

Peltigera (Ascomycota) living in open and shady environments depend on different *Nostoc* photobionts

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Abstract: Species of *Peltigera* (lichen-forming Ascomycota) establish obligate symbioses with several monophyletic groups (phylogroups) of *Nostoc* cyanobacteria. Some of these fungi are strict specialists and only associate with one *Nostoc* phylogroup throughout their range, while others are generalists and associate with many different *Nostoc* phylogroups. We sampled 20 *Peltigera* taxa, mostly representing the *Peltigera* section *Peltigera*, from grassland and forest habitats across Europe. Mycobionts' identities were confirmed using fungal ITS sequences. The *Nostoc* cyanobionts were identified and grouped based on single nucleotide differences in the trnL region and on the phylogenetic analysis of the rbcLX region. Our data confirmed that some *Peltigera* species clearly prefer open habitats, while others are largely confined to shady forest habitats. The two habitat preferences are consistently paired with association with different *Nostoc* groups. As a specific example, two ecologically versatile species, *Peltigera canina* and *P. didactyla*, both growing in open and shady habitats, consistently associated with different groups of *Nostoc* when growing in different habitats. As associations between specific mycobionts and cyanobionts are influenced by habitat conditions, the diversity of both taxon groups should be considered in the context of habitat management and conservation planning.

Keywords: canopy closure, cyanobiont, grassland, ecology, forest, lichen, mycobiont, Peltigerales

INTRODUCTION

Delimiting species in the genus *Peltigera* (lichen-forming Ascomycota) has been a major challenge (e.g., Vitikainen, 1994; Miadlikowska et al., 2003; Jüriado et al., 2017; Magain et al., 2017a,b, 2018, 2023; Timdal & Gjerlaug, 2023). The genus includes eight well supported monophyletic sections (Miadlikowska & Lutzoni, 2000) which all include poorly delimited and/or undescribed taxa, in addition to well established morphological species (Miadlikowska et al., 2003; Jüriado et al., 2017; Magain et al., 2017a, 2018, 2023; Timdal & Gjerlaug, 2023). Several species have a wide or almost cosmopolitan distribution (Martínez et al., 2003; Magain et al., 2017a, 2018, 2023), making them attractive model species for the analysis of symbiont specificity. Some *Peltigera* species are strict habitat specialists and confined to, for example, xerophytic calcareous grasslands (Jüriado et al., 2017), old-growth forests (Nitare, 2000; Botting & Fredeen, 2006), boulders in streams (Miadlikowska et al., 2014), or high-elevation habitats (Kaasalainen et al., 2022). Most

Peltigera species have relatively wide ecological amplitudes, even though their preferences for either xerophytic, mesophytic, or hydrophytic habitat conditions are usually apparent (Goward et al., 1995; Miadlikowska et al., 2003; Jüriado et al., 2017; Kaasalainen et al., 2022).

For most *Peltigera* species, *Nostoc* is their only photosynthetic partner. However, some species have green algae (*Coccomyxa*) as their primary photobiont, and the nitrogen-fixing *Nostoc* cyanobiont is restricted to special structures called cephalodia (Vitikainen, 2007; Rikkinen, 2015, 2017). *Nostoc* is the most common genus of cyanobacteria which is in symbiosis with lichen-forming fungi, especially in the temperate and cool regions of the world (Rikkinen, 2013; Sanders & Masumoto, 2021). Identification of symbiotic *Nostoc* genotypes to specific bacterial species is not feasible at present, mainly because of incongruent phylogenetic signals (Han et al., 2009; Kitahara & Miyazaki, 2013; Kaasalainen et al., 2015; Pardo-De la Hoz et al., 2023). However, in practice, monophyletic groups (phylogroups) of symbiotic and non-symbiotic

Nostoc have often been used as operational taxonomic units (Dvořák et al., 2017; Magain et al., 2017a). Phylogenetic studies have shown that the same lineages of *Nostoc* participate in symbiotic associations with many lichen mycobionts, as well as with some plants (O'Brien et al., 2005, 2013; Svenning et al., 2005; Rikkinen & Virtanen, 2008; Kaasalainen et al., 2021). The ecological adaptation of green-algal photobionts and particular lichenized fungi to specific habitats or habitat conditions has been demonstrated in several studies (e.g., Peksa & Skaloud, 2011; Wagner et al., 2020; Osyczka et al., 2021), but reports on similar phenomena in lichen-symbiotic cyanobacteria have so far been rare (Ortiz-Álvarez et al., 2015; Cardós et al., 2019; Jüriado et al., 2019). In an earlier study, we found that species-specific patterns of photobiont selectivity in *Peltigera* correlate with habitat conditions (Jüriado et al., 2019). Some *Nostoc* variants were only found in *Peltigera* lichens of moist and mesic forest environments, while another set of variants were typically found in *Peltigera* species of xeric habitats. In the present study, we analyse this phenomenon more in detail by comparing the photobiont spectrum of *Peltigera* thalli collected from habitats forming a gradient from well illuminated (open) and relatively dry micro-sites to shady and relatively moist micro-habitats in forest, where the quantity and spectral characteristics of light are altered.

MATERIAL AND METHODS

Taxon sampling

Based on our previous studies on habitat preferences and relationships between mycobiont identity and photobiont specificity, as well as ecological preferences in the lichen genus *Peltigera* (Jüriado et al., 2017, 2019), we selected twenty species for this study. The sampled habitats/substrates range from the ground of dry grasslands to bryophytes on the forest floor, rocks, logs, and tree bases. The compiled data set consists of 282 specimens (Appendix 1). The delimitation of species and the naming of undescribed taxa follow Jüriado et al. (2017) and Magain et al. (2018). Most of the specimens (225) were collected from Estonia. Additionally, 50 specimens were collected from other parts of Europe (e.g., Greece, Italy, Finland, Norway), five specimens from Asia

(Armenia, Japan), and one from Greenland. Most of the Estonian specimens were collected in 2012–2018 from study localities distributed over the whole country, including different types of grasslands (e.g., dry calcareous grasslands, dunes, and roadsides), oligotrophic pine (*Pinus sylvestris*) forests, eutrophic forests with spruce and deciduous trees, and park stands with mature or old temperate broad-leaved tree species (see Jüriado et al., 2017 for details). *Peltigera* specimens from outside Estonia were collected from similar habitats: grasslands, dunes, roadsides, park stands, and forests. The light conditions of the habitats were described using tree canopy closure estimates on a scale from 0 to 1. Based on the canopy closure value, the habitats were further divided into three categories: open habitats (tree canopy closure 0–0.3), shaded semi-open habitats (0.4–0.6), and forest or other closed habitats (0.7–1). For a small set of samples, in case light conditions were not assessed in situ, the canopy cover of the habitat was estimated based on site description, field notes and photos, and digital sources including *Google satellite maps* (<https://www.google.com/maps>) or Google Street Views using the GPS recorded geographical coordinates of the specimen. In addition, three substrate types were distinguished: ground (including bare soil and mossy ground), tree (tree trunks and bases, logs and stumps, often covered with bryophytes), and rock (calcareous and siliceous boulders, usually covered with bryophytes).

