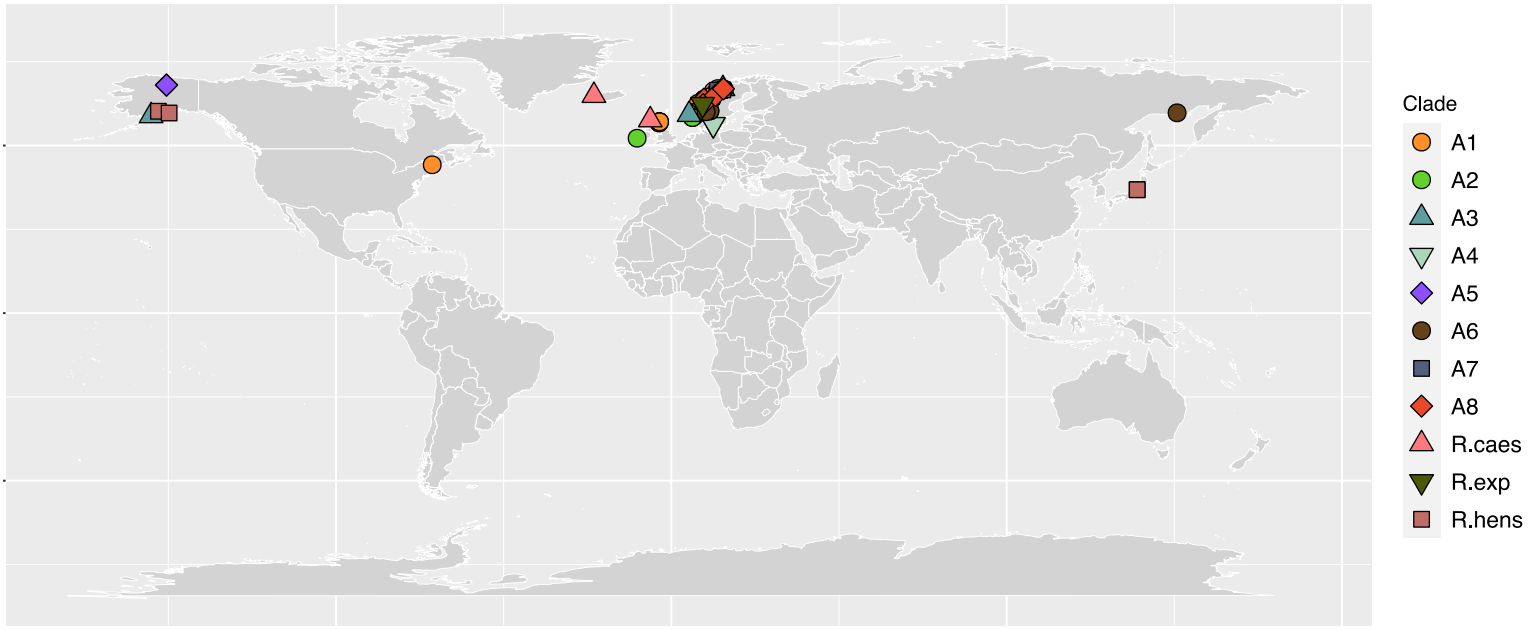

Figure 11. Map of global collections used in this study. Colours correspond to clades in phylogenies (Figs. 1, 2).

4.3 Future perspectives

I have shown that molecular phylogenetics is a powerful tool for discovering diversity, which is perhaps not surprising. When the *R. hochstetteri*-complex was chosen as the focal group in the study, I thought this study would be about cryptic species and phenotypic stasis. Adding more molecular data led to higher resolution in the phylogeny and a better understanding of the diversity in the group. This in turn led to morphological and anatomical patterns gradually appearing. I expected undiscovered diversity, but not to this extent, and all this came from studying a small part of the family. Many more interesting questions and groups remain in the family. *Rhizocarpon geminatum*, *R. eupetraeum* (Nyl.) Arnold and *R. umbilicatum* (Ramond) Flagey are just some species complexes that we considered focusing on. I am also excited to see what will happen to clade C, especially the *R. reductum*-group (clade C2 in this study), when more conserved markers acquired.

In other words, this is by no means the final chapter of Rhizocarpaceae phylogenetics, but it is a step towards a more natural circumscription of the genera and understanding of the diversity in the family. I have no doubt that almost any group in the family would yield interesting results if studied closely. In addition, there were many putative new species that I simply had to look away from in this study, since they were out of my scope and there was not enough time to study them carefully. With the advent of cheap and readily available barcoding and high-throughput sequencing, I believe that the Rhizocarpaceae can be a gold mine for taxonomic and character evolution studies.

References

Alfaro, M. E., Zoller, S., & Lutzoni, F. (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* **20**: 255–266.

Andreev, M. P., Bredkina, L. I., Golubkova, N. S., Dobrysh, A. A., Kotlov, Y. V., Makarova, I. I., Urbanavichene, I- N., Urbanavichus, G. P. (2003) Handbook of the Lichens of Russia. 8. Bacidiaceae, Catillariaceae, Lecanoraceae, Megalariaceae, Mycobilimbiaceae, Rhizocarpaceae, Trapeliaceae. Russian Academy of Sciences, St. Petersburg. 277 pp.

Bendiksby, M. & Timdal, E. (2013) Molecular phylogenetics and taxonomy of *Hypocenomyce* sensu lato (Ascomycota: Lecanoromycetes): extreme polyphyly and morphological/ecological convergence. *Taxon* **62**: 940–956.

Beschel, R. E. (1973) Lichens as a measure of the age of recent moraines. *Arctic and Alpine Research* **5**: 303–309.

Buschbom, J. & Mueller, G. (2004) Resolving evolutionary relationships in the lichen-forming genus Porpidia and related allies (Porpidiaceae, Ascomycota). *Molecular Phylogenetics and Evolution* **32**: 66–82.

Culberson, C. F. (1972) Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* **72**: 113–125.

Culberson, C. F. & Johnson, A. (1982) Substitution of methyl tert.-buthyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* **238**: 483–487.

