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Running head: Symbiont lifestyle determination in *Nostoc* species

A molecular investigation of free-living and lichenized *Nostoc* sp. and symbiotic lifestyle determination

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ABSTRACT. Individual cyanobacterial species can occupy a diversity of symbiotic lifestyles yet how this lifestyle is determined is unknown. In this study we focus on the symbiotic lifestyles of the cyanobacterial genus *Nostoc*, and define their lifestyle based on their symbiotic state: free-living or symbiotic with lichen-forming fungi. Within an evolutionary context, members of one symbiotic lifestyle can be found more closely related to members of another symbiotic lifestyle and do not form monophyletic groups. This pattern can be explained by either deterministic or variable factors, by whether or not switches between lifestyles occur infrequently or frequently, and by limitations on symbiont dispersal. We began this study by focusing within a single habitat in which both free-living and symbiotic *Nostoc* sp. individuals occur in order to test amongst

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hypotheses. Using the *rbc*LX genetic region we placed individuals within a larger phylogenetic context using a worldwide dataset of free-living and symbiotic *Nostoc* sp. Free-living *Nostoc* sp. growing at this site were distinct from co-occurring lichenized strains, but closely related to symbiotic *Nostoc* sp. from other continents. We expanded our sampling to a larger geographic area, including free-living *Nostoc* sp., as well as two additional sites in which free-living and lichenized *Nostoc* sp., co-occurred. We found no evidence of spatial structure on a regional scale. Based on these results, we conclude that symbiotic lifestyles within distinct *Nostoc* sp. lineages are determined by variable factors, and their distributions are constrained and exclude physical proximity. Finally, in the course of identifying the fungal symbionts of *Nostoc* we identified two species of *Peltigera* using ITS rDNA sequences; These species are either under-reported in Wisconsin (*P. ponojensis*) or new to the U.S.A. (*P. islandica)*.

KEYWORDS. *Nostoc,* symbiosis, free-living, lichen, *rbc*LX, ITS rDNA, symbiotic-lifestyle.

It is well known that terrestrial cyanobacteria can occupy a variety of symbiotic lifestyles including in free-living and symbiotic relationships. One important symbiotic lifestyle for terrestrial cyanobacteria is their mutualistic relationship with lichen-forming fungi; terrestrial cyanobacteria form long-lived symbiotic relationships with fungi, and together they form the lichen thallus. In this study we targeted lichen symbiotic relationships between the fungal genera *Leptogium* and *Peltigera*, and members of the cyanobacterial genus *Nostoc*. In these cases, the cyanobacteria are routinely assumed to be of one species called *Nostoc punctiforme*, although this is not necessarily based on taxonomic identification (Rikkinen 2002; Tschermak-Woess,

1988). In addition, it can be difficult to identify *Nostoc* species based on traditional characters such as morphology and physiology as these traits can vary widely depending upon an individual's symbiotic lifestyle (Rikkinen 2002). The nature of bacterial species in general is an unsolved problem (Fraser et al. 2007). However, there have been decades of productive ecological and evolutionary research on bacteria that relate informal naming schemes such as pathovar (Cai et al. 2011), serovar (Chui et al. 2015), RAD type (Lee et al. 2017), etc., to well resolved lineages in phylogenies inferred from the core genome. In the case of *Nostoc*, previous research has studied host association and geographic structuring of clusters defined by the *rbc*LX locus and supported by multi-locus sequence analysis (O'Brien et al. 2013). The *rbc*LX locus targets the intergenic region between *rbc*L (encoding the D-ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit) and *rbc*X (whose product possibly acts as a chaperonin protein) genes using primers anchored in the 3′ end of the *rbc*L gene and the 5′ end of the *rbc*S (encoding the D-ribulose 1,5-bisphosphate carboxylase-oxygenase small subunit] gene found downstream of *rbc*X [Rudi et al. 1998). For these reasons we here refer to the cyanobacterial taxon of our study more broadly as *Nostoc* sp., which includes members of the genus that have previously been found growing across the ecological spectrum from free-living to mutualistic symbiosis in lichens (O'Brien et al. 2005).

We do not understand how the symbiotic lifestyle is determined for *Nostoc* sp. One hypothesis states that symbiotic lifestyle is controlled by deterministic factors that produce an obligatory outcome. With this hypothesis it is possible that switches between symbiotic lifestyles can be infrequent (shown as a phylogeny in **Fig. 1A**) or frequent (**Fig. 1B**). Previous research does not support that infrequent switches occur between symbiotic lifestyles within this hypothetical framework (O'Brien 2005; O'Brien et al. 2013); yet, frequent switches between

lifestyles remain possible. Another hypothesis for symbiotic lifestyle determination states that it is controlled by variable factors that produce a facultative outcome—where individuals are not restricted to a particular symbiotic lifestyle; In this hypothesis the distribution of individuals can either be limited by proximity (shown as a phylogeny in **Fig. 1C**) or by additional factors that vary across ecological and geographical space (**Fig. 1D**).

Figure 1 highlights the difficulties in distinguishing between hypotheses for symbiotic lifestyle determination. Shared locations are shown in bold in **Fig. 1** in order to emphasize that it is not possible to differentiate between hypotheses 1B and 1D, or 1C and 1D without complete sampling of multiple samples per lifestyle per site. Frequently *Nostoc* sp. occupies only one symbiotic lifestyle (lichen-forming versus free-living) per site. Additionally, *Nostoc* sp. lineages that are both free-living and symbiotic with land plants have not been found to co-occur in the same habitat in the wild (Rikkinen & Virtanen 2008; West & Adams 1997; Zimmerman & Bergman 1990; Zimmerman & Rosen 1992). These observations suggest that *Nostoc* sp. individuals can be either symbiotic or free-living but not both, despite *in vitro* evidence that free-living *Nostoc* sp. lineages can be induced to grow symbiotically with land plants (West & Adams 1997). We know of only one study that has used DNA evidence to investigate free-living and lichenized forms of *Nostoc* sp. that co-occur in the same habitat. Oksanen et al. (2002) were able to show that free-living and lichenized forms of *Nostoc* in limestone depressions within a 1m by 1.2 m plot are distinct clonal lineages. However, because these lineages were not placed within a larger phylogenetic context, we cannot tell if these distinct lineages are the result of obligate (**Fig. 1A & B**) or facultative forces (**Fig. 1D**). The current study takes a primary investigation into distinguishing between the hypotheses shown in 1B, 1C and 1D by sampling from multiple individuals per lifestyle at shared locations.

In trying to understand the underlying forces of *Nostoc* evolution it is important to determine if individual clonal lineages of *Nostoc* sp. can occupy both symbiotic and free-living lifestyles. Furthermore, understanding symbiotic lifestyle determination in *Nostoc* can inform us of genetic and/or ecological determinants of *Nostoc* in the lichen-symbiosis. We therefore set out to determine if the patterns of *Nostoc* symbiotic lifestyle within an evolutionary context can be explained by deterministic or varying factors, and if their distributions are limited by physical proximity or additional influences. To do this we used the *rbc*LX genetic region within a phylogenetic context as it has been successfully used to create molecular phylogenies for free-living and lichenized forms of *Nostoc* sp. (O'Brien et al. 2005; O'Brien et al. 2013). We started by using a remarkable habitat in which *Nostoc* sp. occupies both symbiotic and free-living lifestyles in order to differentiate between competing hypotheses, and then expanded our sampling across a wider geographic region.

MATERIALS AND METHODS

Collection area and sampling. We began by collecting 26 free-living and symbiotic samples of *Nostoc* sp. at the UW Milwaukee Field Station (UWMFS; Ozaukee County, WI; +43.385°N, –88.0212°W). UWMFS samples were collected in plots where symbiotic and free-living *Nostoc* sp. were found growing together (**Fig. 2**). Thalli of free-living *Nostoc* sp. over 1 cm wide were collected directly on soil. Collections of *Nostoc* sp. growing symbiotically with the lichen forming fungus *Peltigera* sp. were collected as thalli over 0.5 cm wide and living directly on soil. Distances between adjacent collections were measured in 0.5 cm increments.

We sampled further symbiotic *Nostoc* sp. (with either *Peltigera islandica* or *Leptogium lichenoides*) and free-living *Nostoc* sp. individuals growing from across a broader geographic

region spanning Wisconsin, Minnesota and Michigan (**Table 1** and **Fig. 3**). These collections were made by targeting free-living *Nostoc* sp., as well as two additional sites in the region where free-living and lichenized forms co-occur. One of these sites was found in Waukesha County, WI where *Leptogium lichenoides* co-occurs with free-living forms of *Nostoc* sp. The other site is found in Douglas County, WI, where *P. islandica* co-occurs with free-living *Nostoc* sp. All collections are deposited at the Milwaukee Public Museum Wisconsin State Herbarium (MIL).

In order to observe evolutionary relationships between *Nostoc* sp. individuals as well as possible switches in symbiotic lifestyles, we used the *rbc*LX genetic region within a phylogenetic context. We identified fungal symbiotic partners using either standard DNA or morphological characters. We identified *Peltigera* symbionts using the entire internal-transcribed spacer region (ITS rDNA) sequence within a phylogenetic context; We used this method based on its success in identifying *Peltigera* species (Miadlikowska et al. 2003; O'Brien et al. 2009) and in overcoming inherent difficulties of *Peltigera* identification based on morphology and chemistry. We identified members of the fungal genus *Leptogium* based on morphological characters.

DNA extraction, PCR and sequencing. We extracted DNA from free-living and lichenized *Nostoc* sp. samples using a DNeasy Plant Mini Kit (Qiagen USA, Valencia CA), and amplified portions of the *rbc*X and intergenic spacer region between *rbc*L and *rbc*X (*rbc*LX) for each specimen (Rudi et al. 1998) following the protocol of O'Brien et al. (2013). We amplified and sequenced *rbc*LX using either the published primers *rbcLX*_cx (Rudi et al. 1998) and *rbcLX*_cz (O'Brien et al. 2013), or we used the following redesigned primers. For a Forward primer we used *rbcLX*_1374F which anneals within the last 75 bp of the *rcb*L gene (5′ - CTTGCGAACTGTGGAAAG -3′). For a Reverse primer we used *rbcLX*_cx_20nt, which is a

shortened version of *rbcLX*_cx (5′ - AGGTAAGAAAGGGTTTCGTA -3′). In order to determine the species identity of *Peltigera* samples we amplified the ITS rDNA region following the protocol of O'Brien et al. (2013). We cleaned PCR products using a QIAquick PCR Purification Kit (Qiagen USA, Valencia CA), and sequenced them through either the University of Wisconsin Milwaukee and Great Lakes Genomics Center sequencing facility or through Macrogen.