Molecular data

Peltigera thalli without visible symptoms of fungal infection were selected for molecular analyses. A small thallus fragment was taken from a lobe apex under a dissecting microscope and placed in a 1.5 ml test tube. DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific) following the manufacturer's protocol for gram-negative bacteria (see Jüriado et al., 2017 for details). For 98 *Peltigera* specimens, new sequences were generated. The fungal species' identity was confirmed using the internal transcribed spacer (ITS) region amplified and sequenced with the primer pair ITS0F (Tedersoo et al., 2008) and ITS4R (White et al., 1990). The reaction mix of 25 µl volume contained 3 µl genomic DNA, 16 µl of sterile distilled water, 0.5 µl of each primer (20 mM) and 5 µl of 5x HOT FIREPol® Blend

Master Mix Ready to Load (Solis BioDyne). The heating cycle was the following: 15 min initial denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, with a final extension of 10 min at 72 °C. Since all chosen *Peltigera* species represent established phylogenetic entities (Jüriado et al., 2017; Magain et al., 2018), the species' identities were confirmed by comparing the fungal ITS sequences to the results by Jüriado et al. (2017). The voucher specimens are deposited in the lichenological herbarium of the Natural History Museum at the University of Tartu (TUF), in the mycological collections of the Natural History Museum at the University of Oslo (O), and in the Herbarium at the University of Graz (GZU) (Appendix 1).

Amplification of the cyanobacterial *trnL* was performed with the primer pair *tRNA Leu outF* and *tRNA Leu outR* (Paulsrud & Lindblad, 1998) as described in Jüriado et al. (2019). Additionally, the *Nostoc rbcLX* region was amplified from 52 specimens representing most of the detected *trnL* variants. The amplification of the *rbcLX* region was done using the primers CW (5'-CGTAGCTTCCGGTGGTATCCACGT-3') and CX (5'-GGGGCAGGTAAGAAAGGGTTTCGTA-3'; Rudi et al., 1998). The PCR reaction mix of 25 µl volume contained 2 µl genomic DNA, 17 µl of sterile distilled water, 0.5 µl of each primer (20 mM) and 5 µl of 5x HOT FIREPol® Blend Master Mix Ready to Load with 10 mM MgCl₂ (Solis BioDyne). The heating cycle was the following: 15 min initial denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 1 min at 72 °C, with a final extension of 10 min at 72 °C.

The amplification products were purified using the ExoSAP PCR product purification reaction (Thermo Scientific): 1 µl FastAP Thermosensitive Alkaline Phosphatase (1 U/µl, Thermo Scientific) and 0.5 µl Exonuclease I (20U/µl, Thermo Scientific) were added to 20 µl of the PCR product. Sequencing was performed by Macrogen Inc. (Amsterdam, the Netherlands). The chromatograms of the sequences were checked, edited manually, and assembled using CodonCode Aligner v6.0.2 (CodonCode Corporation, USA). All newly obtained sequences were uploaded in the NCBI GenBank database (Appendix 1). The newly obtained fungal ITS sequences were deposited also in the PlutoF

cloud database (Abarenkov et al., 2010) and are accessible through the public web output UNITE (<http://unite.ut.ee>; Kõljalg et al., 2013; Appendix 1).

Data analyses

The obtained *Nostoc trnL* sequences were divided into variants based on single nucleotide differences and compared with the existing *trnL* variant network published by Jüriado et al. (2019), using the median-joining option of the program Network v5.0.0.1 (Bandelt et al., 1999). The naming of the *trnL* variants follows Jüriado et al. (2019), by dividing the *trnL* variants into 11 further groups identified by letters (A–H, J–L).

The obtained *rbcLX* sequences were aligned together with the sequences downloaded from the NCBI GenBank manually, using PhyDe v0.997 (Müller et al., 2005). For analyses, the intergenic region between *rbcL* and *rbcX*, as well as the long insertions in the intergenic region between *rbcX* and *rbcS* (Rudi et al., 1998) were omitted, which resulted in an alignment of 198 sequences and 710 characters. Maximum likelihood (ML) analysis and the selection of the best fitting partitioning scheme and substitution model(s) for Bayesian analysis were done using IQ-TREE (Nguyen et al., 2015; Chernomor et al., 2016; Kalyaanamoorthy et al., 2017) on an IQ-TREE web server (Trifinopoulos et al., 2016). For the analyses, the data were pre-partitioned according to the region (*rbcL*, *rbcX*, intergenic), as well as according to codon position (1, 2, 3) in the coding regions. For ML analysis, an edge-linked partition model and Auto search for substitution models were selected and bootstrap analysis was performed using ultrafast bootstrap (Minh et al., 2013) with 1000 replicates. For the model and partition selection for Bayesian analysis, partition merging was enabled; the substitution models implemented in MrBayes were selected as candidate models, and the corrected Akaike information criterion was employed to select the best-fit model(s). Based on the IQ-TREE Model Selection results, all data partitions were combined and the K2P+I+G substitution model was used for all data in Bayesian analysis. The analysis was performed using MrBayes v3.2.7a (Ronquist et al., 2003), as described by Olsson et al. (2012), on the CIPRES Science Gateway (Miller et al., 2010) with three parallel runs with four chains each

for 2×10^7 generations. First 25% of the trees were discarded as burn-in and the convergence of the parallel runs was checked using Tracer v1.5 (Rambaut et al., 2018). The 50% majority rule trees were graphed using TreeGraph2 v2.15 (Stöver & Müller, 2010).

Average canopy closure estimates for all *Peltigera* specimens that housed photobionts belonging to the two main *Nostoc* trnL groups, A (n=168) and B (n=83), were compared using Student's t-test in Statistica v10. In addition, to compare the light conditions of particular *Peltigera* species' habitats, the average values of canopy closure estimates for *Peltigera canina* and *P. didactyla* specimens were calculated for calculated for Student's t-test comparison.