Davydov, E. A. & Yakovchenko, L. S. (2017) Rhizocarpon smaragdulum, a new monosporic yellow-thalline species and some additional species of the genus Rhizocarpon from the Altai Mountains (Siberia). *The Lichenologist* **49**: 457–466.

Edgar, R. C. (2004) Muscle: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic acids research* **32**: 1792–97.

Esslinger, T. L. (2018) A Cumulative Checklist for the Lichen-Forming, Lichenicolous and Allied Fungi of the Continental United States and Canada, Version 22. *Opuscula Philolichenum* **17**: 6-268.

Fryday, A. (1996) A provisional re-assessment of the non-yellow species of Rhizocarpon occuring in the British Isles. *British Lichen Society Bulletin* **78**: 29–40.

Fryday, A. M. (2002) A revision of the species of the Rhizocarpon hochstetteri group occurring in the British Isles. *The Lichenologist* **34**: 451–477.

Fryday, A. M. & Kantvilas, G. (2012) Rhizocarpon austroamphibium Rhizocarpaceae, lichenized Ascomycota, a new species from Tasmania. *Australasian Lichenology* **71**: 12–17.

Fryday, A. (2019) Lichens of the Southern Subpolar Region. http://herbarium.msu.edu/SSP/SSP_Checklists.html

Galloway, D. (2007) *Flora of New Zealand Lichens. Revised Second Edition Including Lichen-Forming and Lichenicolous Fungi. Volumes 1 and 2*. Manaaki Whenua Press, Lincoln, New Zealand. 2261 pp.

Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular ecology* **2**: 113-118.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic biology* **59**: 307–321.

Hafellner, J. (1984) Studien in Richtung einer natürlichen Gliederung der Sammelfamilien Lecanoraceae und Lecideaceae. *Beiheft zur Nova Hedwigia* **79**: 241–371.

Hafellner, J. (1995) A new checklist of lichens and lichenicolous fungi of insular LauriMacaronesia including a lichenological bibliography for the area. *Fritschiana* **5**: 1–132

Holtan-Hartwig, J. & Timdal, E. (1987) Notes on some parasitic Rhizocarpon species. *The Lichenologist* **19**: 335-338.

Honegger, R. (1980) The ascus apex in lichenized fungi II. The Rhizocarpon-type. *The Lichenologist* **12**: 157–172.

Ihlen, P. G. (2004) Taxonomy of the Non-yellow Species of Rhizocarpon (Rhizocarpaceae, Lichenized Ascomycota) in the Nordic Countries, with Hyaline and Muriform Ascospores. *Mycological Research* **108**: 533–570.

Ihlen, P. G. & Ekman, S. (2002) Outline of Phylogeny and Character Evolution in Rhizocarpon (Rhizocarpaceae, Lichenized Ascomycota) Based on Nuclear ITS and Mitochondrial SSU Ribosomal DNA Sequences. *Biological Journal of the Linnean Society* **77**: 535–546.

Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**: 4453-4455.

Kristinsson, H., Zhurbenko, M. & Hansen, E. S. (2010) *Panarctic checklist of Lichens and Lichenicolous Fungi. CAFF Technical Report No. 20*. CAFF International Secretariat, Akureyri, Iceland. 120 pp.

Körber, G. W. (1861) *Parerga Lichenologica.* Trewendt, Breslau. 501 pp.

Lamarck, J-B. & De Candolle, A. P. (1805) *Flore française ou descriptions succinctes de toutes les plantes qui croissent naturellement en France. ed. 3. Vol. 2*. 367 pp.

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular biology and evolution* **29**: 1695–1701.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., Calcott, B. (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution formolecular and morphological phylogenetic analyses. *Molecular biology and evolution* **34**: 772–773.

Larsson, A. (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* **30**: 3276–3278.

Linnaeus, C. (1753) *Species Plantarum, Tomus II*. Stockholm: L. Salvius.

Lutzoni, F., Pagel, M., & Reeb, V. (2001) Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* **411**: 937-940.

Marthinsen, G., Rui, S., & Timdal, E. (2019) OLICH: A reference library of DNA barcodes for Nordic lichens. *Biodiversity Data Journal* **7**: e36252.

Masson, D. & Magain, N. (2020) Ascospore size declines with elevation in two tropical parmelioid lichens. *Plant and Fungal Systematics* **65**: 227–237.

Mayer, B. & Printzen, C. (2000) Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. *The Lichenologist* **32**: 571-583.

McCarthy, P. & Elix, J. (2014) The lichen genus Rhizocarpon in mainland Australia. *Telopea* **16**: 195–211.

McCarthy, P. M. (2018) Checklist of the Lichens of Australia and its Island Territories. http://https://www.anbg.gov.au/abrs/lichenlist/introduction.html

McCune, B., Timdal, E., & Bendiksby, M. (2016) Rhizocarpon quinonum, a new anthraquinone-containing species from the Alaska Peninsula. *The Lichenologist* **48**: 367–375.

Menlove, J. E. (1974) Thin-Layer Chromatography for the Identification of Lichen Substances. *British Lichen Society Bulletin* **34**: 3–5.

Miadlikowska, J., Kauff, F., Högnabba, F., Oliver, J. C., Molnár, K., Fraker, E., ... & Stenroos, S. (2014) A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Molecular phylogenetics and evolution* **79**: 132–168.

Nimis, P. L. & Martellos, S. (2019) ITALIC - The Information System on Italian Lichens. Version 5.0. University of Trieste, Dept. of Biology. http://dryades.units.it/italic

Nimis, P. L., Hafellner, J., Roux, C., Clerc, P., Mayrhofer, H., Martellos, S., Bilovitz, P. O. (2018) The lichens of the Alps - An annotated checklist. *MycoKeys* **31**: 1–634.

Ohmura, Y, Kashiwadani, H (2018) Checklist of lichens and allied fungi of Japan. National Museum of Nature and Science Monographs **49**: 1-140

Poelt, J. (1990) Parasitische Arten der Flechtengattung Rhizocarpon: eine weitere Übersicht. *Mitteilungen der Botanische Staatssammlung München* **29**: 515–538

Rambaut, A. (2012) Figtree v. 1.4. https://github.com/rambaut/figtree

Rambaut A., Drummond A. J., Xie D., Baele G. & Suchard M. A. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 901.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... & Huelsenbeck, J. P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* **61**: 539-542.