Phylogenetic analysis. We assembled sequences using UGENE (Okonechnikov et al. 2012). MAFFT (Katoh et al. 2002) was used to align *rbc*LX sequences to a reference set of sequences from O'Brien et al. (2013) and to align ITS rDNA sequences to reference sequences from GenBank identified using Blast. Maximum likelihood trees were inferred using PhyML (Guindon et al. 2010), and branch support was assessed with approximate likelihood ratio tests. Sequence metadata was stored in a MySQL database (accessible at www.PhotobiontDiversity.org and downloadable at https://github.com/hobrien/PhotobiontDiversity/tree/master/DB) and mapped onto the tree using the ETE toolkit (Huerta-Cepas et al. 2016). Associations between membership of sequences in the five labeled lineages in **Fig. 4** (plus the singleton at the bottom of the figure) and collection location (UWMFS vs. the broader sampling) and symbiotic lifestyle (free-living vs. *Peltigera* vs. *Leptogium*) with generalized linear modeling and likelihood ratio tests using the MASS library in R (Venables & Ripley 2002). We tested associations between lineage and location, conditioned on Symbiotic lifestyle (loglm(~ Lineage + Location + Symbiotic lifestyle + Lineage * Symbiotic lifestyle + Location * Symbiotic lifestyle)) and between lineage and Symbiotic lifestyle, conditioned on location (loglm(~ Lineage + Symbiotic lifestyle + Location + Lineage * Location + Symbiotic lifestyle * Location)).

RESULTS

Collection area and sampling. Initial UWMFS isolates were collected in four plots, each less than 2 m in diameter. Plot sizes and relative distance between plots are shown in **Supplementary Figure S1**, and distances between adjacent samples are recorded in **Supplementary Table S1**. Within plots, distances between samples ranged from 4 cm to 131cm. Additional symbiotic and free-living *Nostoc* individuals were sampled from a broader geographic area across Michigan, Minnesota and Wisconsin, and their collection information is included in **Table 1**.

Sequencing (both *rbc*LX and ITS rDNA). We generated 37 new *rbc*LX sequences for free-living *Nostoc* sp., eight new *rbc*LX sequences of *Nostoc* sp. lichenized with *Leptogium lichenoides*, and 14 new *rbc*LX sequences of *Nostoc* sp. lichenized with either *Peltigera ponojensis* or *P. islandica* (**Table 1**). We generated 14 new ITS rDNA sequences for these *Peltigera* species (**Table 1**). Sequence results for both *rbc*LX and ITS rDNA loci are deposited at GenBank (Accession numbers MF067303–MF067361 and MF067362–MF067375, respectively).

Phylogenetic analysis. The top Blast hit for all fungal ITS rDNA sequences except SLJ4349 were sequences from *Peltigera ponojensis* (**Supplementary Table S2**). SLJ4349 was 100% identical to a sequence from *P. islandica* (KC139759). The 13 sequences whose ITS rDNA sequences most closely resembled *P. ponojensis* based upon top BLAST hits were aligned with 16 *P. ponojensis, P. monticola* and *P. scotteri,* reference sequences, producing an alignment with 586 positions, of which 83 were variable and 53 were parsimony-informative. All sequences from this study were nested within the *P. ponojensis* clade (**Supplementary Figure**

S2). The final *rbc*LX alignment consisted of 59 new sequences plus 321 distinct reference sequences and 835 unambiguous nucleotide positions, of which 505 are polymorphic. The tree consisted of 12 *Nostoc* sequences on long branches at the base of the tree, and a clade of 16 *Nodularia* sequences sister to a large clade of 352 *Nostoc* sequences. Within the large *Nostoc* clade, there was a large, well-supported subclade of Nephromataceae/Lobariaceae symbionts, a smaller clade consisting of symbionts of *Sticta hypochra*, four subclades of plant symbionts (two of which were sister, but without support), eight subclades of Collemataceae symbionts, and 11 subclades of *Peltigera* symbionts. There was also a well-supported clade that consisted of a mixture of symbionts of plants, *Peltigera* and Collemataceae, as well as free-living strains and a number of sequences that did not cluster into well-supported clades.

In the original UWMFS study site in which both lichenized and free-living *Nostoc* sp. individuals co-occurred at four plots, we found distinct evolutionary lineages occupying the free-living and lichen-symbiotic lifestyles, and we found that members of each symbiotic lifestyle belong to one of one or two possible lineages per lifestyle (**Fig. 4**). In addition, within a large evolutionary context these lineages are associated with additional lifestyle specific isolates from around the world (**Fig. 5**). For example, free-living UWMFS members of Clades A and B (**Fig. 5A & B**) are related to free-living *Nostoc* sp. from additional sites in Wisconsin, Minnesota, and Michigan (See **Table 1** for location information of isolates from this study, and **Supplementary Table S3** for location information of GenBank isolates). Lichenized UWMFS members of Clades C, D, and E (**Fig. 5C, D & E**) are related to lichenized *Nostoc* sp. from additional Wisconsin sites as well as from Canada and from Europe. Sampling locality (UWMFS vs. other) was not associated with lineage membership (p=0.667), but there was a strong association between lineage membership and lifestyle (free-living vs. *Peltigera* vs. *Leptogium*, p= 2.65e-09).

This indicates that there is no geographical structure at this scale, but that symbiotic lifestyle membership is highly non-random within each clade. Finally, although each clade in **Fig. 5** is strongly represented by one *Nostoc* sp. symbiotic lifestyle, both free-living and lichenized forms are present in four out of the five clades. For example, clades dominated by free-living forms also contain symbiotic individuals that form lichens with *Collema* and *Leptogium* (**Fig. 5A & B**). Clades C and D are dominated by symbionts of the lichen genus *Peltigera* but each clade contains one free-living form (Z94891 and AJ632030, respectively). This is true of all clades except for Clade E in which only symbiotic *Nostoc* sp. forms are found, collected from Wisconsin and Europe.

DISCUSSION

Symbiotic lifestyle diversity across the phylogeny of *Nostoc* sp. is broad, including as free-living individuals and in intimate symbiotic relationships with vascular plants, non-vascular plants, and fungi (O'Brien et al. 2005, 2013). Individuals from a single lifestyle do not form monophyletic groups within a global evolutionary scale, and it has been an open question if symbiotic lifestyle is evolutionarily labile and controlled by deterministic factors, or if symbiotic lifestyle is controlled by variable factors. It was not possible to answer this question until differing symbiotic lifestyles could be sampled from one location, thereby ruling out location as a confounding factor. In this study we sampled a remarkable habitat at the UWMFS in which both lichenized and free-living *Nostoc* sp. individuals co-occur. We found that members are genetically distinct from each other based on symbiotic lifestyle, and that these lifestyle-specific lineages are not each other's closest relatives (**Fig. 5**). Our results are consistent with the only other known studies in which symbiotic (with land plants) and free-living *Nostoc* sp. were

sampled from the same habitat using phylogenetics; In each case different symbiotic lifestyles contain distinct evolutionary lineages (Rikkinen & Virtanen 2008; West & Adams 1997). Although a larger sample size could uncover closely related lineages occupying both symbiotic lifestyles at the UWMFS site, the presence of independent lineages by lifestyles suggests that this is not the general trend; From the UWMFS site there are two separate symbiotic lineages, and two separate free-living lineages.

Based on the distribution of our samples and their symbiotic state within a large evolutionary context, we conclude that lifestyle determination in *Nostoc* sp. is controlled by variable factors (**Fig. 5**). Not only do all of our free-living and symbiotic forms fail to form lifestyle-specific clades, but within each clade that contains our samples both symbiotic and free-living lifestyles can be found. Symbiotic forms can be found within the overwhelmingly free-living clades in Clades A and B (**Fig. 5A & B**), and free-living forms can be found in the overwhelmingly symbiotic clades in Clades C and D (**Fig. 5C & D**). Because not all free-living or lichenized genotypes can be found in every available habitat, these results also support the hypothesis that the distribution of *Nostoc* sp. is not constrained by physical proximity but instead by additional factors such as the interactions of genetics with environment. These interactions could take place in the form of selective advantages for particular genotypes in particular environmental conditions, or particular genotypes in pairing with symbiotic partner genotypes. The molecular mechanisms for these determinations are poorly understood in *Nostoc* (Adams & Dugan 2008; Honegger 1998). Further studies can help clarify this by investigating differences in genomics and gene-expression profiles in lifestyle-specific lineages across a broad geographic and environmental landscape.

In the course of identifying fungal symbionts of *Nostoc* in this study we uncovered two *Peltigera* species which are either under-reported from Wisconsin (*P. ponojensis*), or completely new to the U.S.A. (*P. islandica*). Regarding *P. ponojensis*, this discrepancy is most likely a result of inconsistent taxonomic treatments for the state. While Thomson's Lichens of Wisconsin (2003) does not include *P. ponojensis* in the state's lichen-flora, Brodo et al. (2001) show *P. ponojensis* as occurring throughout the Northern North American continent including Wisconsin and surrounding states. Historically, *Peltigera* taxonomy based on morphological and chemical traits has been hampered by overlapping phenotypic plasticity between species (Miadlikowska et al. 2003). Some species have only recently been resolved using molecular phylogenetics, including *P. ponojensis* (Miadlikowska et al. 2003; O'Brien et al. 2009). Based on shared characteristics in thallus tomentum, color and morphology, *P. ponojensis* specimens previously collected in Wisconsin were most likely identified as either *P. canina* or *P. rufescens* according to the original species description and the *Peltigera* key and descriptions in Thomson (2003). A molecular phylogeny using DNA from herbarium collections should be able to determine if *P. ponojensis* already exists in herbarium collections. All three of these closely-related species belong to the "*Peltigera canina* group" as defined by Miadlikowska et al. 2003. We were able to find only two reports of *P. ponojensis* occurring in Wisconsin in the Consortium of North American Lichen Herbaria (accessed 10 August 2017); Both collections were made by J. W. Thomson in 1945, and later identified as *P. ponojensis* by additional researchers.

Regarding *Peltigera islandica*, this species has previously been reported only from Iceland as well as from a single isolate collected in southern British Columbia, Canada (Manoharan-Basil et al. 2016). The authors of this species suggest that its geographic range may be larger than this, a possibility that can be verified only by additional field and herbarium

sampling (Manoharan-Basil et al. 2016). It also belongs to the "*Peltigera canina* group," and was successfully delimited from other members using molecular phylogenetics (Manoharan-Basil et al. 2016). It is predicted that further exploration of *Peltigera* species in Wisconsin at the molecular level will uncover additional populations of *P. islandica*. If previous collections of *P. islandica* exist in herbaria, they are most likely identified as either *P. canina* or *P. membranacea* based on their shared characteristics in thallus and rhizine morphology and color. A molecular phylogeny using DNA extractions from herbarium collections will likely be able to confirm this. Due to the limited nature of finding free-living and lichenized forms of *Nostoc* sp. co-occurring in the same habitat (and within 2m of each other), we came across this species only once (SLJ4349). This specimen was found growing within 5 mm of a free-living form of *Nostoc* sp. (SLJ4350). Even with our limited sampling of *Peltigera* species, we have greatly expanded the ranges of *P. islandica* and *P. ponojensis*. It stands to reason that by adopting the use of molecular markers in identifying *Peltigera* species, our understanding of *Peltigera* biodiversity in Wisconsin will change.