RESULTS

Mycobiont species

Altogether the 282 *Peltigera* specimens represent 20 taxa (Table 1). Of these, 15 belong to the *Peltigera* section *Peltigera*, including nine widely recognized species (in the parentheses the name used in Jüriado et al. 2017, 2019), *Peltigera canina* (*P. canina* II & III), *P. degenii*, *P. didactyla* (*P. didactyla* I, II & III), *P. extenuata*, *P. lepidophora*, *P. membranacea*, *P. ponojensis* (*P. ponojensis* I & II), *P. praetextata*, and *P. rufescens*, one recently described species (*P. islandica*), and at least five undescribed taxa: *P. canina* 2 (*P. canina* I), *P. "fuscoponojensis"*, *P. "neocanina"* (*P. aff. neocanina*), *P. "neorufescens"*, and *P. neorufescens* *agg.*, of which the last may encompass up to three undescribed species closely related to *P. "neorufescens"* (Jüriado et al., 2017). Additionally, five species represent other sections of the genus *Peltigera*, including *P. collina* and *P. neckeri* (section *Horizontales*), *P. hymenina* and *P. polydactylon* (section *Polydactylon*), and *P. aphthosa* (section *Peltidea*).

The two most abundant *Peltigera* species in our material were *P. canina* (75 specimens) and *P. rufescens* (36 specimens). All other taxa were represented by 20 or less specimens (Table 1). *Peltigera canina* was sampled from the widest geographical area with specimens from eight countries across Europe. *Peltigera didactyla*, *P. praetextata*, *P. membranacea*, *P. ponojensis*, and *P. rufescens*, as well as *P. "neocanina"*, were collected from up to five countries in Europe. The

other taxa were mainly collected from Estonia and Finland (Table 1). However, *Peltigera "neocanina"* is reported here for the first time from Austria, Finland, and Italy and *P. islandica* for the first time from Finland.

Of the *Peltigera* specimens 63% were collected from the ground, 21% from trees, and 16% from moss covered rocks. Six of the studied taxa (*P. canina*, *P. degenii*, *P. didactyla*, *P. membranacea*, *P. "neocanina"*, *P. praetextata*) were found in all three substrate types, while several taxa, including *P. canina* 2, *P. ponojensis* and *P. "neorufescens"*, were collected only from the ground (Appendix 1).

Nostoc cyanobionts

The cyanobionts of the studied *Peltigera* specimens included 33 different *Nostoc* trnL variants listed in Jüriado et al. (2019), and three new trnL variants (Appendix 1). Most of the trnL sequences (269) had a Class 2 repeat motif in the P6b region (Costa et al., 2002; Kaasalainen et al., 2015), while eight trnL sequences representing four different trnL variants had a Class 1 repeat motif of the P6b region. Eighty-three *Peltigera* specimens had a cyanobiont belonging to *Nostoc* trnL group B and 159 specimens had a cyanobiont belonging to *Nostoc* trnL group A, the most common variants being A1 and A2.

The phylogenetic analysis of the rbcLX region placed most of the studied *Nostoc* cyanobionts within two clades, Clade A and Clade B (Appendix 2). Of these, Clade B is well supported (BS = 100, PP = 0.999) and only includes specimens with a trnL variants of group B. Clade A includes all the cyanobionts with a trnL variants of group A but additionally also the single variants from trnL groups G and D. However, Clade A was only poorly supported (BS = 68) by the ML analysis and not recovered by the Bayesian analysis. Also, most cyanobionts with a trnL variant of group C formed a well-supported (BS = 98, PP = 1) clade, mainly with *P. hymenina* cyanobionts from different parts of the world.

The *Nostoc* cyanobionts belonging to trnL group B were recovered from seven *Peltigera* species from southern and northern Europe as well as from Japan. They were most commonly found in *P. canina* (34 specimens), *P. polydactylon* (16), *P. "neocanina"* (13) and *P. praetextata* (13). More rarely, it was found as a cyanobiont

Table 1. The number of *Peltigera* specimens analysed and their countries of origin. Abbreviations of the taxa used in Fig. 2. Abbreviations: Arm – Armenia, Aus – Austria, Den – Denmark, Est – Estonia, Fin – Finland, Green – Greenland, Jap – Japan, Lith – Lithuania, Nor – Norway, Pol – Poland.

Taxa	Abbreviation of taxa	Total No	Country
<i>Peltigera apthosa</i> (L.) Willd.		5	Est, Fin
<i>Peltigera canina</i> (L.) Willd.	P. cani	75	Aus, Den, Est, Fin, Greece, Lith, Nor, Pol
<i>Peltigera canina</i> 2	P. cani2	10	Est
<i>Peltigera collina</i> (Ach.) Schrad.		1	Est
<i>Peltigera degenii</i> Gyeln.		5	Est, Jap
<i>Peltigera didactyla</i> (With.) J.R.Laundon	P. dida	19	Aus, Est, Fin, Pol
<i>Peltigera extenuata</i> (Nyl. ex Vain.) Lojka		5	Est, Fin, Nor
<i>Peltigera "fuscoponjensis"</i>	P. fusc	5	Est
<i>Peltigera hymenina</i> (Ach.) Delise		3	Est
<i>Peltigera islandica</i> Goward & Manoharan-Basil		2	Est, Fin
<i>Peltigera lepidophora</i> (Vain.) Bitter.		2	Est
<i>Peltigera membranacea</i> (Ach.) Nyl.		11	Aus, Est, Fin, Green, Nor
<i>Peltigera neckeri</i> Hepp ex Müll.Arg.	P. neck	10	Est
<i>Peltigera "neocanina"</i>	P. neoc	14	Aus, Est, Fin, Italy
<i>Peltigera "neorufescens"</i>	P. neor	16	Est
<i>Peltigera "neorufescens" agg.</i>	P. neor agg	10	Est
<i>Peltigera polydactylon</i> (Neck.) Hoffm.	P. poly	16	Est
<i>Peltigera ponojensis</i> Gyeln.	P. pono	19	Arm, Est, Fin, Greece, Nor
<i>Peltigera praetextata</i> (Flörke ex Sommerf.) Zopf	P. prae	18	Est, Jap
<i>Peltigera rufescens</i> (Weiss) Humb.	P. rufe	36	Est, Fin, Greece, Nor
Total		282	

Table 2. Presence of *Nostoc* photobionts of trnL groups A and B in twelve *Peltigera* taxa collected from habitats with different canopy closure conditions. Canopy closure values from the best lit habitat to the most shaded closed canopy habitat: open (0 – 0.3), shaded (0.4 – 0.6) and closed (0.7 – 1). Abbreviations: Sub. – substratum, gr – ground, ro – rock, tr – tree.