Rosenwinkel, S., Korup, O., Landgraf, A., & Dzhumabaeva, A. (2015) Limits to lichenometry. *Quaternary Science Reviews* **129**: 229–238.

Runemark, H. (1956) Studies in Rhizocarpon. I. Taxonomy of the yellow species in Europe. *Opera Botanica* **2, 1**: 1–152.

Schmitt, I., Crespo, A., Divakar, P. K., Fankhauser, J. D., Herman-Sackett, E., Kalb, ... & Lumbsch, H. T. (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **23**: 35.

Schneider, K., Resl, P., & Spribille, T. (2016) Escape from the cryptic species trap: lichen evolution on both sides of a cyanobacterial acquisition event. *Molecular ecology* **25**: 3453–3468.

Smith, C. W., Aptroot, A., Coppins, B. J., Fletcher, A., Gilbert, O. L., James, P. W., & Wolseley, P. A. (eds) (2009) *The Lichens of Great Britain and Ireland*. British Lichen Society, London. 1046 pp.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihoodbased phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Timdal, E., Holtan-Hartwig, J. (1988) A preliminary key to Rhizocarpon in Scandinavia. *Graphis Scripta* **2**: 41–54

Timdal, E. (1986) Rhizocarpon santessonii, a new parasitic lichen from Norway. *Lichenologist* **18**: 317–320.

Westberg, M., Moberg, R., Myrdal M., Nordin, A., & Ekman, S. (2021) *Santesson's Checklist of Fennoscandian Lichen-Forming and Lichenicolous Fungi*. Museum of Evolution, Uppsala University.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **18:** 315-322.

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org.

Widhelm, T. J., Grewe, F., Huang, J. P., Mercado-Díaz, J. A., Goffinet, B., Lücking, R., … & Lumbsch, H. T. (2019) Multiple historical processes obscure phylogenetic relationships in a taxonomically difficult group (Lobariaceae, Ascomycota). *Scientific reports* **9**: 1–16.

Zoller, S., Scheidegger, C., & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* **31**: 511-516.

Øvstedal, D.O. & Lewis Smith, R.I. (2001) *Lichens of Antarctica and South Georgia - A Guide to their Identification and Ecology*. Cambridge University Press, 411 pp.

Appendix 1.

Table 1. Specimens included in the study. Gray row colour indicates that the specimen was in both the FO and MO phylogenies, while white row colour means it was in only MO phylogeny. The **DNA Extr.** column refers to the extraction ID or GenBank ID. The species column indicates what species the specimen is determined to before sequenceing. Some collectors' surnames are abbreviated to save space: E.T. = E. Timdal, R.H = R. Haugan, M.B. = M. Bendiksby, M.F. = M. Fjelde, E.M. = E. Möller, V.K. = V. Kinneberg, J.T.K. = J.T. Klepsland**. Coll.No** refers to the collection number. The **TLC** column indicates what secondary metabolites have been observed, an empty field means that TLC has not been conducted. **GUID** refers to the herbarium ID of the specimen. SX numbers refer to ID the Oslo herbarium gives to loaned specimens. The **ITS**, **mtSSU** and **MCM7** columns refer to the PCR ID (PCR_tube) or GenBank ID of the respective markers. *The *R. oederi* accession was a chimeric sequence, consisting of an MCM7 from GenBank and ITS+mtSSU from NHM database. **364 & 365 did not have voucher information in the database, but both appeared sister to their respective conspecifics, and were kept in the study. *** 6112 (*R. grande*) is a wrongly determined *R. lavatum.* ****Two specimens were first determined to *Lecidea sp*. but blasted to *Rhizocarpon* and turned out to be part of clade A3 in the MO phylogeny. *****An erroneously determined *R. polycarpum*, a common mistake made by myself and every other herbarium we loaned material from. The two species differ mainly in prescence/absence of the apothecial marginal rim and presence/absence of the sharply delimited pigmented paraphyse

DNA Extr.	*Species*	Country	Coll.Date	Collector	Coll.No	TLC	GUID	ITS	mtSSU	MCM7
208	*R. inarense* (Vain.) Vain.	Norway	6/25/2011	E.T.	12074	norstictic acid, rhizocarpic acid	O-L-170574	40_1	42_3	x
209	*R. distinctum*	Norway	9/12/2010	E.T.	11817		O-L-169125	40_2	42_4	x
210	*R. furfurosum* H. Magn. & Poelt	Norway	5/27/2011	R.H., E.T.	11858	stictic acid	O-L-169766	40_3	42_5	x
211	*R. norvegicum*	Norway	8/4/2010	E.T.	11507		O-L-163568	40_4	42_6	x
212	*R. alpicola* (Fr.) Rabenh.	Norway	6/25/2011	E.T.	12041	psoromic acid, rhizocarpic acid	O-L-170541	40_5	42_7	x
213	*R. suomiense*	Norway	5/29/2011	R.H., E.T.	11910	norstictic acid	O-L-169818	40_6	42_8	x
214	*R. santessonii*	Norway	5/29/2011	R.H., E.T.	11897		O-L-169805	40_7	42_9	x
215	*R. intermediellum* Räsänen	Norway	8/1/2007	E.T.	10615	psoromic acid, rhizocarpic acid	O-L-149126	40_8	42_10	x
216	*R. superficiale* (Schaer.) Malme	Norway	5/23/2010	E.T.	11351	hypostictic acid, rhizocarpic acid, stictic acid	O-L-163412	40_9	42_11	x
218	*R. oederi**	Norway	11/16/2007	R.H.	7579	no lichen substances	O-L-151401	40_11	42_13	MN437622
219	*R. umbilicatum*	Norway	9/12/2010	E.T.	11815		O-L-169123	40_12	42_14	x
220	*R. lecanorinum* Anders	Norway	4/2/2011	S. Rui, E.T.	11822		O-L-169130	40_13	42_15	x
221	*R. amphibium*	Norway	8/3/2010	E.T.	11467	no lichen substances	O-L-163528	40_14	42_16	x