The results of this study highlight that the secrets of *Nostoc* sp. lichenization and the ability to form long-lasting symbiotic relationships with lichen-forming fungi cannot be found by comparing deterministic genomic elements of the *Nostoc* sp. alone. Instead, the place to look for informative clues on symbiotic lifestyle determination can be found by comparing gene expression profiles within members of a *Nostoc* sp. lineage that occupy both free-living and symbiotic states. To untangle the phenotypically-plastic nature of how the *Nostoc* sp. symbiotic lifestyle is determined we will need to be able to quantitatively differentiate among environmental influences, gene expression levels, and the availability of symbiotic-partners.

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Supplementary documents online:

Supplementary Figure S1. Size of, and distances between, four collection plots at UWMFS. Distances within clusters are known (see Supplementary Table S1), while distances between clusters are approximate.

Supplementary Figure S2. ML phylogeny of a subset of *Peltigera* species including *P. ponojensis* based on ITS rDNA alleles. Numbers represent approximate likelihood-ratio test proportions. Reference sequences are labeled by species name and GenBank accession number. New sequences from this study are labeled by collection number.

Supplementary Figure S3. ML phylogeny of free-living and symbiotic *Nostoc* sp. based on *rbc*LX alleles. Circles indicate branches with ≥90% a LRT support. Yellow (from UWMFS) and orange (non-UWMFS) highlighting indicates sequences from this study, which are labeled with collection numbers and host info or *Nostoc* sp. for free-living strains. Reference sequences are labeled with GenBank accession numbers for unique sequence and group numbers for duplicate sequences. Sequences from symbionts are also labeled with host information and sequences from free-living strains are labeled with cyanobacterial nomenclature. Numbers indicate multiple identical sequences from the same host species or cyanobacterial taxon. References sequences are colour coded by host family (purple: Stereocaulaceae, brown: Lobariaceae, red: Peltigeraceae, blue: Collemetaceae, orange: Nephromataceae, pink: Pannariaceae, green: plant hosts, cyan: free-living). See Supplementary Table S3 for GenBank accession numbers and collection locations of all group members.

Supplementary Table S1. Distances between lichenized and free-living *Nostoc* samples from four UWMFS collection sites. Collections marked by "N" for *Nostoc* sp., or "P" for *Peltigera ponojensis*, followed by the collection number. Measurements given in cm, measured in 0.5 cm increments.

Supplementary Table S2. Top BLAST hit of sequences generated in this study, excluding unidentified sequences

Supplementary Table S3. Collection information for Figure 4 isolates.

Figure 1. Comparison of competing symbiotic-state determination hypotheses. **A.** Symbiotic state controlled by deterministic factors, and switches between lifestyles are rare. **B.** Symbiotic state controlled by deterministic factors, and switches between lifestyles are frequent. **C.** Symbiotic state controlled by variable factors and individuals have limited dispersal. **D.** Symbiotic state controlled by variable factors, and the distribution of individual lineages are limited by factors such as genetic background, symbiotic partner availability, and environment. Bolded text refers to isolates sampled from the same location. Triangles refer to multiple individuals within the corresponding lifestyle state/s.

Figure 2. Sample of lichenized and free-living *Nostoc* sp. growing near to each other. The larger circle on the left surrounds *Peltigera ponojensis* thalli, and the smaller circle on the right surrounds a free-living *Nostoc* sp. thallus. Measurement marks in 0.5 cm increments.

Figure 3. Map of free-living and lichenized *Nostoc* sp. collection sites (black dots) identified in Table 1. Collections are from locations in Michigan state (MI), Minnesota state (MN) and Wisconsin state (WI).

Figure 4. Simplified phylogeny of free-living and symbiotic *Nostoc* sp. based on *rbc*LX alleles. See Figure S3 for details. Circles indicate branches with ≥90% a LRT support. Highlighted names represent new sequences from this study: Yellow highlights are for isolates from UWMFS, and orange highlights are from non-UWMFS sites. Triangles represent clades with ≥90% a LRT support from a single host lineage (red: Peltigeraceae, blue: Collemataceae, brown:

Nephromataceae/Lobariaceae, green: plants, turquoise: free living *Nodularia*), or from a mixture of free-living strains and symbionts of Peltigeraceae, Collemataceae and plants (unfilled). Composition of the labeled clades is provided in Figure 5.

Figure 5. Clades containing *Nostoc* sp. samples from this study, based on Figure 4. **A.** Clade of mainly free-living *Nostoc* sp. from across Wisconsin, Minnesota and Michigan, with two lichenized forms from Europe. **B.** Clade of free-living *Nostoc* sp. from Wisconsin, Minnesota and North Carolina, with symbiotic forms from Europe (both with fungi and with a plant). **C.** Clade of lichenized *Nostoc* sp. from Wisconsin, Canada and Europe, and one free-living strain. **D.** Clade of symbiotic *Nostoc* sp. from Wisconsin, Canada and Europe, and one aquatic form from Europe. **E.** Clade of lichenized *Nostoc* sp. from Wisconsin and Europe. (red: Peltigeraceae, blue: Collemataceae, brown: Nephromataceae/Lobariaceae, green: plants, turquoise: free living*)*. Yellow (from UWMFS) and orange (non-UWMFS) highlighting indicates sequences from this study. See Supplementary Table S3 for collection locations of previously published samples.

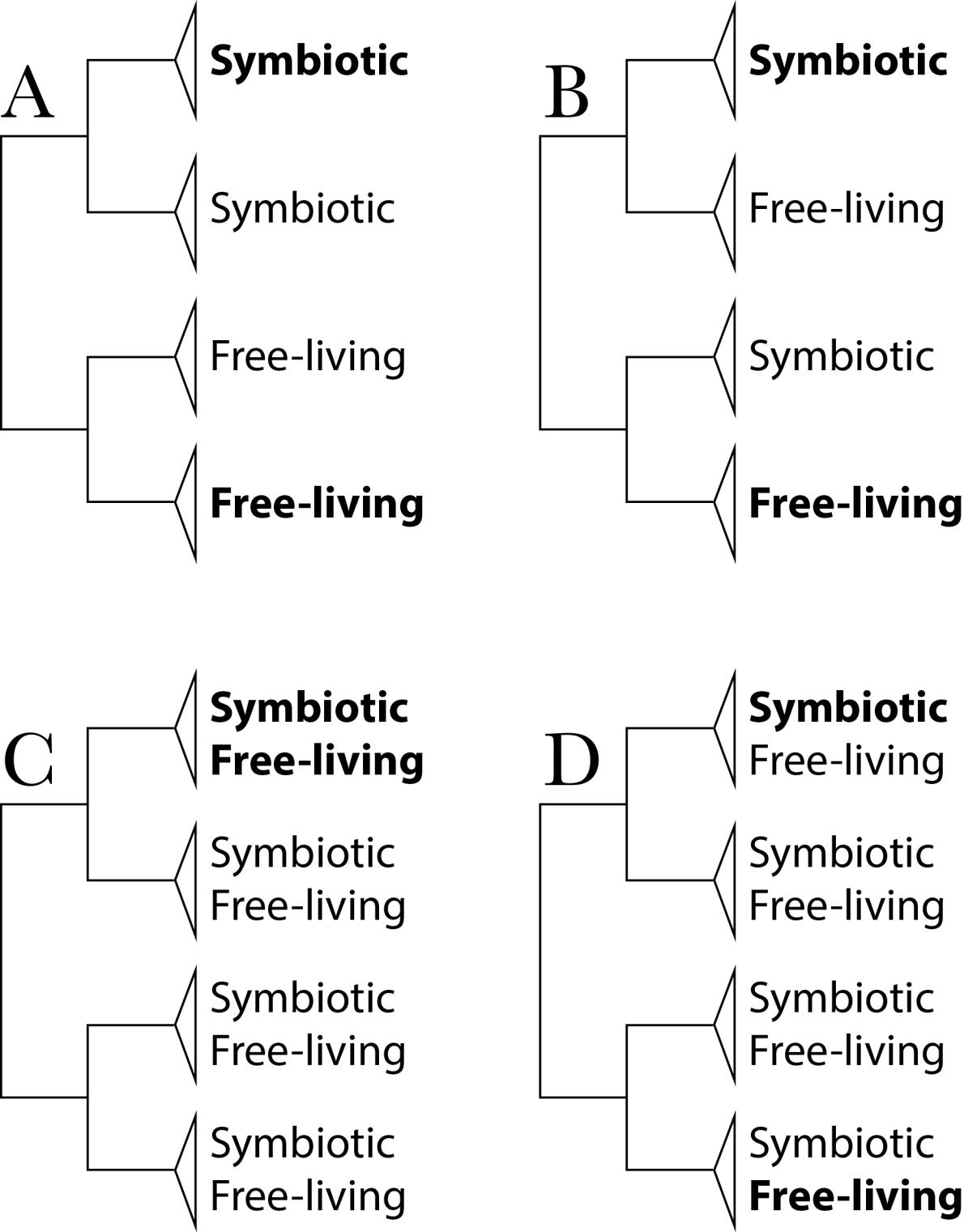
Table 1. Collection information and GenBank accession numbers for *Nostoc* sp. *rbc*LX sequences, and new *Leptogium* and *Peltigera* ITS sequences generated for this study. "Site" refers to UWMFS locations shown in Supplementary Figure S1. **A.** Collections from original site at UWMFS. **B.** Collections from a broader geographic region spanning Wisconsin, Minnesota and Michigan. Niche/Mycobiont: individuals were found growing either as free-living thalli ("Soil"), or in a lichenized state (with either *Peltigera* spp. or *Leptogium lichenoides*).