<i>Peltigera</i> taxa	<i>Nostoc</i> trnL group A				Sub.	<i>Nostoc</i> trnL group B				
	Canopy closure					open	shaded	closed	mean (\pm SD)	Sub.
	open	shaded	closed	mean (\pm SD)						
<i>P. canina</i>	27	10		0.24 (\pm 0.19)	gr, ro	6	28	0.84 (\pm 0.20)	gr, ro, tr	
<i>P. canina</i> 2	5	5			gr					
<i>P. didactyla</i>	13	2		0.22 (\pm 0.17)	gr, ro	1	3	0.73 (\pm 0.23)	gr, ro, tr	
<i>P. "fuscoponjensis"</i>	5				gr					
<i>P. neckeri</i>	7	3			gr, ro					
<i>P. "neocanina"</i>						2	11		ro, tr	
<i>P. "neorufescens"</i>	15				gr					
<i>P. "neorufescens" agg.</i>	9	1			gr, ro					
<i>P. polydactylon</i>						1	15		gr, tr	
<i>P. ponojensis</i>	18	1			gr					
<i>P. praetextata</i>		2	2		gr, ro, tr		13		ro, tr	
<i>P. rufescens</i>	22	12			gr, ro	1			gr	

in *P. didactyla* or *P. rufescens* (Table 2). The cyanobionts belonging to trnL group A were found in various *Peltigera* species representing mainly the section *Peltigera*, most commonly in *P. canina* (39 specimens) and *P. rufescens* (35), as well as in *P. ponojensis*, *P. didactyla* and *P. neckeri* (Table 2). Also, the undescribed taxa of section *Peltigera*, e.g., *P. "fuscoponojensis"*, *P. "neorufescens"* and *P. canina* 2 were associated with *Nostoc* symbionts of this group.

Differences between the *Nostoc* groups in open and shady habitats

A total of 17 *Peltigera* taxa were found to house photobionts belonging to *Nostoc* trnL groups A and B (Appendix 1), twelve of them with ten or more specimens sampled (Table 2). The average canopy closure estimates of all *Peltigera* specimens housing the photobionts belonging to *Nostoc* group A were clearly lower (average closure estimate 0.21, SD±0.206, n=158) compared with the estimates for specimens housing the photobionts belonging to *Nostoc* group B (0.85, SD±0.19, n=81; Student's t-value=-23.26, df=237, p<0.00001). The *Peltigera* species with *Nostoc* photobionts of group A were mostly growing on the ground in open habitats (Fig. 1 & Table 2). The *Peltigera* species with *Nostoc* photobionts of group B were collected from closed habitats (Fig. 1), where they were most commonly growing on the ground in parklands and forests, and on mossy tree bases or rocks (Table 2).

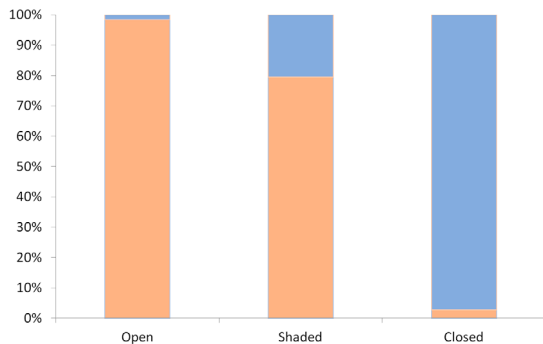


Fig. 1. Occurrence of *Nostoc* photobionts of trnL group A (orange) and group B (blue) in habitats with different degrees of shading.

The *Nostoc* cyanobionts of both groups (A and B) were found in the different specimens of four *Peltigera* species (Table 2 & Fig. 2). *Peltigera canina* housed photobionts of both groups almost evenly, while *P. didactyla* housed more frequently photobionts of group A, and *P. praetextata*, those of group B. With only one exception, *P. rufescens* specimens housed *Nostoc* photobionts of group A (Table 2).

Comparison of the canopy closure estimates for the specimens of a single *Peltigera* species, such as *P. canina* or *P. didactyla*, revealed that the specimens housing photobionts belonging to *Nostoc* group A were growing in habitats with lower canopy closure estimates compared with those belonging to *Nostoc* group B (Student's t-test for *P. canina* specimens: t-value=-12.43, df=67, p<0.00001, and for *P. didactyla* specimens: t=-4.51, df=17, p=0.00031; Table 2).

DISCUSSION

In lichens, the patterns of association between mycobionts and their photosynthetic partners (green algae or/and cyanobacteria) are often discussed in terms of specificity and selectivity. In this study, specificity refers to the taxonomic spectrum of acceptable partners and selectivity to the frequency of association between compatible partners (Rambold et al., 1998; Yahr et al., 2004, 2006). The level of specificity of symbiotic partners can be expressed using a number of different scales, e.g., taxonomic, temporal, or environmental, with the underlying mechanisms often remaining unclear (Paracer & Ahmadjian, 2000; Yahr et al., 2006, Magain et al., 2017a). For example, a mycobiont that associates with several unrelated lineages of photobionts (exhibiting low photobiont specificity) may still show high partner selectivity, meaning that it is mainly associated with only one of these lineages (Yahr et al., 2004). It has been suggested that the wide geographical distribution and broad ecological niches of many lichenized fungi may in part be linked with symbiotic plasticity between mycobiont species and their algal or cyanobacterial partners (Muggia et al., 2014; Rolshausen et al., 2018). Clarifying the influence of individual ecological factors on mycobiont selectivity is always complicated by intercorrelations between various driving factors

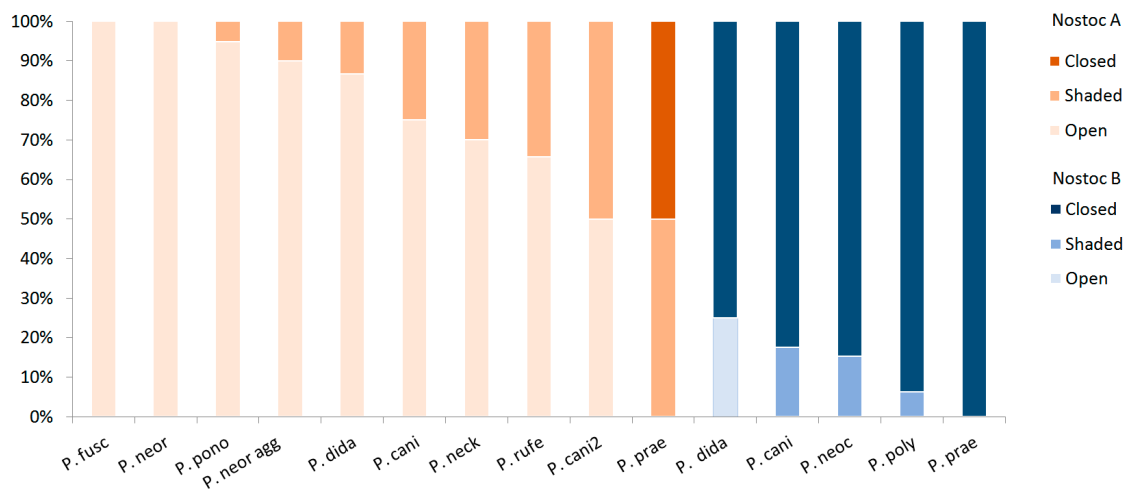


Fig. 2. Frequency of *Peltigera* species with *Nostoc* photobionts of trnL group A (*Nostoc* A) and group B (*Nostoc* B) in open, shaded, and closed habitats. For abbreviations of the *Peltigera* taxa, see Table 1.