223	*R. rittokense* (Hellb.) Th. Fr.	Norway	8/12/2011	H. Bratli, T. Myhre, E.T.	8051	barbatic acid	O-L-175915	40_16	42_18	x
224	*R. chioneum* (Norman) Th. Fr.	Norway	8/10/2011	M.B., H. Bratli, R.H., T. Myhre, E.T.	12444		O-L-175723	40_17	42_19	x
225	*R. polycarpum*	Norway	8/7/2011	M.B., T. Myhre, E.T.	12460		O-L-175739	40_18	42_20	x
226	*R. geographicum*	Norway	8/10/2011	M.B., H. Bratli, R.H., T. Myhre, E.T.	12438		O-L-175717	40_19	42_21	x
227	*R. ferax* H. Magn.	Norway	8/9/2011	M.B., H. Bratli, R.H., T. Myhre, E.T.	12420		O-L-175699	40_20	42_22	x
228	*R. vorax* Poelt & Hafellner	Norway	7/6/1995	R.H., E.T.	4753		O-L-20249	40_21	42_23	x
230	*R. caesium*	Iceland	7/23/2009	E.T.	11208	no lichen substances	O-L-160733	40_23	42_25	x
232	*R. dinothetes* Hertel & Leuckert	Norway	8/12/2011	H. Bratli, T. Myhre, E.T.	12788		O-L-184631	40_25	x	x
233	*R. leptolepis* Anzi	Norway	7/20/1987	E.T.	4944	friesiic acid, unknown substance	O-L-12539	40_26	42_28	x
236	*R. timdalii* Ihlen & Fryday	Norway	1989-02	J. Holtan-Hartwig, E.T.	7212	fatty acid	O-L-71409	40_29	42_31	x
335	*R. cinereovirens*	Norway	8/17/2011	R.H.	10536	norstictic acid, stictic acid	O-L-174113	46_8	50_26	x
358	*R. pycnocarpoides*	Norway	6/16/2012	M.B., R.H., J.T.K., E.T., N. Valland, M. Westberg	12636		O-L-179560	48_1	49_1	x
363	*R. sublavatum*	Sweden	6/29/2003	P.G. Ihlen	1283		O-L-177918	48_6	49_6	x
364	*R. lavatum***						X	48_7	49_7	x
365	*R. roridulum* Th. Fr.**						X	48_8	49_8	x
667	*R. norvegicum*	Norway	8/4/2010	E.T.	11507		O-L-163568	OLICH450_13	x	x
1327	*R. hochstetteri*	Norway	7/11/2013	E.T.	13021	unknown, unknown, unknown	O-L-184434	OLICH770_13	x	x
1389	*E. scabrosus*	Norway	8/8/2009	J.T.K.	JK09-L398		O-L-164936	OLICH832_13	x	x

1717	*E. glauconigellus*	Norway	7/16/2012	S. Rui, E.T.	12669		O-L-179907	OLICH1100_14	x	x
1718	*E. glauconigellus*	Norway	8/9/2003	R.H.	skib03-7-26		O-L-141634	OLICH1101_14	x	x
1719	*E. scabrosus*	Norway	7/27/2012	S. Rui, E.T.	12717		O-L-179955	OLICH1102_14	x	x
2172	*R. hochstetteri*	Norway	10/20/2010	R.H.	9001	no lichen substances	O-L-165653	OLICH040_11	x	x
2173	*R. hochstetteri*	Norway	7/27/2010	E.T.	11417	no lichen substances	O-L-163478	OLICH041_11	x	x
2175	*R. hochstetteri*	Norway	8/9/2010	E.T.	11725	no lichen substances	O-L-163778	OLICH043_11	x	x
2176	*R. hochstetteri*	Norway	7/28/2010	E.T.	11450	stictic acid	O-L-163511	OLICH044_11	x	x
3613	*P. cacuminum*	Austria	8/3/2004	J. Hafellner	63791		SX-16563	LT12_26	x	x
3614	*P. cacuminum*	Austria	8/5/2004	J. Hafellner	63781		SX-16564	LT12-27	x	x
3948	*R. hochstetteri*	Norway	8/4/2015	E.T., et al.	WG1-1453	unknown (white), unknown	O-L-201249	OLICH2674_16	x	x
6109	*R. geographicum*	USA	9/23/2015	B. McCune	36194	psoromic acid, rhizocarpic acid	SX-15411	241_21	240_21	x
6110	*R. grande* (Flörke ex Flot.) Arnold	USA	9/4/2015	B. McCune	35937	gyrophoric acid, stictic acid, unknown	SX-15412	241_22	240_22	x
6112	*R. grande****	USA	7/7/2015	B. McCune	36539	no lichen substances	SX-15414	241_24	240_24	x
6113	*R. hensseniae*	USA	7/17/2014	B. McCune	35464	stictic acid	SX-15415	241_25	240_25	x
6114	*R. hensseniae*	USA	7/9/2015	B. McCune	36273	stictic acid	SX-15416	241_26	240_26	x
6116	*R. infernulum*	USA	7/27/2013	B. McCune	32921	no lichen substances	SX-15418	241_28	240_28	x
6117	*R. jemtlandicum* Malme	USA	7/20/2012	B. McCune	34078		SX-15419	241_29	x	x
6119	*R. lavatum*	USA	7/11/2014	B. McCune	35070	no lichen substances	SX-15421	241_31	240_31	x
6121	*R. lavatum*	USA	7/16/2014	B. McCune	35424	no lichen substances	SX-15423	241_33	240_33	x
6122	*R. lecanorinum*	USA	7/9/2015	B. McCune	36309	norstictic acid, rhizocarpic acid, stictic acid, unknown	SX-15426	241_34	240_34	x
6123	*R. macrosporum* Räsänen	USA	--	B. McCune	32022	norstictic acid, rhizocarpic acid, stictic acid, unknown	SX-15427	241_35	240_35	x
6125	*R. polycarpum*	USA	7/7/2015	B. McCune	36183		SX-15430	241_37	240_37	x