A. Niche/Mycobiont	Collection Number	State	County	Latitude	Site	Longitude	*rbc*LX GenBank Accession Number	ITS GenBank Accession Number
Peltigera ponojensis	SLJ4214	WI	Ozaukee	43.38510	1	-88.02128	MF067344	MF067370
Peltigera ponojensis	SLJ4217	WI	Ozaukee	43.38510	1	-88.02128	MF067352	MF067369
Peltigera ponojensis	SLJ4221	WI	Ozaukee	43.38510	1	-88.02128	MF067353	MF067364
Peltigera ponojensis	SLJ4222	WI	Ozaukee	43.38510	1	-88.02128	MF067341	MF067366
Peltigera ponojensis	SLJ4225	WI	Ozaukee	43.38525	2	-88.02120	MF067349	MF067373
Peltigera ponojensis	SLJ4226	WI	Ozaukee	43.38525	2	-88.02120	MF067350	MF067365
Peltigera ponojensis	SLJ4227	WI	Ozaukee	43.38525	2	-88.02120	MF067342	MF067374
Peltigera ponojensis	SLJ4228	WI	Ozaukee	43.38525	2	-88.02120	MF067343	MF067375
Peltigera ponojensis	SLJ4252	WI	Ozaukee	43.38508	3	-88.02126	MF067351	MF067371
Peltigera ponojensis	SLJ4280	WI	Ozaukee	43.38508	4	-88.02129	MF067339	MF067368
Peltigera ponojensis	SLJ4281	WI	Ozaukee	43.38508	4	-88.02129	MF067345	MF067363
Peltigera ponojensis	SLJ4282	WI	Ozaukee	43.38508	4	-88.02129	MF067348	MF067367
Peltigera ponojensis	SLJ4283	WI	Ozaukee	43.38508	4	-88.02129	MF067346	MF067372
Soil	SLJ4216	WI	Ozaukee	43.38510	1	-88.02128	MF067328	—
Soil	SLJ4223	WI	Ozaukee	43.38510	1	-88.02128	MF067319	—
Soil	SLJ4229	WI	Ozaukee	43.38525	2	-88.02120	MF067313	—
Soil	SLJ4231	WI	Ozaukee	43.38525	2	-88.02120	MF067314	—
Soil	SLJ4237	WI	Ozaukee	43.38525	2	-88.02120	MF067327	—
Soil	SLJ4258	WI	Ozaukee	43.38508	3	-88.02126	MF067338	—
Soil	SLJ4268	WI	Ozaukee	43.38508	3	-88.02126	MF067337	—
Soil	SLJ4269	WI	Ozaukee	43.38508	3	-88.02126	MF067331	—
Soil	SLJ4288	WI	Ozaukee	43.38508	4	-88.02129	MF067330	—
Soil	SLJ4290	WI	Ozaukee	43.38508	4	-88.02129	MF067325	—
Soil	SLJ4294	WI	Ozaukee	43.38508	4	-88.02129	MF067326	—

Soil	SLJ4296	WI	Ozaukee	43.38508	4	-88.02129	MF067324	—
Soil	SLJ4298	WI	Ozaukee	43.38508	4	-88.02129	MF067329	—

B. Niche/Mycobiont	**Collection Number**	**State**	**County**	**Latitude**		**Longitude**	***rbcX* GenBank Accession Number**	**ITS GenBank Accession Number**
Leptogium lichenoides	SLJ4300	WI	Waukesha	43.029594	—	-88.386095	MF067354	—
Leptogium lichenoides	SLJ4302	WI	Waukesha	43.029594	—	-88.386095	MF067358	—
Leptogium lichenoides	SLJ4303	WI	Waukesha	43.029594	—	-88.386095	MF067356	—
Leptogium lichenoides	SLJ4304	WI	Waukesha	43.029594	—	-88.386095	MF067355	—
Leptogium lichenoides	SLJ4305	WI	Waukesha	43.029594	—	-88.386095	MF067357	—
Leptogium lichenoides	SLJ4306	WI	Waukesha	43.029594	—	-88.386095	MF067360	—
Leptogium lichenoides	SLJ4309	WI	Waukesha	43.029594	—	-88.386095	MF067361	—
Leptogium lichenoides	SLJ4310	WI	Waukesha	43.029594	—	-88.386095	MF067359	—
Peltigera islandica	SLJ4349	WI	Douglas	46.533520	—	-92.118590	MF067347	MF067362
Soil	SLJ4240	WI	Waukesha	43.031826	—	-88.393888	MF067312	—
Soil	SLJ4311	WI	Waukesha	43.031826	—	-88.393888	MF067304	—
Soil	SLJ4312	WI	Waukesha	43.031826	—	-88.393888	MF067310	—
Soil	SLJ4313	WI	Waukesha	43.031826	—	-88.393888	MF067309	—
Soil	SLJ4314	WI	Waukesha	43.031826	—	-88.393888	MF067305	—
Soil	SLJ4316	WI	Waukesha	43.031826	—	-88.393888	MF067308	—
Soil	SLJ4317	WI	Waukesha	43.031826	—	-88.393888	MF067307	—
Soil	SLJ4318	WI	Waukesha	43.031826	—	-88.393888	MF067306	—
Soil	SLJ4321	MI	Manastique	45.946865	—	-86.232593	MF067315	—
Soil	SLJ4323	WI	Waukesha	43.029846	—	-88.26815	MF067335	—
Soil	SLJ4324	WI	Ozaukee	43.340938	—	-87.885485	MF067333	—
Soil	SLJ4325	WI	Ozaukee	43.340938	—	-87.885485	MF067317	—
Soil	SLJ4331	WI	Waukesha	43.016319	—	-88.436920	MF067334	—
Soil	SLJ4332	WI	Waukesha	43.017713	—	-88.438793	MF067332	—

Soil	SLJ4333	WI	Jefferson	43.011662	—	-88.590265	MF067311	—
Soil	SLJ4335	WI	Waukesha	43.003129	—	-88.370324	MF067303	—
Soil	SLJ4336	WI	Waukesha	43.002699	—	-88.370233	MF067321	—
Soil	SLJ4337	MN	Lake	47.035683	—	-91.665931	MF067318	—
Soil	SLJ4338	MN	Lake	47.035153	—	-91.666262	MF067336	—
Soil	SLJ4340	MN	St. Louis	46.840121	—	-92.006726	MF067316	—
Soil	SLJ4348	WI	Douglas	46.533520	—	-92.118590	MF067322	—
Soil	SLJ4350	WI	Douglas	46.533520	—	-92.118590	MF067323	—
Soil	SLJ4351	WI	Douglas	46.533520	—	-92.118590	MF067340	—
Soil	SLJ4353	WI	Douglas	46.533520	—	-92.118590	MF067320	—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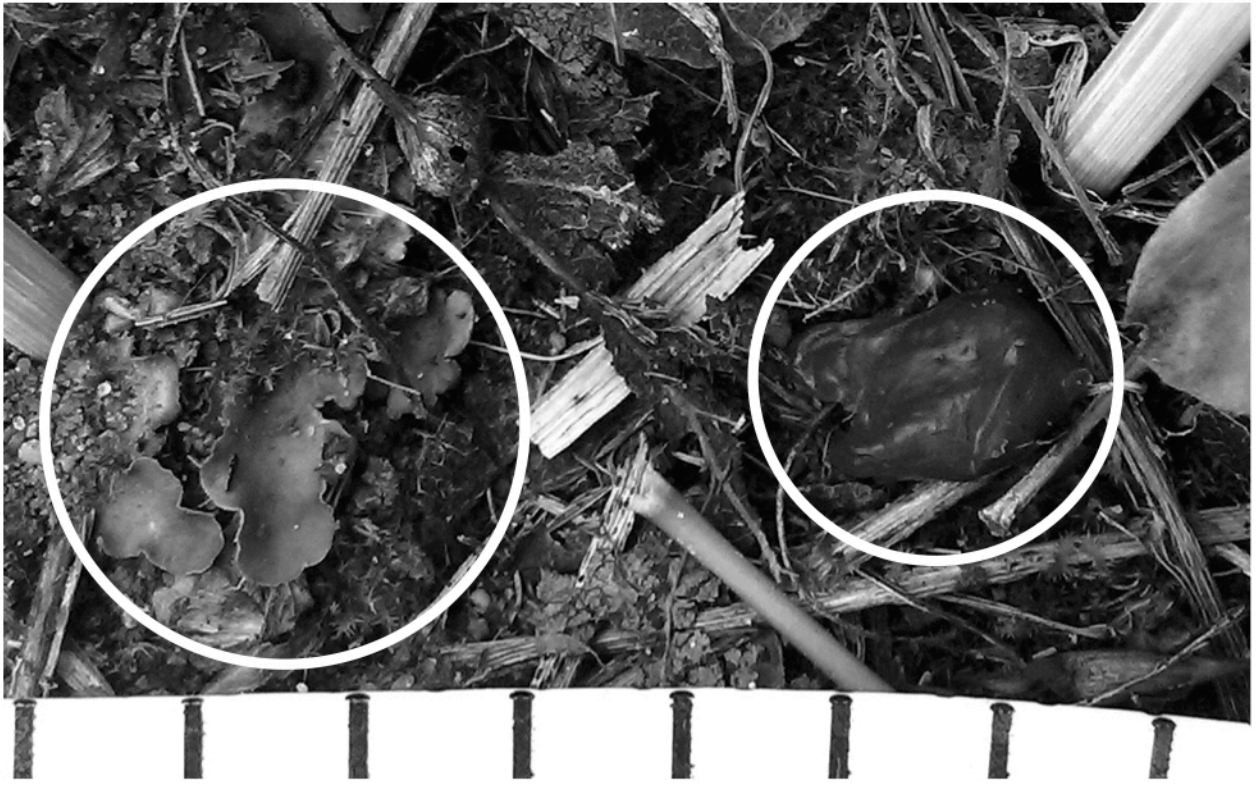