(Yahr et al., 2006; Peksa & Škaloud, 2011; Werth & Sork, 2014).

In the lichen genus *Peltigera*, most mycobiont taxa associate with several, sometimes up to eight, different *Nostoc* phylogroups (O'Brien et al., 2005, 2013; Magain et al., 2017a, 2018). In particular, this is the case when *Peltigera* species are analysed on a global scale, as many widely distributed *Peltigera* species associate with different *Nostoc* phylogroups in different parts of their range (Manoharan-Basil et al., 2016; Magain et al., 2017a, 2018). Conversely, on a local scale, strict specificity is often detected at the species level (O'Brien et al., 2005; Lu et al., 2018, Jüriado et al., 2019). It has been suggested that this phenomenon could reflect specialization of different photobiont lineages to different ecological conditions (Yahr et al., 2006; O'Brien et al., 2013; Muggia et al., 2014).

In this study, we found clear correlation between photobiont choice and environmental conditions. Some *Peltigera* species from open and well-illuminated habitats were associated with a particular *Nostoc* group in all cases, while other taxa from shady forest habitats were associated with another group. In the case of two *Peltigera* species (*P. canina* and *P. didactyla*), a similar distinction was seen also at the species level: lichen thalli of the same species collected

from the two different habitat types housed different *Nostoc* photobionts.

All *Nostoc* cyanobionts encountered in our study are known to be widely distributed. In phylogenetic analysis, most of the sequences were placed within the *Nostoc* phylogroups defined by Magain et al. (2017a, 2018). For example, the rbcLX Clade B corresponds to phylogroup V, while the rbcLX Clade A includes cyanobionts from several different phylogroups, including XXV, XXIX, XXX, XXXI, XXXII, and XXXIII. Most of these *Nostoc* phylogroups have an extensive longitudinal range, but a relatively narrow latitudinal distribution, except for the phylogroup V which is cosmopolitan (Magain et al., 2017a, 2018; Pardo-De la Hoz et al., 2018). Photobionts of the *Nostoc* phylogroup V have been identified from 20 different species of the section *Peltigera* (Magain et al., 2018) and from several species of the section *Polydactylon*, as well as from some additional species of three other sections (Magain et al., 2017a; 2023). According to our previous study in Estonia (Jüriado et al., 2019), this *Nostoc* photobiont was characteristically associated with *Peltigera* species in shady forest habitats. In this study, the same *Nostoc* photobionts were found in additional lichen specimens from different parts of Europe and also from Japan. It associated most frequently with *P. canina*,

P. "neocanina" and *P. praetextata* (sect. *Peltigera*), and *P. polydactylon* (sect. *Polydactylon*). The *Peltigera* species associating with this *Nostoc* phylogroup are mesophytic, being most common in herb-rich deciduous or mixed forests and growing mostly on mossy tree bases, logs, mossy rocks and less frequently on the ground (Goward et al., 1995; Miadlikowska et al., 2003; Jüriado et al., 2017). On the other hand, the phylogroups corresponding to our rbcLX Clade A (e.g., phylogroups XXV, XXIX, XXX, XXXI, XXXII, XXXIII in Magain et al., 2018) and some other closely related phylogroups were found in *Peltigera* species that generally prefer more open habitats (Magain et al., 2018). For example, *Peltigera rufescens*, *P. didactyla*, *P. neckeri*, *P. ponjensis* associate with these *Nostoc* variants all across Europe. In the boreal region, they represent xerophytic species typically found at exposed and often disturbed sites including dunes, calcareous grasslands, and roadsides, but also in oligotrophic pine forests with relatively open canopies (Jüriado et al., 2017, 2019).

We were particularly interested in *Peltigera canina* and other species that have previously been shown to associate with different groups of *Nostoc* (Magain et al., 2018; Jüriado et al., 2019). Recent phylogenetic analyses have shown that *P. canina* actually includes at least two species, *P. canina* s. str. and *P. canina* 2 (Magain et al., 2018). Both of them exhibit low symbiont specificity and represent generalists on the global scale. Up to now, *P. canina* 2 has predominantly been known from North America, and from some places in northern Europe including Iceland (Magain et al., 2018; Timdal & Gjerlaug, 2023) and Estonia (referred to as *P. canina* 1 in Jüriado et al., 2017). In Estonia, *P. canina* 2 grows on the ground in xerophytic habitats like open-canopy parks, roadsides and pastures and associates with the group A *Nostoc*. *Peltigera canina* s. str., on the other hand, is a common species with a wider habitat range and occurs in many parts of Europe. It grows in several different habitat types such as acid soils of dunes, heaths and dry grasslands, as well as on bryophytes on forest floor, rocks and tree bases (Jüriado et al., 2017). Globally, it associates with several different *Nostoc* phylogroups (Magain et al., 2018). According to the present study, its preference of *Nostoc*

photobionts is also linked with habitat's light conditions. Thus, it associates with *Nostoc* group A, when growing in open habitats, and with *Nostoc* group B in closed-canopy forests. A similar pattern of photobiont selectivity was also exhibited by *Peltigera didactyla*. This widespread species, reported from six continents (Martinez et al., 2003), represents a complex of at least three putative species (Goffinet et al., 2003; Jüriado et al., 2017) whose habitat or cyanobiont preferences are still unclear.

Both, the preference for oceanic regions with high precipitation, shown by several cyanolichen species, as well as the distinct preference for shady woodland habitats, shown by epiphytic cyanolichens in more continental areas, are well established (e.g. Goward & Arsenault, 2000; Richardson & Cameron, 2004). These general habitat preferences have been proposed to be linked to the need for liquid water for activating photosynthesis in cyanolichens (Lange et al., 1986). Moreover, Almer et al. (2022) demonstrated differences in the temperature-mediated gene expression of green algal and cyanobacterial photobionts in *Peltigera britannica* photosymbiodemes and proposed that the green algal partner may be more tolerant of high temperatures than the cyanobacterial or fungal partners of the symbiosis. The occurrence of *Peltigera* species both in well-lit and occasionally very hot habitats (e.g., alvars) and in shady and cool forests indicates that there are probably considerable differences in the environmental optima of different symbiotic cyanobacteria (Rikkinen, 1995).

Our results emphasize the importance of considering the diversity of different lichen components in conservation planning and underpinning of conservation measures of particular species. The observed habitat specific variation in cyanobiont composition indicates that various habitats within the distribution range of a species should be protected and/or included in the habitat management plan.