6129	*R. reductum*	USA	3/16/2016	B. McCune	36587	stictic acid, unknown, unknown	SX-15433	241_41	240_41	x
6138	*R. sulphurosum* (Tuck. ex Willey) Lendemer	USA	5/20/2016	B. McCune	36737	rhizocarpic acid, unknown	SX-15443	241_50	240_50	x
6139	*R. superficiale*	USA	9/7/2012	B. McCune	32287	hypostictic acid, rhizocarpic acid, stictic acid	SX-15444	241_51	240_51	x
6145	*R. kakurgon* Poelt	Austria	9/4/1991	J. Hafellner			O-L-195024	241_57	x	x
6147	*R. roridulum*	Norway	9/2/2016	E.T.	16309	no lichen substances	O-L-201497	241_59	240_59	x
6691	*R. badioatrum* (Flörke ex Spreng.) Th. Fr.	Russia	7/27/1992	R.H., E.T.	YAK27/06	no lichen substances	O-L-19248	293_16	292_16	x
6693	*R. badioatrum*	Japan	6/8/2003	H. Shimizu	3000	confriesiic acid	O-L-129708	293_18	293_18	x
7702	*Rhizocarpon*	Norway	8/8/2010	E.T.	11666	no lichen substances	O-L-163727	OLICH6280_19	x	x
7893	*R. hochstetteri*	Norway	7/10/2018	R.H.	180447		O-L-225093	OLICH5311_19 |	x	x
8606	*R. hochstetteri*	Norway	4/27/2018	E.T.	16784	unknown (white)	O-L-225431	OLICH6510_19	x	x
9088	*Lecidea* Ach. ****	Norway	6/27/2018	R.H.	180356		O-L-224564	OLICH5927_19	x	x
9242	*R. alpicola*	Canada	7/21/2018	E.T., S. Rui	18111	psoromic acid, rhizocarpic acid	O-L-223755	403_1	403_33	463_8
9244	*R. disporum*	Canada	7/8/2018	E.T., S. Rui	18005	no lichen substances	O-L-223649	403_3	x	x
9245	*R. effiguratum* (Anzi) Th. Fr.	Canada	7/16/2018	E.T., S. Rui	18085	psoromic acid, rhizocarpic acid	O-L-223731	403_4	403_36	463_10
9246	*R. geographicum*	Canada	7/16/2018	E.T., S. Rui	18086		O-L-223732	403_5	403_37	463_11
9247	*R. geographicum*	Canada	7/11/2018	E.T., S. Rui	18020		O-L-223664	403_6	403_38	463_12
9248	*R. geographicum*	Canada	7/15/2018	E.T., S. Rui	18067		O-L-223711	403_7	403_39	463_13
9258	*R. pusillum* Runemark	Canada	7/15/2018	E.T., S. Rui	18073	norstictic acid	O-L-223718	403_17	403_49	463_14
9263	*Rhizocarpon*	Canada	7/25/2018	E.T., S. Rui	18124	no lichen substances	O-L-223768	403_22	403_54	463_16
9271	*R. parvum* Runemark	Norway	9/15/2018	E.T.	18273		O-L-225584	403_30	403_62	463_17
9330	*R. saurinum*	USA	9/14/2013	T. Wheeler	6026p.p.		SX-16500	415_12	423_4	463_1
9652	*R. hochstetteri*	USA	7/23/2012	T. Wheeler	4284	no lichen substances	SX-16434	424_3	x	463_6
9653	*R. hochstetteri*	USA	7/23/2012	T. Wheeler	4314	no lichen substances	SX-16435	424_4	x	463_7
10096	*R. eupetraeum*	Norway	7/10/2019	M.F., E.M.	19102		O-L-225894	x	x	458_21

10103	*R. hochstetteri*	Norway	8/19/2019	M.F., R.H., E.M., E.T.	19178	stictic acid	O-L-225964	431_15	432_15	458_26
10104	*R. hochstetteri*	Norway	8/19/2019	M.F., R.H., E.M., E.T.	19179	stictic acid	O-L-225965	431_16	x	x
10107	*R. hochstetteri*	Norway	8/19/2019	M.F., R.H., E.M., E.T.	19186	no lichen substances	O-L-225971	431_19	432_19	458_28
10108	*R. hochstetteri*	Norway	8/19/2019	M.F., R.H., E.M., E.T.	19187	no lichen substances	O-L-225972	431_20	432_20	458_29
10186	*R. hochstetteri*	Norway	10/14/2010	R.H.	9081	no lichen substances	O-L-165731	435_1	x	458_32
10188	*R. hochstetteri*	Norway	6/14/2012	M.B., R.H., J.T.K., E.T., M.W.	12529	no lichen substances	O-L-179453	435_3	x	458_33
10190	*R. hochstetteri*	Norway	10/20/2010	R.H.	9001	no lichen substances	O-L-165653	435_5	x	458_35
10191	*R. hochstetteri*	Norway	8/19/2012	R.H.	11097	stictic acid	O-L-182299	435_6	x	x
10195	*R. hochstetteri*	Norway	5/21/1998	R.H.	7935	no lichen substances	O-L-155279	435_10	436_10	458_37
10197	*R. hochstetteri*	Norway	7/28/2010	E.T.	11450	stictic acid	O-L-163511	435_12	x	x
10198	*R. hochstetteri*	Norway	7/27/2010	E.T.	11415	no lichen substances	O-L-163476	435_13	x	x
10199	*R. hochstetteri*	Norway	7/27/2010	E.T.	11416	no lichen substances	O-L-163477	435_14	x	x
10200	*R. hochstetteri*	Norway	10/3/2010	R.H.	9174	unknown (white), unknown	O-L-165824	435_15	436_15	x
10203	*R. hochstetteri*	Norway	8/8/2012	R.H.	11286	stictic acid	O-L-183846	435_18	436_18	458_41
10205	*R. hochstetteri*	Norway	8/15/2011	R.H.	10478	unknown (white), unknown	O-L-174055	435_20	436_20	x
10206	*R. hochstetteri*	Norway	7/8/2016	J.T.K.	JK16-543	no lichen substances	O-L-222653	435_21	436_21	458_43
10423	*Lecidea*****	Norway	8/10/2020	E.T.	18731		O-L-227832	OLICH7582_20	x	x
10515	*C. wahlenbergii*	Norway	7/27/2019	E.T.	18445		O-L-226139	442_1	462_1	458_46
10516	*R. hochstetteri*	Norway	7/27/2019	E.T.	18437	unknown (white)	O-L-226131	442_2	x	x
10517	*R. hochstetteri*	Norway	4/27/2018	E.T.	16786	no lichen substances	O-L-225433	442_3	x	x
10518	*R. hochstetteri*	Norway	7/27/2019	E.T.	18432	no lichen substances	O-L-226126	442_4	x	x
10519	*C. wahlenbergii*	Norway	7/12/2019	M.B., E.T.	18422		O-L-226116	442_5	462_5	458_47
10524	*R. hochstetteri*	Sweden	8/16/2017	E.T.	16464	stictic acid	O-L-208154	442_10	x	x
10525	*R. hochstetteri*	Russia	7/30/1992	R.H., E.T.	YAK32/23	no lichen substances	O-L-19364	442_11	x	x