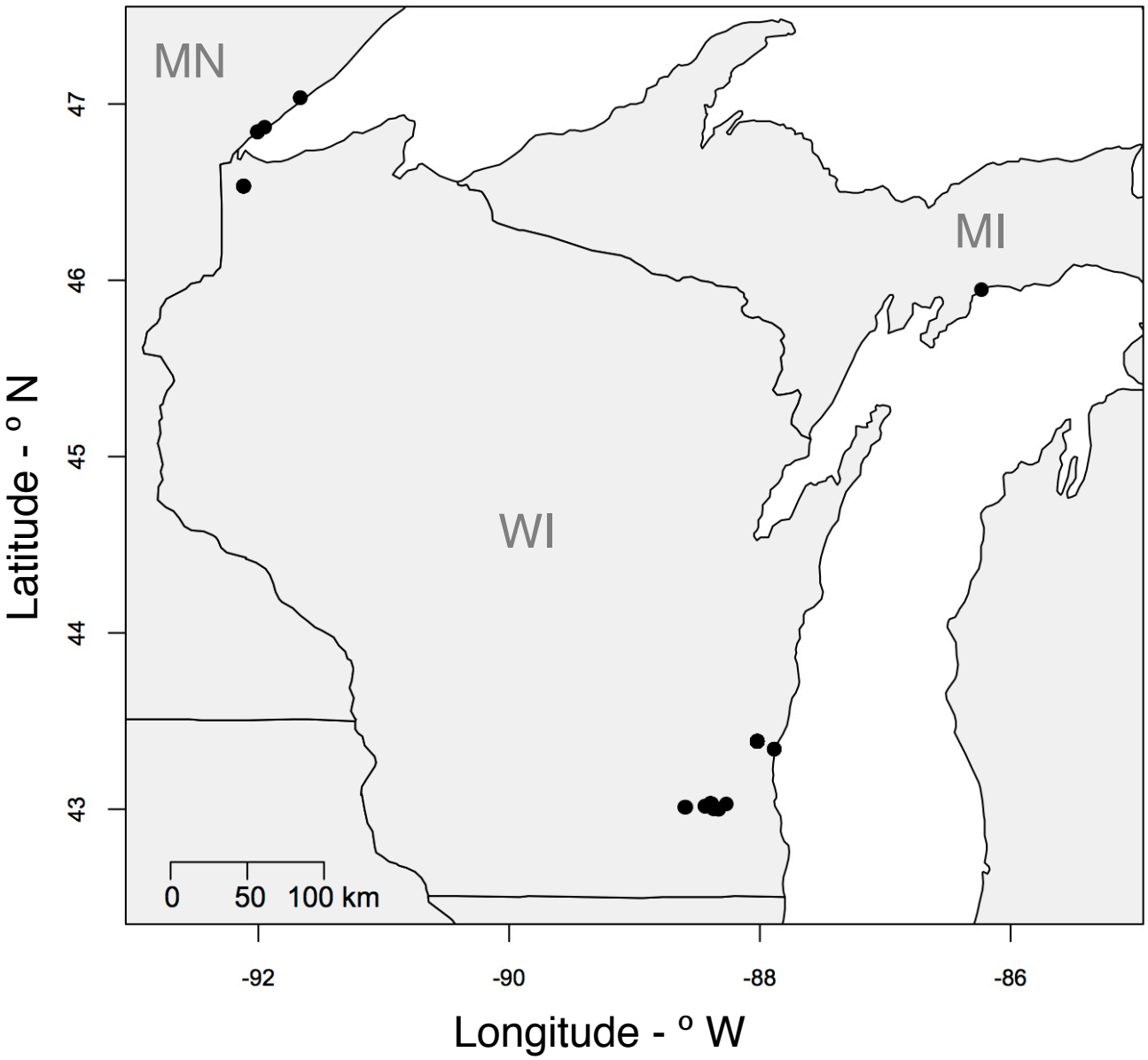
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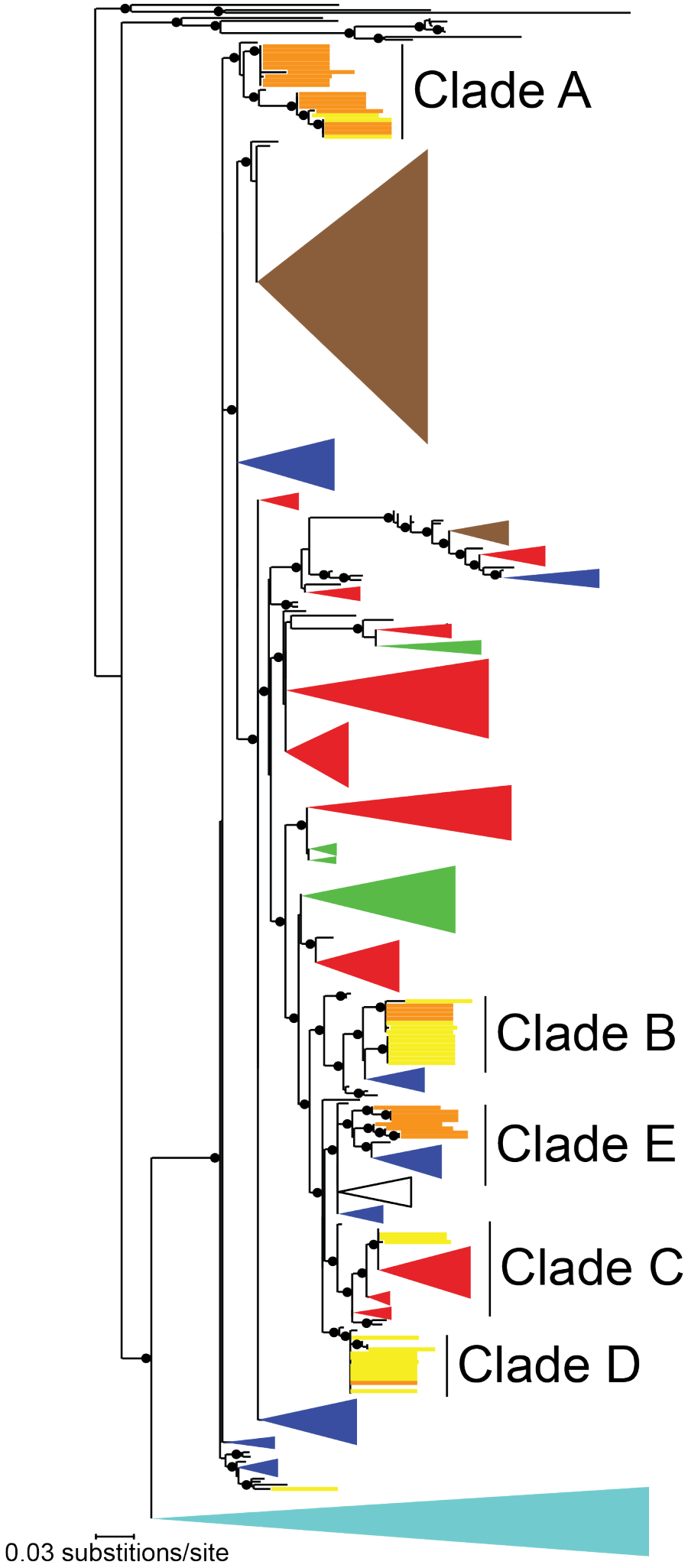
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Clade B
Clade E
Clade C
Clade D
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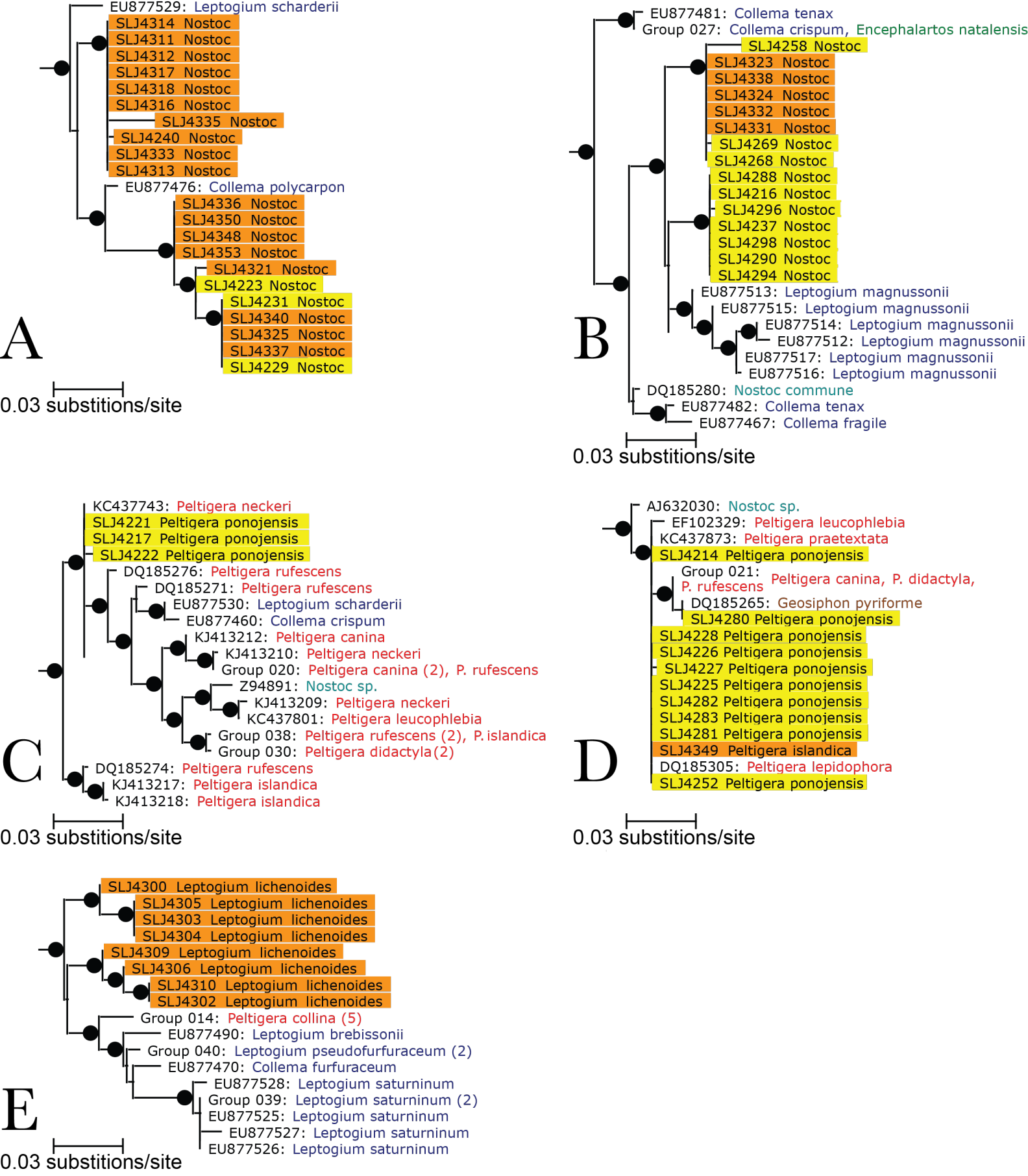

A
EU877529: Leptogium scharderii
SLJ4314 Nostoc
SLJ4311 Nostoc
SLJ4312 Nostoc
SLJ4317 Nostoc
SLJ4318 Nostoc
SLJ4316 Nostoc
SLJ4335 Nostoc
SLJ4240 Nostoc
SLJ4333 Nostoc
SLJ4313 Nostoc
EU877476: Collema polycarpon
SLJ4336 Nostoc
SLJ4350 Nostoc
SLJ4348 Nostoc
SLJ4353 Nostoc
SLJ4321 Nostoc
SLJ4223 Nostoc
SLJ4231 Nostoc
SLJ4340 Nostoc
SLJ4325 Nostoc
SLJ4337 Nostoc
SLJ4229 Nostoc
0.03 substitions/site
B
EU877481: Collema tenax
Group 027: Collema crispum, Encephalartos natalensis
SLJ4258 Nostoc
SLJ4323 Nostoc
SLJ4338 Nostoc
SLJ4324 Nostoc
SLJ4332 Nostoc
SLJ4331 Nostoc
SLJ4269 Nostoc
SLJ4268 Nostoc
SLJ4288 Nostoc
SLJ4216 Nostoc
SLJ4296 Nostoc
SLJ4237 Nostoc
SLJ4298 Nostoc
SLJ4290 Nostoc
SLJ4294 Nostoc
EU877513: Leptogium magnussonii
EU877515: Leptogium magnussonii
EU877514: Leptogium magnussonii
EU877512: Leptogium magnussonii
EU877517: Leptogium magnussonii
EU877516: Leptogium magnussonii
DQ185280: Nostoc commune
EU877482: Collema tenax
EU877467: Collema fragile
0.03 substitions/site
C
KC437743: Peltigera neckeri
SLJ4221 Peltigera ponojensis
SLJ4217 Peltigera ponojensis
SLJ4222 Peltigera ponojensis
DQ185276: Peltigera rufescens
DQ185271: Peltigera rufescens
EU877530: Leptogium scharderii
EU877460: Collema crispum
KJ413212: Peltigera canina
KJ413210: Peltigera neckeri
Group 020: Peltigera canina (2), P. rufescens
Z94891: Nostoc sp.
KJ413209: Peltigera neckeri
KC437801: Peltigera leucophlebia
Group 038: Peltigera rufescens (2), P. islandica
Group 030: Peltigera didactyla (2)
DQ185274: Peltigera rufescens
KJ413217: Peltigera islandica
KJ413218: Peltigera islandica
0.03 substitions/site
D
AJ632030: Nostoc sp.
EF102329: Peltigera leucophlebia
KC437873: Peltigera praetextata
SLJ4214 Peltigera ponojensis
Group 021: Peltigera canina, P. didactyla, P. rufescens
DQ185265: Geosiphon pyriforme
SLJ4280 Peltigera ponojensis
SLJ4228 Peltigera ponojensis
SLJ4226 Peltigera ponojensis
SLJ4227 Peltigera ponojensis
SLJ4225 Peltigera ponojensis
SLJ4282 Peltigera ponojensis
SLJ4283 Peltigera ponojensis
SLJ4281 Peltigera ponojensis
SLJ4349 Peltigera islandica
DQ185305: Peltigera lepidophora
SLJ4252 Peltigera ponojensis
0.03 substitions/site
E
SLJ4300 Leptogium lichenoides
SLJ4305 Leptogium lichenoides
SLJ4303 Leptogium lichenoides
SLJ4304 Leptogium lichenoides
SLJ4309 Leptogium lichenoides
SLJ4306 Leptogium lichenoides
SLJ4310 Leptogium lichenoides
SLJ4302 Leptogium lichenoides
Group 014: Peltigera collina (5)
EU877490: Leptogium brebissonii
Group 040: Leptogium pseudofurfuraceum (2)
EU877470: Collema furfuraceum
EU877528: Leptogium saturninum
Group 039: Leptogium saturninum (2)
EU877525: Leptogium saturninum
EU877527: Leptogium saturninum
EU877526: Leptogium saturninum
0.03 substitions/site