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Appendix 1. List of the studied *Peltigera* taxa with GenBank accession numbers or UNITE codes for the ITS and cyanobacterial trnL and rbcLX sequences. Personal specimen ID numbers of I. Jüriado are presented. Voucher specimens are deposited in the herbaria of TUF, GZU, O or stored in private collections. *Nostoc* trnL group = *Nostoc* trnL group A or B. *Nostoc* trnL variant = *Nostoc* variant according to Jüriado et al. (2019), * denotes new trnL variants. Information about collection site (Country), substratum and canopy closure estimates is presented. Canopy closure: “Open” = open habitats, tree canopy closure 0–0.3, “Shaded” = shaded semi-open habitats, tree canopy closure 0.4–0.6 and “Closed” = forest or other closed habitats, tree canopy closure 0.7–1.

Specimen ID (I. Jüriado)	Taxa	Voucher Specimen	ITS GenBank Accession/ UNITE codes	trnL GenBank Accession	rbcLX GenBank Accession	<i>Nostoc</i> trnL group	<i>Nostoc</i> trnL variant	Country	Canopy closure	Substratum
173	<i>Peltigera aphthosa</i>	TUF052275	LT852805	LS999033			E	Estonia	Closed	Ground
443	<i>Peltigera aphthosa</i>	TUF073090	LT852806	LS999034			E	Estonia	Closed	Ground
468	<i>Peltigera aphthosa</i>	TUF045233	LT853036	LS999031			E	Estonia	Closed	Rock
484	<i>Peltigera aphthosa</i>	TUF52250	LT853037	LS999032			E	Estonia	Closed	Ground
244	<i>Peltigera aphthosa</i>	K23414 (private coll. J. Rikkinen)	OR397255	OR501925			E	Finland		Ground
13	<i>Peltigera canina</i>	TUF085017	LT852816	LS998813	OR413267	B	B	Estonia	Shaded	Ground
14	<i>Peltigera canina</i>	TUF085018	LT852817	LS998814		A	A2	Estonia	Shaded	Ground
124	<i>Peltigera canina</i>	TUF085030	LT852818	LS998810		B	B	Estonia	Closed	Rock
125	<i>Peltigera canina</i>	TUF085031	LT852819	LS998811		A	A5	Estonia	Open	Ground
126	<i>Peltigera canina</i>	TUF085032	LT852820	LS998812		A	A1	Estonia	Open	Ground
146	<i>Peltigera canina</i>	TUF085033	LT852821	LS998815		B	B	Estonia	Closed	Tree
170	<i>Peltigera canina</i>	TUF052272	LT852822	LS998816		A	A2	Estonia	Open	Ground
174	<i>Peltigera canina</i>	TUF052276	LT852823	LS998817		B	B	Estonia	Closed	Tree
179	<i>Peltigera canina</i>	TUF085036	LT852824	LS998818		A	A2	Estonia	Open	Ground
206	<i>Peltigera canina</i>	TUF052281	LT852825	LS998819		B	B	Estonia	Shaded	Ground
426	<i>Peltigera canina</i>	TUF058785	LT852826	LS998820		B	B	Estonia	Closed	Rock
434	<i>Peltigera canina</i>	TUF039796	LT852827	LS998821		B	B	Estonia	Closed	Tree
37	<i>Peltigera canina</i>	TUF085019	LT852828	LS998833		A	A1	Estonia	Shaded	Ground
68	<i>Peltigera canina</i>	TUF085021	LT852829	LS998845		A	A2	Estonia	Open	Ground
69	<i>Peltigera canina</i>	TUF085022	LT852830	LS998846		A	A2	Estonia	Open	Ground
71	<i>Peltigera canina</i>	TUF085023	LT852831	LS998847		A	A2	Estonia	Open	Ground
72	<i>Peltigera canina</i>	TUF085024	LT852832	LS998848		A	A2	Estonia	Open	Ground
86	<i>Peltigera canina</i>	TUF085026	LT852833	LS998849		A	A1	Estonia	Open	Ground
111	<i>Peltigera canina</i>	TUF085027	LT852834	LS998825	OR413264	A	A2	Estonia	Shaded	Ground
121	<i>Peltigera canina</i>	TUF085028	LT852835	LS998826		B	B	Estonia	Closed	Rock
123	<i>Peltigera canina</i>	TUF085029	LT852836	LS998827		B	B	Estonia	Closed	Rock
150	<i>Peltigera canina</i>	TUF085034	LT852837	LS998828		B	B	Estonia	Closed	Tree

Specimen ID (L. Jüriado)	Taxa	Voucher Specimen	ITS GenBank Accession/ UNITE codes	trnL GenBank Accession	rbclX GenBank Accession	<i>Nostoc</i> trnL group	<i>Nostoc</i> trnL variant	Country	Canopy closure	Substratum
168	<i>Peltigera canina</i>	TUF052270	LT852838	LS998829		A	A2	Estonia	Open	Ground
171	<i>Peltigera canina</i>	TUF052273	LT852839	LS998830		B	B	Estonia	Closed	Ground
178	<i>Peltigera canina</i>	TUF085035	LT852840	LS998831		A	A1	Estonia	Open	Ground
180	<i>Peltigera canina</i>	TUF085037	LT852841	LS999035	OR413271		H	Estonia	Open	Ground
192	<i>Peltigera canina</i>	TUF052279	LT852842	LS998832		A	A1	Estonia	Open	Ground
419	<i>Peltigera canina</i>	TUF052224	LT852843	LS998834		B	B	Estonia	Closed	Tree
424	<i>Peltigera canina</i>	TUF052285	LT852844	LS998835		B	B	Estonia	Closed	Tree
425	<i>Peltigera canina</i>	TUF052286	LT852845	LS998836		B	B	Estonia	Closed	Tree
432	<i>Peltigera canina</i>	TUF046221	LT852846	LS998837		B	B	Estonia	Closed	Tree
433	<i>Peltigera canina</i>	TUF039795	LT852847	LS998838		B	B	Estonia	Closed	Tree
453	<i>Peltigera canina</i>	TUF052225	LT852848	LS998839	OR413284	A	A19	Estonia	Shaded	Rock
473	<i>Peltigera canina</i>	TUF047639	LT853041	LS998840		B	B	Estonia	Closed	Ground
477	<i>Peltigera canina</i>	TUF052216	LT853042	LS998822		A	A9	Estonia	Open	Ground
478	<i>Peltigera canina</i>	TUF052237	LT853043	LS998823		B	B	Estonia	Closed	Tree
479	<i>Peltigera canina</i>	TUF052220	LT853044	LS998824		B	B	Estonia	Closed	Tree
482	<i>Peltigera canina</i>	TUF052287	LT853046	LS998841		A	A2	Estonia	Open	Rock
486	<i>Peltigera canina</i>	TUF052289	LT853047	LS998842		A	A2	Estonia	Shaded	Ground
490	<i>Peltigera canina</i>	TUF052292	LT853048	LS998843		B	B	Estonia	Closed	Tree
594	<i>Peltigera canina</i>	TUF053010	LT853049	LS998844		A	A2	Estonia	Open	Ground
480	<i>Peltigera canina</i>	TUF052239	LT853045	OR501929	OR413290			Estonia	Shaded	Ground
495	<i>Peltigera canina</i>	TUF090819		OR501930		A	A5	Finland	Shaded	Rock
503	<i>Peltigera canina</i>	TUF090818		OR501936		B	B	Finland	Shaded	Ground
651	<i>Peltigera canina</i>	TUF090812		OR501943		B	B	Finland	Closed	Tree
667	<i>Peltigera canina</i>	TUF090804	OR397206 UDB07674536 JL_667 ITS4	OR501951		B	B	Finland	Shaded	Rock
671	<i>Peltigera canina</i>	TUF090806		OR501952		A	A2	Finland	Open	Rock
678	<i>Peltigera canina</i>	TUF047696	OR397208 UD- B07674536 JL_678 ITS4	OR501954		B	B	Estonia	Shaded	Tree
679	<i>Peltigera canina</i>	TUF047791		OR501955		B	B	Estonia	Closed	Rock
680	<i>Peltigera canina</i>	TUF073091	UDB023747 TK98_its	OR501956		B	B	Estonia	Shaded	Tree
681	<i>Peltigera canina</i>	TUF039651	OR397209 UD- B07674537 JL_681 ITS4	OR501957		B	B	Estonia	Closed	Tree