10526	*R. hochstetteri*	Norway	8/11/2020	E.T.	18745	no lichen substances	O-L-227846	442_12	x	x
10528	*C. wahlenbergii*	Norway	8/8/2020	E.T.	18687		O-L-227789	442_14	462_14	458_49
10529	*C. wahlenbergii*	Norway	7/29/2020	E.T.	18597		O-L-227703	442_15	462_15	458_50
10530	*R. hochstetteri*	Norway	8/11/2020	E.M., E.T.	19460		O-L-228013	443_1	x	x
10534	*"R. expallescens"*	Norway	8/14/2020	E.M., E.T.	19484		O-L-228038	443_5	x	x
10535	*R. hochstetteri*	Norway	8/12/2020	E.M., E.T.	19482	no lichen substances	O-L-228036	443_6	x	x
10536	*R. hochstetteri*	Norway	8/12/2020	E.M., E.T.	19480	no lichen substances	O-L-228034	443_7	x	458_12
10537	*R. hochstetteri*	Norway	8/8/2020	M.F., R.H., V.K., E.M., E.T.	19386B	no lichen substances	O-L-227941	443_8	462_22	469_13
10538	*R. hochstetteri*	Norway	8/8/2020	M.F., R.H., V.K., E.M., E.T.	19386	stictic acid	O-L-227940	443_9	462_23	469_14
10542	*R. hochstetteri*	Norway	8/6/2020	M.F., R.H., V.K., E.M., E.T.	19362	stictic acid	O-L-227917	x	446_27	471_3
10543	*R. hochstetteri*	Norway	8/6/2020	M.F., R.H., V.K., E.M., E.T.	19358	no lichen substances	O-L-227913	443_14	462_28	471_4
10544	*R. hochstetteri*	Norway	8/6/2020	M.F., R.H., V.K., E.M., E.T.	19357	no lichen substances	O-L-227912	443_15	462_29	471_5
10545	*R. hochstetteri*	Norway	8/5/2020	M.F., R.H., V.K., E.M., E.T.	19351	no lichen substances	O-L-227906	443_16	462_30	471_6
10546	*R. hochstetteri*	Norway	8/5/2020	M.F., R.H., V.K., E.M., E.T.	19350	no lichen substances	O-L-227905	443_17	462_31	471_7
10547	*R. hochstetteri******	Norway	8/5/2020	M.F., R.H., V.K., E.M., E.T.	19342		O-L-227898	443_18	462_32	471_8
10548	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19452	unknown (white), unknown	O-L-228005	443_19	462_33	471_9
10549	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19451	stictic acid, unknown (white), unknown	O-L-228004	443_20	x	x
10550	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19450		O-L-228003	x	462_35	471_11
10551	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19449	no lichen substances	O-L-228002	443_22	462_36	471_12

10552	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19448	unknown (white), unknown	O-L-228001	443_23	462_37	471_13
10553	*R. hochstetteri*	Norway	8/10/2020	M.F., R.H., V.K., E.M., E.T.	19432	no lichen substances	O-L-227985	443_24	x	x
10554	*R. hochstetteri*	Norway	8/10/2020	M.F., R.H., V.K., E.M., E.T.	19431	stictic acid	O-L-227984	443_25	x	x
10555	*R. hochstetteri*	Norway	8/10/2020	M.F., R.H., V.K., E.M., E.T.	19430		O-L-227983	443_26	462_40	471_16
10556	*R. intersitum*	Norway	8/9/2020	M.F., R.H., V.K., E.M., E.T.	19419		O-L-227971	443_27	462_41	x
10557	*R. hensenniae*	Japan	10/4/2017	R.H., E.T.	16731		O-L-209874	443_28	462_42	471_18
10558	*R. hensenniae*	Japan	10/3/2017	R.H., E.T.	16714		O-L-209863	443_29	462_43	471_19
10559	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19457	unknown (white),	O-L-228010	443_30	462_44	471_20
10560	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19456	stictic acid	O-L-228009	443_31	462_45	471_21
10561	*R. atroflavescens*	Norway	8/15/2020	R.H., E.M., E.T.	19492		O-L-228046	443_32	462_46	x
10563	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19454	stictic acid	O-L-228007	443_34	462_48	471_24
11039	*R. hochstetteri*	Norway	8/11/2020	R.H., E.M., E.T.	19455	norstictic acid	O-L-228008	x	470_21	469_5
11041	*R. infernulum* f. *sylvaticum*	Ireland	4/26/1994	A. Fryday (& O. Gilbert)	5185			495_33	x	x
11043	*R. infernulum*	USA	27/8/2008	A. Fryday	9154		MSC0086678	x	470_41	x
11044	*R. hochstetteri*	USA	27/8/2008	A. Fryday	9195		MSC0086709	x	470_42	x
11065	*R. caesium*	Scotland	8/6/1991	A. Fryday	2448		E00456329	495_14	x	x
11097	*Rhizocarpon*	Norway	4/6/2021	E.M.	19519			496_13	497_13	x
11098	*Rhizocarpon*	Norway	4/6/2021	E.M.	19521			496_14	497_14	x
AF483173	*P. cerebrina*	Austria	--	--	--	--	--	AF483606.1	AF483173	x
AF483607	*R. hochstetteri*	Norway	7/19/1990	R.H.	1622	no lichen substances	O-L-119602	AF483607.1	x	x
EDNA16-0046365	*R. hochstetteri*	Scotland	9/9/2016	R. Yahr	6088		E0046365	EDNA16-0046365	x	x
EDNA16-0046444	*R. hochstetteri*	Scotland	9/20/2015	R. Yahr	5767		E0046444	EDNA16-0046444	x	x