Supplementary Table 1. Distances between lichenized and free-living *Nostoc* samples from four UWMFS collection sites. Collections marked by "N" for *Nostoc* sp., or "P" for *Peltigera ponojensis*, followed by the collection number. Measurements given in cm, measured in 0.5 cm increments.

Site 1	N4216	N4223	P4214	P4217	P4221	P4222
N4216	—	41.0	14.0	21.0	36.0	37.5
N4223	—	—	28.0	—	13.0	4.0
P4214	—	—	—	20.0	22.0	24.0
P4217	—	—	—	—	28.5	—
P4221	—	—	—	—	—	10.0
P4222	—	—	—	—	—	—

Site 2	N4229	N4231	N4237	P4225	P4226	P4227	P4228
N4229	—	22.0	—	—	—	10.5	—
N4231	—	—	—	—	—	14.0	—
N4237	—	—	—	—	29.5	58.0	19.0
P4225	—	—	—	—	17.5	60.5	17.5
P4226	—	—	—	—	—	46.0	27.0
P4227	—	—	—	—	—	—	39.0
P4228	—	—	—	—	—	—	—

Site 3	N4258	N4268	N4269	P4252
N4258	—	—	—	—
N4268	—	—	—	23.5
N4269	—	—	—	—
P4252	—	—	—	—

Site 4	N4288	N4290	N4294	N4296	N4298	P4280	P4281	P4282	P4283
N4288	—	24.0	—	—	—	10.0	—	—	—
N4290	—	—	—	—	—	15.5	—	—	—
N4294	—	—	—	—	100.5	—	—	9.0	30.5
N4296	—	—	—	—	—	—	21.5	—	—
N4298	—	—	—	—	—	—	—	109.5	131.0
P4280	—	—	—	—	—	—	—	—	—
P4281	—	—	—	—	—	—	—	—	—
P4282	—	—	—	—	—	—	—	—	21.5
P4283	—	—	—	—	—	—	—	—	—

Table S2: Top blast hit of sequences generated in this study, excluding unidentified sequences

Query	Length	Hit Species	Hit Accession	Query Cover (%)	Identity (%)	Score	E-value
SLJ4214	682	P. ponojensis	KF957622	86	99	1055	0
SLJ4217	444	P. ponojensis	KF957622	99	99	787	0
SLJ4225	613	P. ponojensis	KF957622	94	99	1007	0
SLJ4227	658	P. ponojensis	KF957622	91	99	1092	0
SLJ4222	657	P. ponojensis	KF957622	89	99	1062	0
SLJ4252	627	P. ponojensis	KF957622	92	99	1051	0
SLJ4282	558	P. ponojensis	KF957622	98	99	1011	0
SLJ4283	571	P. ponojensis	KF957622	100	99	1027	0
SLJ4221	627	P. ponojensis	KF957622	95	99	1042	0
SLJ4222	582	P. ponojensis	KF957622	100	98	1022	0
SLJ4226	320	P. ponojensis	AY257883	98	96	534	0
SLJ4228	492	P. ponojensis	KF957622	100	99	893	0
SLJ4280	443	P. ponojensis	AY257883	97	99	784	0
SLJ4281	538	P. ponojensis	KF957622	100	98	952	0
SLJ4349	551	P. islandica*	KC139759	94	100	959	0

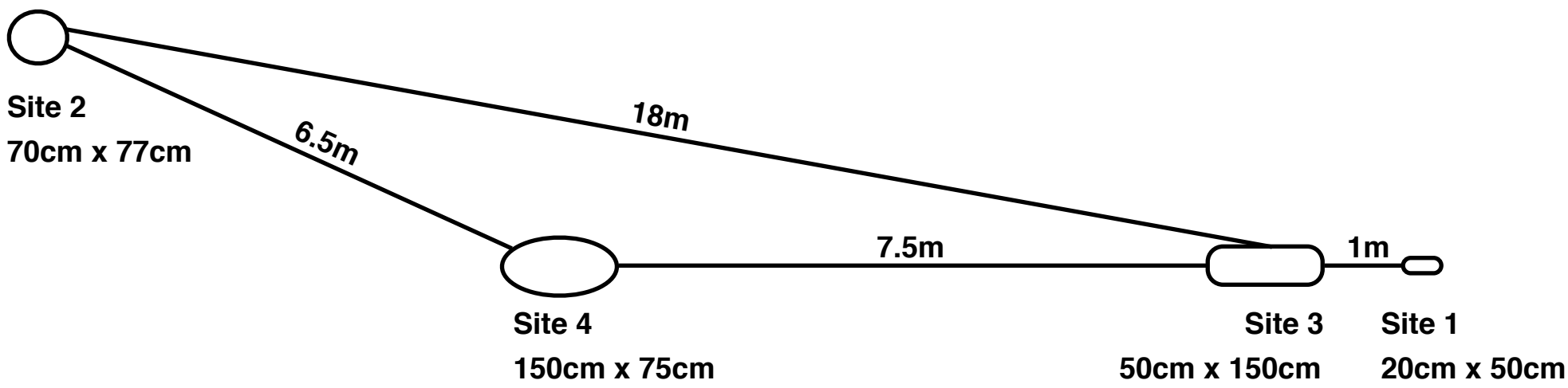
* Three other Peltigera sequences (KX354694, KX354693 and FJ708907) have higher bit scores, but < 100% identity

Supplementary material for: Joneson, S. & H. O'Brien. A molecular investigation of free-living and lichenized *Nostoc* sp. and symbiotic lifestyle determination. The Bryologist 120: XX–XX.

Supplementary Table S3. Collection information for Figure 4 isolates.

Clade	Group	Accession #	Host	Location	Reference
A		EU877529	Leptogium scharderii	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
A		EU877476	Collema polycarpon	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU877481	Collema tenax	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B	Group 027	DQ185267	Encephalartos natalensis	Italy	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
B	Group 027	DQ185273	Collema crispum	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
B		EU877513	Leptogium magnussonii	Denmark	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU877515	Leptogium magnussonii	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU877514	Leptogium magnussonii	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU811512	Leptogium magnussonii	Sweden	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU877517	Leptogium magnussonii	Sweden	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU877516	Leptogium magnussonii	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		DQ185280	Free-living	USA	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
B		EU877482	Collema tenax	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
B		EU877467	Collema fragile	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
C		KC437743	Peltigera neckeri	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
C		DQ185276	Peltigera rufescens	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C		DQ185271	Peltigera rufescens	Unknown	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C		EU877530	Leptogium scharderii	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
C		EU877460	Collema crispum	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
C		KJ413212	Peltigera canina	Iceland	Manoharan-Basi Lichenologist (Lond.) 485 (5), 451-467 (2016)
C		KJ413210	Peltigera neckeri	Iceland	Manoharan-Basil et al. Lichenologist (Lond.) 485 (5), 451-467 (2016)
C	Group 020	DQ185279	Peltigera canina	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C	Group 020	DQ185296	Peltigera canina	Poland	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C	Group 020	DQ185308	Peltigera rufescens	Poland	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C		Z94891	Free-living	Svalbard	
C		KJ413209	Peltigera neckeri	Iceland	Manoharan-Basil et al. Lichenologist (Lond.) 485 (5), 451-467 (2016)
C		KC437801	Peltigera leucophlebia	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
C	Group 038	DQ185278	Peltigera rufescens	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C	Group 038	KJ413208	Peltigera islandica	Iceland	Manoharan-Basil et al. Lichenologist (Lond.) 485 (5), 451-467 (2016)
C	Group 038	KJ413211	Peltigera rufescens	Iceland	Manoharan-Basil et al. Lichenologist (Lond.) 485 (5), 451-467 (2016)
C	Group 030	DQ185272	Peltigera didactyla	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C	Group 030	DQ185277	Peltigera didactyla	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C		DQ185274	Peltigera rufescens	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
C		KJ413217	Peltigera islandica	Iceland	Manoharan-Basil et al. Lichenologist (Lond.) 485 (5), 451-467 (2016)
C		KJ413218	Peltigera islandica	Iceland	Manoharan-Basil et al. Lichenologist (Lond.) 485 (5), 451-467 (2016)
D		AJ632030	Aquatic	Finland	Rajaniemi et al. Int. J. Syst. Evol. Microbiol. 55 (PT 1), 11-26 (2005)