Specimen ID (L. Jüriado)	Taxa	Voucher Specimen	ITS GenBank Accession/ UNITE codes	trnL Gen-Bank Accession	rbclX Gen-Bank Accession	<i>Nosoc</i> trnL group	<i>Nosoc</i> trnL variant	Country	Canopy closure	Substratum
682	<i>Peltigera canina</i>	TUF070439	OR397210 UD-B07674538 J_682_ITS4	OR501958		A	A2	Estonia	Open	Ground
683	<i>Peltigera canina</i>	TUF029215	OR397211 UD-B07674540 J_683_ITS	OR501959	OR413295		J	Estonia	Closed	Rock
684	<i>Peltigera canina</i>	TUF029216	OR397212 UD-B07674541 J_684_ITS	OR501926	OR413296	A	A*	Estonia	Open	Ground
685	<i>Peltigera canina</i>	TUF029212	OR397213 UD-B07674542 J_685_ITS4	OR501927	OR413297		H	Estonia	Shaded	Rock
686	<i>Peltigera canina</i>	TUF029213	OR397214 UD-B07674543 J_686_ITS	OR501960	OR413297	A	A15	Estonia	Shaded	Rock
687	<i>Peltigera canina</i>	TUF029218	OR397215 UD-B07674544 J_687_ITS4	OR501961		A	A1	Estonia	Open	Rock
689	<i>Peltigera canina</i>	TUF029654	OR397217 UD-B07674546 J_689_ITS	OR501963		B	B	Estonia	Closed	Rock
690	<i>Peltigera canina</i>	TUF029361	OR397218 UD-B07674547 J_690_ITS	OR501964		B	B	Estonia	Closed	Tree
691	<i>Peltigera canina</i>	TUF085038	OR397219 UD-B07674548 J_691_ITS4	OR501965	OR413299	B	B1*	Estonia	Closed	Tree
692	<i>Peltigera canina</i>	TUF085040	OR397220 UD-B07674549 J_692_ITS	OR501966		B	B	Estonia	Closed	Tree
708	<i>Peltigera canina</i>	GZU66-2014	OR397223 UD-B07674520 J_708_ITS	OR501969		A	A1	Greece	Open	Rock
709	<i>Peltigera canina</i>	GZU08-2003	OR397224 UD-B07674515 J_709_ITS4	OR501970		B	B	Austria	Closed	Rock
710	<i>Peltigera canina</i>	GZU-58964	OR397225 UD-B07674516 J_711_ITS4	OR501971		B	B	Austria	Closed	Rock
711	<i>Peltigera canina</i>	GZU48-2012	OR397225 UD-B07674516 J_711_ITS4	OR501928	OR413301		L*	Austria	Open	Ground
713	<i>Peltigera canina</i>	GZU43-2011	OR397226 UD-B07674517 J_713_ITS	OR501972		A	A2	Denmark	Open	Ground

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714	<i>Peltigera canina</i>	GZU1-2016	OR397227 UD-B07674551 JJ_714_ITS	OR501973		A	A1	Denmark	Open	Ground
740	<i>Peltigera canina</i>	Oslo 101557	OR397228 UD-B07674556 JJ_740_ITS	OR501976		A	A1	Norway	Open	Rock
747	<i>Peltigera canina</i>	TUF060662	OR397230 UD-B07674554 JJ_747_ITS4	OR501977		A	A1	Denmark	Shaded	Ground
748	<i>Peltigera canina</i>	TUF010706	OR397231 UD-B07674555 JJ_748_ITS4	OR501978		A	A1	Denmark	Open	Ground
749	<i>Peltigera canina</i>	TUF061570	OR397254	OR501979	OR413302	A	A1	Denmark	Open	Ground
751	<i>Peltigera canina</i>	TUF081318	UDB07674560 JJ_751_ITS4	OR501980		B	B	Lithuania	Closed	Tree
765	<i>Peltigera canina</i>	TUF084690	UDB07674561 JJ_765_ITS4	OR501981		A	A1	Poland	Shaded	Ground
808	<i>Peltigera canina</i>	TUF085328	UD-B07674567 JJ_808_ITS		OR413308	A		Estonia	Open	Ground
809	<i>Peltigera canina</i>	TUF090799			OR413309	A		Estonia	Open	Ground
4	<i>Peltigera canina</i> 2	TUF085179	LT852807	LS998807		A	A2	Estonia	Open	Ground
15	<i>Peltigera canina</i> 2	TUF085180	LT852808	LS998801		A	A2	Estonia	Shaded	Ground
26	<i>Peltigera canina</i> 2	TUF085181	LT852809	LS998806		A	A1	Estonia	Open	Ground
40	<i>Peltigera canina</i> 2	TUF085182	LT852818	LS998808		A	A2	Estonia	Shaded	Ground
41	<i>Peltigera canina</i> 2	TUF085183	LT852811	LS998809		A	A2	Estonia	Shaded	Ground
199	<i>Peltigera canina</i> 2	TUF052280	LT852812	LS998802		A	A2	Estonia	Open	Ground
200	<i>Peltigera canina</i> 2	TUF085184	LT852813	LS998803		A	A2	Estonia	Open	Ground
207	<i>Peltigera canina</i> 2	TUF052282	LT852814	LS998804		A	A1	Estonia	Shaded	Ground
208	<i>Peltigera canina</i> 2	TUF052283	LT852815	LS998805		A	A1	Estonia	Shaded	Ground
688	<i>Peltigera canina</i> 2	TUF029219	OR397216 UD-B07674545 JJ_688_ITS	OR501962		A	A8	Estonia	Open	Ground
445	<i>Peltigera collina</i>	TUF045667	LT852850	LS998850	OR413281		K	Estonia	Open	Rock
436	<i>Peltigera degenii</i>	TUF058786	LT852851	LS998851			C2	Estonia	Shaded	Rock
437	<i>Peltigera degenii</i>	TUF045213	LT852852	LS998852			C2	Estonia	Closed	Ground
457	<i>Peltigera degenii</i>	TUF052244	LT852853	LS998853	OR413285		C2	Estonia	Shaded	Rock
784	<i>Peltigera degenii</i>	TUF053888	MN752236	OR501984	OR413305	B	B1*	Japan	Closed	Tree