EDNA16-0046445	*R. hochstetteri*	Scotland	9/20/2015	R. Yahr	5886		E0046445	EDNA16-0046445	x	x
EDNA16-0046579	*R. hochstetteri*	Scotland	9/21/2016	R. Yahr	6136		E0046579	EDNA16-0046579	x	x
EDNA16-0046580	*R. hochstetteri*	Scotland	8/18/2016	R. Yahr	5940		E0046580	EDNA16-0046580	x	x
EDNA16-0046588	*R. infernulum* f. *infernulum*	Scotland	9/21/2016	R. Yahr	6144		E0046588	EDNA16-0046588	x	x
U3427	*Rhizocarpon*	Sweden	10/11/2020	U. Arup	U3427	norstictic acid	L20089	U3427	x	x
U3428	*Rhizocarpon*	Sweden	10/11/2020	U. Arup	U3428	norstictic acid	L20096	U3428	x	x

Appendix 2. Gene trees.

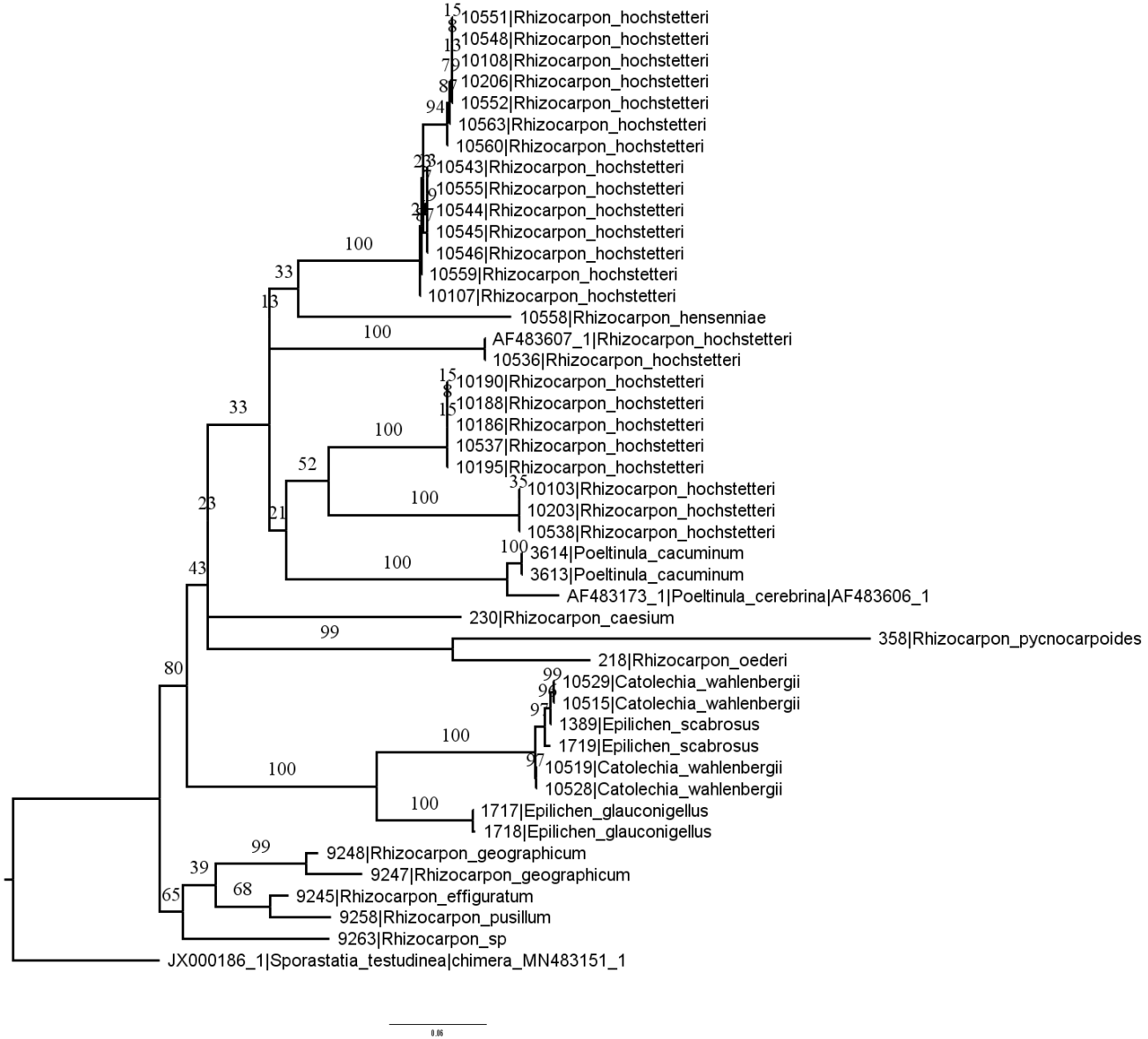

Figure 1. Maximum likelihood gene tree of ITS marker for the FO phylogeny. Bootstrap support indicated on branches. JX000186 is used as root.