D		EF102329	Peltigera leucophlebia	Finland	Myllys et al. New Phytol. 173 (3), 621-629 (2007)
D		KC437873	Peltigera praextata	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
D	Group 021	DQ185261	Peltigera canina	Unknown	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
D	Group 021	DQ185262	Peltigera rufescens	England	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
D	Group 021	DQ185263	Peltigera didactyla	Iceland	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
D		DQ185265	Geosiphon pyriforme	Germany	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
D		DQ185305	Peltigera lepidophora	Canada	O'Brien et al. Eur. J. Phycol. 40 (4), 363-378 (2005)
E	Group 014	KC437882	Peltigera collina	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
E	Group 014	KC437889	Peltigera collina	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
E	Group 014	KC437890	Peltigera collina	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
E	Group 014	KC437893	Peltigera collina	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
E	Group 014	KC437895	Peltigera collina	Canada	O'Brien et al. New Phytol. 198 (2), 557-566 (2013)
E		EU877490	Leptogium brebissonii	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E	Group 040	EU877518	Leptogium pseudofurfurac	USA	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E	Group 040	EU877519	Leptogium pseudofurfurac	USA	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E		EU877470	Collema furfuraceum	Croatia	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E		EU877528	Leptogium saturninum	USA	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E	Group 039	DQ266037	Leptogium saturninum	Finland	stenroos et al. Cladistics 22 (3), 230-238 (2006)
E	Group 039	DQ266038	Leptogium saturninum	Finland	stenroos et al. Cladistics 22 (3), 230-238 (2006)
E		EU877525	Leptogium saturninum	Canada	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E		EU877527	Leptogium saturninum	Spain	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)
E		EU877526	Leptogium saturninum	France	Otalora et al. Mol. Phylogenet. Evol. 56 (3), 1089-1095 (2010)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18m
7.5m
1m
Site 4
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Site 3
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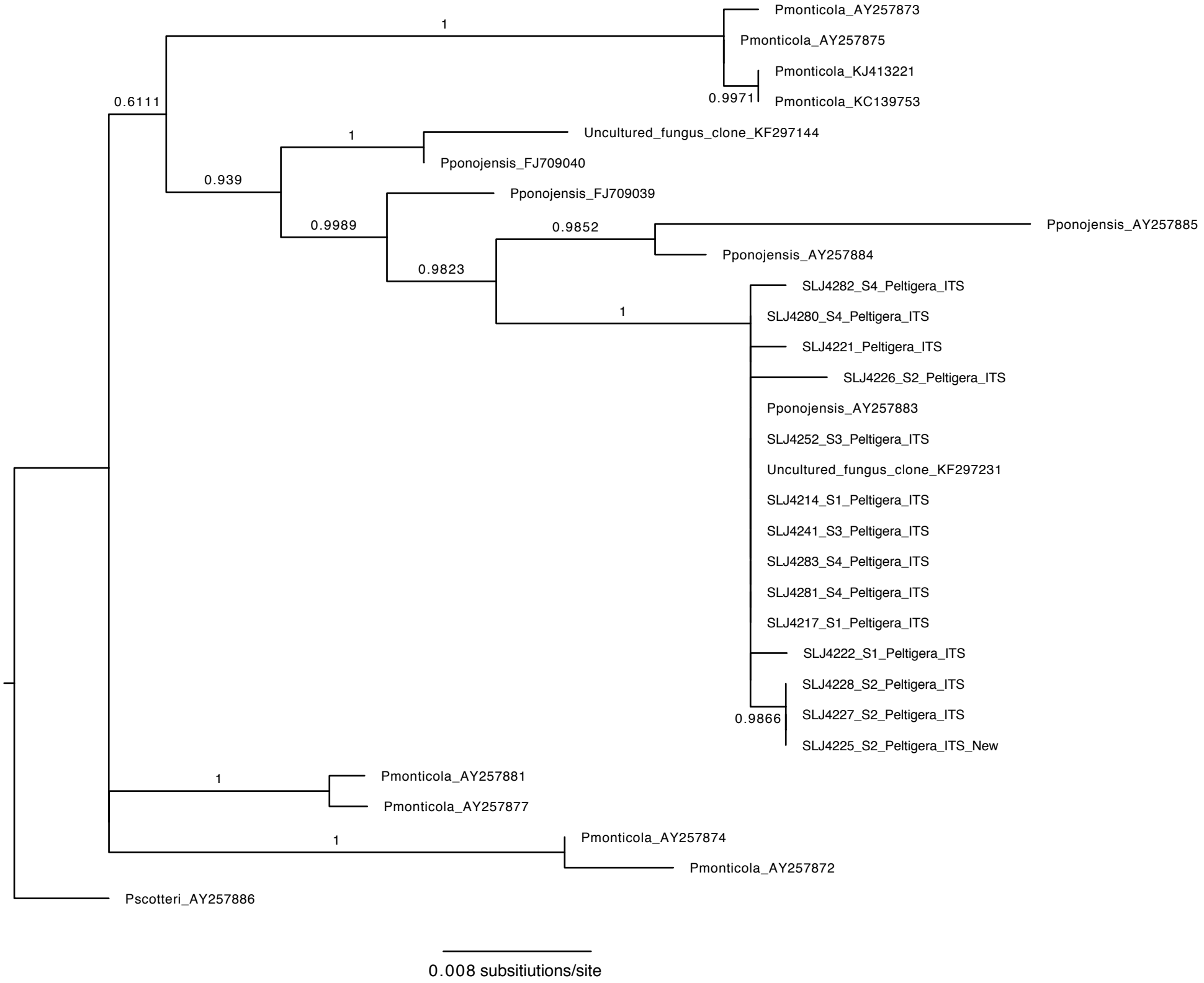
Site 1
20cm x 50cm


Pmonticola_AY257873
Pmonticola_AY257875
Pmonticola_KJ413221
Pmonticola_KC139753
Uncultured_fungus_clone_KF297144
Pponojensis_FJ709040
Pponojensis_FJ709039
Pponojensis_AY257885
Pponojensis_AY257884
SLJ4282_S4_Peltigera_ITS
SLJ4280_S4_Peltigera_ITS
SLJ4221_Peltigera_ITS
SLJ4226_S2_Peltigera_ITS
Pponojensis_AY257883
SLJ4252_S3_Peltigera_ITS
Uncultured_fungus_clone_KF297231
SLJ4214_S1_Peltigera_ITS
SLJ4241_S3_Peltigera_ITS
SLJ4283_S4_Peltigera_ITS
SLJ4281_S4_Peltigera_ITS
SLJ4217_S1_Peltigera_ITS
SLJ4222_S1_Peltigera_ITS
SLJ4228_S2_Peltigera_ITS
SLJ4227_S2_Peltigera_ITS
SLJ4225_S2_Peltigera_ITS_New
Pmonticola_AY257881
Pmonticola_AY257877
Pmonticola_AY257874
Pmonticola_AY257872
Pscotteri_AY257886
1
0.9971
0.6111
1
0.939
0.9989
0.9852
0.9823
1
0.9866
1
1
0.008 subsitiutions/site

A363266: Nostoc ellipsosporum
DQ266029: Stereocaulon exutum
Group 045: Protopannaria pezizioides, Stereocaulon fronduliferum
DQ260033: Fuscopannaria leucophaea
DQ185264: Peltigera didactyla
HQ313213: Nostoc sp.
EU672830: Peltigera didactyla
EU672828: Peltigera didactyla
EU672829: Peltigera didactyla
HQ335214: Nostoc sp.
EU877497: Leptogium cyanescens
EU877487: Leptogium azureum
EU877529: Leptogium scharderii
SL34314 Nostoc
SL34311: Nostoc
SL34312: Nostoc
SL34317: Nostoc
SL34316: Nostoc
SL34315: Nostoc
SL34335: Nostoc
SL34340: Nostoc
SL34333: Nostoc
SL34313: Nostoc
EU877476: Collema polycarpon
SL34336: Nostoc
SL34350: Nostoc
SL34348: Nostoc
SL34353: Nostoc
SL34331: Nostoc
SL34323: Nostoc
SL34321: Nostoc
SL34324: Nostoc
SL34325: Nostoc
SL34337: Nostoc
SL34329: Nostoc
EU877480: Leptogium azureum
KC437912: Peltigera neopolydactyla
DQ266003: Pseudocyphellaria crathata
EU877480: Collema subnigrescens
DQ266021: Pseudocyphellaria crocata
EU877474: Collema nigrescens
EU877464: Collema fasciculare
EF102304: Lobaria pulmonaria
EF102303: Lobaria pulmonaria
Group 128: Lobaria pulmonaria, Nephroma parile (2)
Group 129: Lobaria pulmonaria (3)
EF102302: Lobaria pulmonaria
EF102315: Nephroma parile
EF102317: Nephroma parile
EU877495: Leptogium cyanescens
DQ266019: Pseudocyphellaria crocata
KC437909: Nephroma resupinatum
Group 218: Nephroma bellum (2), Parmeliella triptophylla
Group 026: Parmeliella triptophylla, Nephroma bellum (3), N. resupinatum, N. parile
EF102296: Lobaria pulmonaria
EF102297: Lobaria pulmonaria
EF102297: Lobaria pulmonaria
Group 080: Nephroma bellum (3), N. resupinatum
EF102319: Nephroma resupinatum
EF102322: Parmeliella triptophylla
EF102309: Nephroma bellum
EF102300: Lobaria pulmonaria
EF102299: Lobaria pulmonaria
DQ185286: Sticta beauvoisii
DQ266020: Pseudocyphellaria intricata
DQ266013: Pseudocyphellaria intricata
DQ266022: Pseudocyphellaria corifolia
DQ266007: Pseudocyphellaria hirsuta
DQ266008: Pseudocyphellaria crocata
DQ185295: Nephroma compitum
DQ266014: Pseudocyphellaria hirsuta
DQ266004: Pseudocyphellaria mallota
DQ266042: Pseudocyphellaria lechleri
DQ266011: Pseudocyphellaria crocata
DQ266010: Pseudocyphellaria crocata
DQ266041: Pseudocyphellaria scabrosa
DQ266017: Pseudocyphellaria pilosella
DQ266000: Lobaria amplissima
Group 155: Degelia plumbea, Nephroma laevigatum (2)
JX126718: Degelia plumbea
JX126713: Degelia plumbea
DQ266018: Pseudocyphellaria crocata
DQ266002: Pseudocyphellaria crocata
DQ266016: Pseudocyphellaria corifolia
DQ266012: Pseudocyphellaria crocata
DQ266009: Pseudocyphellaria crocata
DQ266015: Pseudocyphellaria crocata
DQ266008: Pseudocyphellaria scabrosa
DQ266005: Pseudocyphellaria corifolia
JX126716: Degelia plumbea
Group 031: Degelia plumbea (8)
Group 094: Degelia plumbea, Degelia atlantica (38), D. plumbea (75),
DQ185290: Lobaria amplissima
JX126712: Degelia plumbea
Group 236: Degelia plumbea (2), Nephroma helveticum
EF102347: Pseudocyphellaria crocata
DQ266018: Pseudocyphellaria mallota
DQ266001: Lobaria amplissima
EF102295: Degelia plumbea
JX126714: Degelia plumbea
EF102320: Nephroma tangerinae
KC437913: Lobaria kurkawe
KC437915: Lobaria kurkawe
EU877472: Collema furfuraceum
EU877468: Leptogium corticola
EU877471: Collema furfuraceum
EU877475: Collema nigrescens
EU877478: Collema subnigrescens
EF102301: Lobaria pulmonaria
EU877463: Collema fasciculare
EU877469: Collema furfuraceum
EU877489: Leptogium brebissonii
DQ185291: Lobaria halli
EU877494: Leptogium corticola
EU877493: Leptogium corticola
DQ185307: Peltigera membranacea
JX876663: Peltigera membranacea
EF102336: Peltigera neopolydactyla
Group 024: P. neopolydactyla, Peltigera degenii, P. membranacea,
Group 025: Peltigera aphthosa (3)
Group 010: Peltigera aphthosa (6), P. leucophlebia
Group 008: P. leucophlebia (2)
Peltigera aphthosa (3), P. britannica (3),
KC437880: Peltigera aphthosa
KC437883: Peltigera malacea
KC437886: Nephroma arcticum
DQ266027: Sticta gausulchadda
DQ266024: Sticta hypochra
DQ266028: Sticta hypochra
DQ266025: Sticta hypochra
DQ266024: Sticta hypochra
DQ266023: Sticta hypochra
KC437916: Peltigera malacea
EF102333: Peltigera malacea
Group 002: Peltigera malacea (11)
Group 013: Peltigera malacea (5)
KC437896: Peltigera malacea
Group 015: Peltigera malacea (5)
KC437885: Nephroma arcticum
EF102321
EU877508: Leptogium lichenoides
Group 043: Leptogium lichenoides (2)
EU877507: Leptogium lichenoides
EU877509: Leptogium lichenoides
GQ184605: Leptogium lichenoides
DQ185292: Massalongia carnosa
DQ266036: Massalongia carnosa
DQ185289: Leptogium gelatinosum
KC437914: Peltigera neopolydactyla
DQ266034: Peltigera degenii
Group 136: Peltigera degenii, P. membranacea (2)
EF102337: Peltigera neopolydactyla
AJ632064: Nostoc calciola
AJ632063: Nostoc sp.
294893: Nostoc commune var. flagelliforme
DQ185281: Peltigera neopolydactyla
Group 042: Nephroma arcticum, Peltigera neopolydactyla
KM006015: Peltigera hydrothyria
KM006016: Peltigera hydrothyria
KM006009: Peltigera hydrothyria
Group 009: Peltigera hydrothyria (7)
Group 037: Gunnera magellanica (2)
KF142708: Gunnera magellanica
Group 152: Gunnera magellanica (2)
KF142705: Gunnera magellanica
KC437911: Peltigera leucophlebia
DQ185275: Peltigera rufescens
KC437826: Peltigera leucophlebia
Group 029: Peltigera praetextata (2)
Group 005: Peltigera canina (3), P. cinnamomea (3),
P. fuscopraetextata, P. leucophlebia, P. polydactylon
Group 016: Peltigera britannica, P. leucophlebia (2),
P. venosa
Group 033: Peltigera aphthosa (2)
DQ266031: Stereocaulon tomentosum
DQ266035: Peltigera neopolydactyla
KC437910: Peltigera neopolydactyla
KC437876: Peltigera neopolydactyla
Group 004: Peltigera britannica (2), P. fuscopraetextata,
P. kristinssonii, P. leucophlebia (2), P. praetextata (2),
P. sp.
DQ185312: Peltigera aphthosa
Group 006: Peltigera aphthosa (13), P. britannica (9),
P. canina (14), P. fuscopraetextata (11), P. kristinssonii (19),
P. leucophlebia (58), P. ponojensis (2), P. praetextata (2),
P. sp., P. venosa (2)
Group 041: P. praetextata (2), Peltigera horizontalis, P. leucophlebia,
Group 056: Peltigera leucophlebia (2)
EF102343: Peltigera praetextata
EF102342: Peltigera praetextata
DQ185306: Peltigera membranacea
Group 263: Peltigera leucophlebia, P. praetextata
EF102339: Peltigera praetextata
EF102338: Peltigera praetextata
Group 047: Peltigera horizontalis (2)
Group 001: Peltigera aphthosa, P. canina (2),
P. leucophlebia (2), P. praetextata (2), P. membranacea (5),
P. neocanina (6), P. polydactylon (2)
EF102345: Peltigera praetextata
EF102332: Peltigera leucophlebia
EF102330: Peltigera leucophlebia
Group 019: Peltigera aquatica (3)
KM006025: Peltigera aquatica
KM006022: Peltigera aquatica
Group 046: Peltigera aquatica (2)
Group 250: Peltigera gowardii (2)
KM006001: Peltigera gowardii
KM006006: Peltigera hydrothyria
KM006012: Peltigera hydrothyria
Group 049: Peltigera hydrothyria (2)
Group 032: Peltigera hydrothyria (2)
KM005998: Peltigera gowardii
Group 012: Peltigera gowardii (6)
CP001037: Macrozamia sp.
DQ185316: Geosiphon pyriforme
DQ185268: Blasia pusilla
DQ185315: Gunnera manicata
Group 044: Blasia pusilla, Peltigera didactyla
KF142695: Gunnera magellanica
KF142704: Gunnera magellanica
KF142691: Gunnera magellanica (7)
KF142691: Gunnera magellanica
KF142697: Gunnera magellanica
Group 023: Gunnera magellanica (3)
KF142692: Gunnera magellanica
KF142690: Gunnera magellanica
KF142681: Gunnera magellanica
Group 028: Gunnera magellanica (2)
KF142682: Gunnera magellanica
KF142687: Gunnera magellanica
KF142683: Gunnera magellanica
Group 150: Gunnera magellanica (2)
KF142684: Gunnera magellanica
KF142679: Gunnera magellanica
DQ185270: Anthoceros sp.
EF102335: Peltigera membranacea
EF102346: Peltigera sp.
JX876601: Peltigera membranacea
Group 035: Peltigera membranacea (2)
JX876594: Peltigera membranacea
Group 022: Peltigera membranacea (3)
JX876599: Peltigera membranacea
JX876596: Peltigera membranacea
JX876584: Peltigera membranacea
EU297407: Peltigera membranacea
Group 036: Peltigera membranacea (9)
Group 038: Peltigera membranacea (2)
EU877481: Collema tenax
Group 027: Collema crispum, Encephalartos natalensis
SL34258 Nostoc
SL34323 Nostoc
SL34338 Nostoc
SL34324 Nostoc
SL34332 Nostoc
SL34331 Nostoc
SL34209 Nostoc
SL34268 Nostoc
SL34266 Nostoc
SL34216 Nostoc
SL34296 Nostoc
SL34237 Nostoc
SL34298 Nostoc
SL34290 Nostoc
SL34294 Nostoc
EU877513: Leptogium magnussonii
EU877515: Leptogium magnussonii
EU877514: Leptogium magnussonii
EU877512: Leptogium magnussonii
EU877517: Leptogium magnussonii
EU877516: Leptogium magnussonii
DQ185280: Nostoc punctiforme
EU877482: Collema tenax
EU877467: Collema fragil
EU877466: Collema fragile
Group 007: Peltigera britannica, P. leucophlebia (4),
P. neckeri, P. neocanina (2)
SL34300: Leptogium lichenoides
SL34305: Leptogium lichenoides
SL34303: Leptogium lichenoides
SL34304: Leptogium lichenoides
SL34309: Leptogium lichenoides
SL34306: Leptogium lichenoides
SL34310: Leptogium lichenoides
EU877479: Collema nigrescens
Group 014: Peltigera canina (5)
EU877490: Leptogium brebissonii
Group 040: Leptogium pseudofurfuraceum (2)
EU877470: Collema furfuraceum
EU877528: Leptogium saturninum
Group 039: Leptogium saturni um (2)
EU877525: Leptogium saturninum
EU877527: Leptogium saturninum
EU877526: Leptogium saturninum
DQ185317: Nostoc punctiforme
EU877473: Collema nigrescens
294889: Nostoc sp.
DQ185284: Peltigera polycarpon
DQ185287: Peltigera canina
294892: Nostoc commune
DQ185266: Anthoceros sp.
DQ185314: Blasia pusilla
Group 099: Peltigera canina, P. membranacea
294890: Nostoc sp.
GQ184606: Collema flaccidum
DQ266040: Collema flaccidum
DQ266039: Collema flaccidum
EU877465: Collema flaccidum
EU877466: Collema flaccidum
Group 018: Peltigera neckeri (4)
Group 017: Peltigera sp.
SL34221 Peltigera ponojensis
SL34217 Peltigera ponojensis
SL34222 Peltigera ponojensis
DQ185276: Peltigera rufescens
DQ185271: Peltigera rufescens
EU877530: Leptogium scharderii
EU877468: Collema crim
KM13212: Peltigera canina
KM13218: Peltigera neckeri
Group 020: Peltigera canina (2), P. rufescens
294891: Nostoc sp.
KM13209: Peltigera neckeri
KM13201: Peltigera leucophlebia
Group 038: Peltigera rufescens (2), P. sp.
Group 030: Peltigera didactyla, P. rufescens
DQ185274: Peltigera rufescens
KM13217: Peltigera sp.
KM13218: Peltigera sp.
KM13219: Peltigera sp.
KC437900: Peltigera neocanina
Group 011: Nostoc sp. (2), Peltigera sp. (16)
DQ185313: Nostoc muscorum
Q185310: Peltigera rufescens
AJ632030: Nostoc sp.
EF102339: Peltigera leucophlebia
KC437877: Peltigera leucophlebia
SL34214 Peltigera ponojensis
Group 021: P. rufescens, Peltigera canina, P. didactyla,
DQ185265: Geosiphon pyriforme
SL34200 Peltigera ponojensis
SL34228 Peltigera ponojensis
SL34226 Peltigera ponojensis
SL34227 Peltigera ponojensis
SL34225 Peltigera ponojensis
SL34282 Peltigera ponojensis
SL34283 Peltigera ponojensis
SL34281 Peltigera ponojensis
SL34349 Peltigera neckeri
DQ185305: Peltigera lepidophora
SL34252 Peltigera ponojensis
EU877502: Leptogium furfuraceum
EU877504: Leptogium furfuraceum
EU877501: Leptogium furfuraceum
EU877499: Leptogium furfuraceum
Group 117: Leptogium furfuraceum (2)
EU877498: Leptogium furfuraceum
EU877486: Leptogium azureum
EU877484: Leptogium austroamericanum
EU877483: Leptogium austroamericanum
EU877505: Leptogium gelatinosum
EU877496: Leptogium cyanescens
EU877473: Collema tenax
EU877462: Collema aurriforme
AJ632065: Nostoc edaphicum
EU877506: Leptogium gelatinosum
EU877491: Leptogium corniculatum
EU877461: Collema aurriforme
EU877520: Leptogium pseudofurfuraceum
EU877521: Leptogium pseudofurfuraceum
EU877492: Leptogium corniculatum
EU877522: Leptogium pseudofurfuraceum
EU877523: Leptogium pulvinatum
EU877523: Leptogium pulvinatum
DQ185309: Lyngbya circinalis
SL34251 Nostoc
AF783697: Nodularia harveyana
AF783698: Nodularia spumigena
Group 083: Nodularia sphaerocarpa (2)
AF783685: Nodularia spumigena
AF783684: Nodularia spumigena
Group 050: Nodularia spumigena (6)
Group 090: Nodularia spumigena (4)
AF783694: Nodularia sphaerocarpa
AF783696: Nodularia spumigena
HM246943: Nodularia spumigena
AF783691: Nodularia sphaerocarpa
DQ185288: Nodularia harveyana
AF783702: Nodularia harveyana
AF783701: Nodularia harveyana
AF783700: Nodularia harveyana
AF783699: Nodularia harveyana
0.03