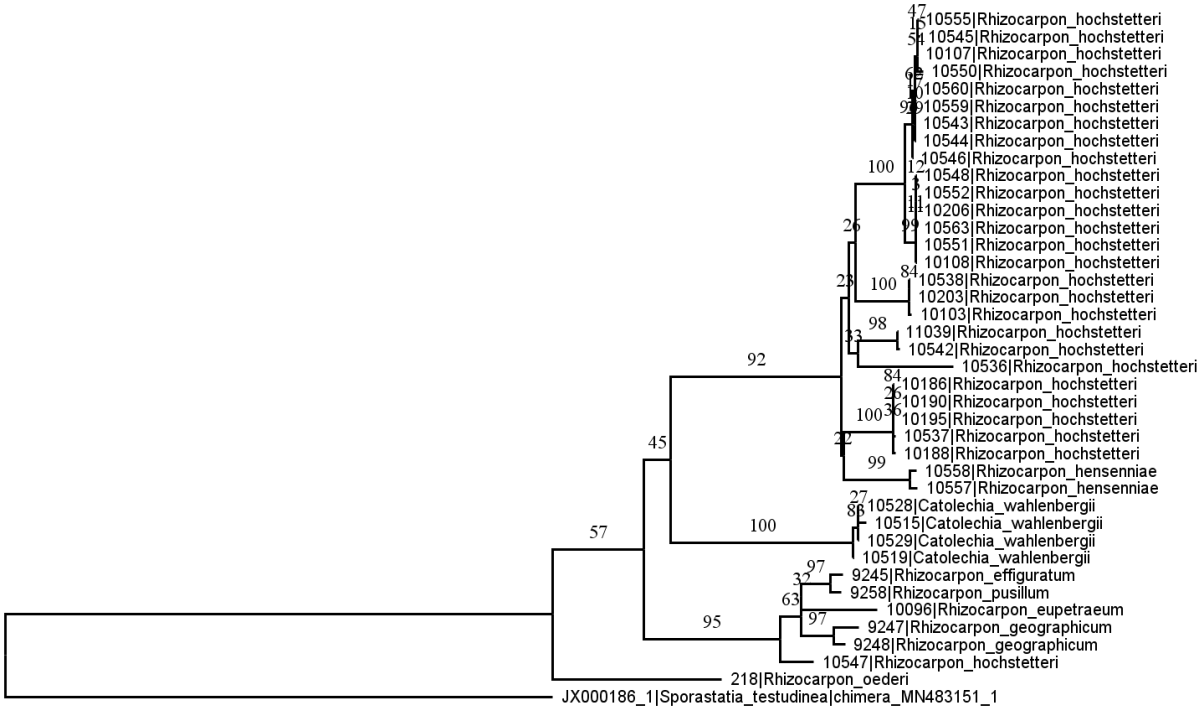

Figure 2. Maximum likelihood gene tree of MCM7 locus for the FO and the MO phylogenies. JX000186 is used as root.

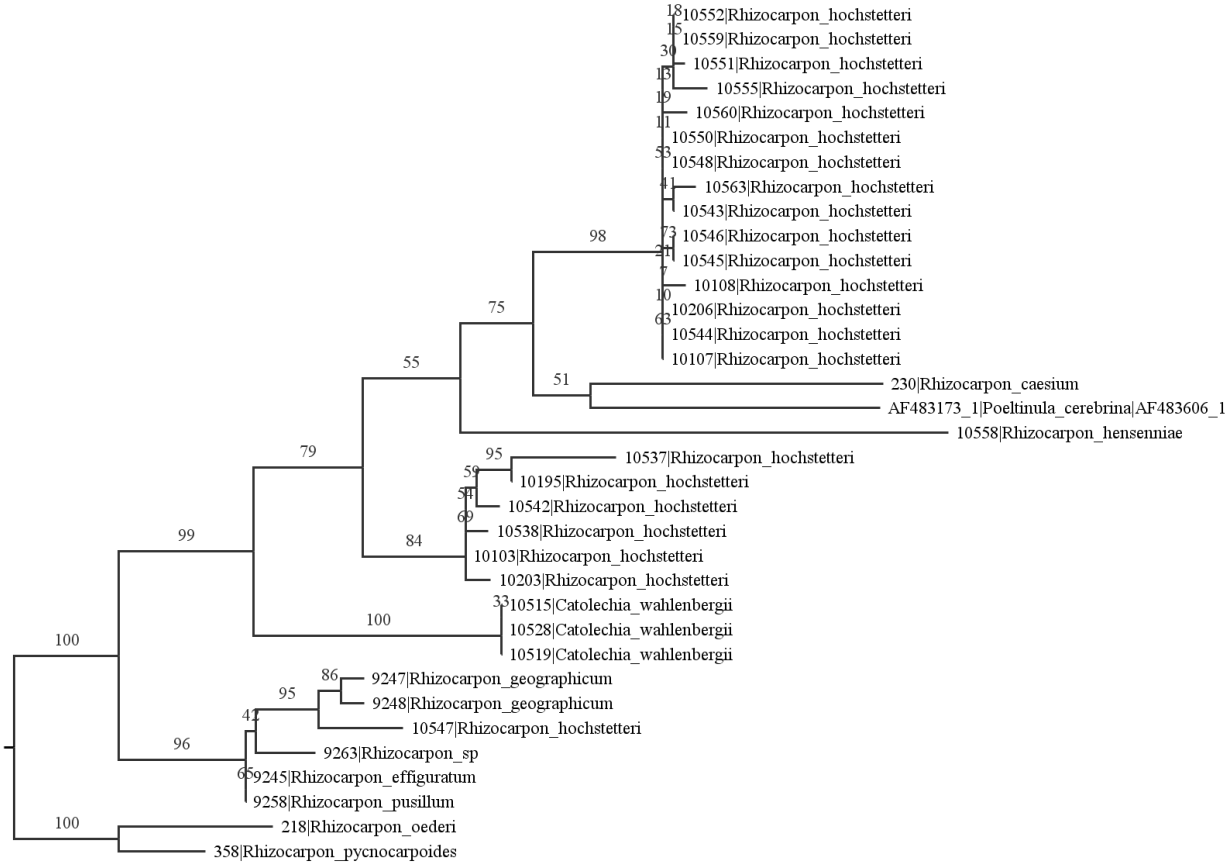

Figure 3. Maximum likelihood gene tree of the mtSSU locus for the FO phylogeny. 218/358-clade is used as root.

Figure 4. Maximum likelihood gene tree of the ITS locus for the MO phylogeny. Bootstrap support indicated on branches. 218/358-clade is used as root.

Figure 5. Maximum likelihood gene tree of the mtSSU locus fr the MO phylogeny. Bootstrap support indicated on branches. 218/358-clade is used as root.