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791	<i>Peltigera degenerii</i>	TUF053878	OR397237 UD-B07674565 J_791L ITS	OR501985	OR413306	B	B	Japan	Closed	Tree
38	<i>Peltigera didactyla</i>	TUF052915	LT852854	LS998856		A	A1	Estonia	Shaded	Ground
193	<i>Peltigera didactyla</i>	TUF052940	LT852855	LS998854		A	A12	Estonia	Open	Ground
195	<i>Peltigera didactyla</i>	TUF052942	LT852856	LS998855	OR413274	A	A12	Estonia	Open	Ground
421	<i>Peltigera didactyla</i>	TUF052242	LT852857	LS998857		A	A2	Estonia	Open	Ground
83	<i>Peltigera didactyla</i>	TUF052917	LT852858	LS998863		A	A2	Estonia	Open	Ground
184	<i>Peltigera didactyla</i>	TUF052935	LT852859	LS998858		B	B	Estonia	Open	Ground
194	<i>Peltigera didactyla</i>	TUF052941	LT852869	LS998859		A	A2	Estonia	Open	Ground
420	<i>Peltigera didactyla</i>	TUF052228a	LT852861	LS998860		B	B	Estonia	Closed	Tree
458	<i>Peltigera didactyla</i>	TUF052253	LT852862	LS998861		B	B	Estonia	Closed	Rock
464	<i>Peltigera didactyla</i>	TUF052229	LT852863	LS998862		B	B	Estonia	Closed	Tree
43	<i>Peltigera didactyla</i>	TUF052916	LT852864	LS998864		A	D	Estonia	Shaded	Ground
85	<i>Peltigera didactyla</i>	TUF052918	LT852865	LS998865		A	A2	Estonia	Open	Ground
87	<i>Peltigera didactyla</i>	TUF052919	LT852866	LS998866		A	A2	Estonia	Open	Ground
499	<i>Peltigera didactyla</i>	TUF090822		OR501934		A	A13	Finland	Open	Ground
642	<i>Peltigera didactyla</i>	TUF045715	OR397199 UD-B07674523 J_642L ITS	OR501940		A	A1	Estonia	Open	Ground
643	<i>Peltigera didactyla</i>	TUF045422	OR397200 UD-B07674524 J_643L ITS	OR501941		A	A2	Estonia	Open	Ground
655	<i>Peltigera didactyla</i>	TUF090817	OR397203 UD-B07674530 J_655L ITS	OR501947		A	A13	Finland	Open	Rock
715	<i>Peltigera didactyla</i>	GZU48-2012	OR397229 UD-B07674518 J_715L ITS	OR501974		A	A1	Austria	Open	Ground
766	<i>Peltigera didactyla</i>	TUF084642	OR397234 UD-B07674562 J_766L ITS	OR501982	OR413304	A	A1	Poland	Open	Ground
20	<i>Peltigera extenuata</i>	TUF052912	LT852867	LS998870			D	Estonia	Shaded	Ground
114	<i>Peltigera extenuata</i>	TUF052931	LT852870	LS998867			D	Estonia	Open	Ground
185	<i>Peltigera extenuata</i>	TUF052936	LT852871	LS998868	OR413272		D	Estonia	Open	Ground
645	<i>Peltigera extenuata</i>	TUF090875	OR397249 UD-B07674526 J_645L ITS	OR501999			D	Finland	Open	Ground

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656	<i>Peltigera extenuata</i>	TUF090871	OR397250 UD-B07674531 J_656_ITS5	OR502000			D	Norway	Open	Ground
65	<i>Peltigera "fuscoponjensis"</i>	TUF052962	LT852875	LS998878	OR413293	A	A7	Estonia	Open	Ground
74	<i>Peltigera "fuscoponjensis"</i>	TUF052964	LT852877	LS998880		A	A2	Estonia	Open	Ground
100	<i>Peltigera "fuscoponjensis"</i>	TUF052970	LT852878	LS998874	OR413262	A	A10	Estonia	Open	Ground
130	<i>Peltigera "fuscoponjensis"</i>	TUF052932	LT852879	LS998875		A	A1	Estonia	Open	Ground
463	<i>Peltigera "fuscoponjensis"</i>	TUF052251	LT852880	LS998876		A	A2	Estonia	Open	Ground
162	<i>Peltigera hymenina</i>	TUF052217	LT852881	LS998881	OR413269		C3	Estonia	Closed	Ground
189	<i>Peltigera hymenina</i>	TUF052983	LT852882	LS998882	OR413273		C1	Estonia	Open	Ground
446	<i>Peltigera hymenina</i>	TUF071624	LT852883	LS998883			C1	Estonia	Closed	Rock
167	<i>Peltigera islandica</i>	TUF052269	LT852849	LS998884		A	A4	Estonia	Open	Ground
649	<i>Peltigera islandica</i>	TUF090801	OR397239 UD-B07674528 J_649_ITS			A	A5	Finland	Closed	ground
450	<i>Peltigera lepidophora</i>	TUF066438	LT852884	LS998885	OR413282	A	A21	Estonia	Open	Ground
451	<i>Peltigera lepidophora</i>	TUF045690	LT852885	LS998886		A	A4	Estonia	Open	Ground
427	<i>Peltigera membranacea</i>	TUF058684	LT852896	LS998892			C1	Estonia	Shaded	Ground
428	<i>Peltigera membranacea</i>	TUF058678	LT852897	LS998893			C1	Estonia	Closed	Ground
430	<i>Peltigera membranacea</i>	TUF058679	LT852898	LS998894	OR413280		C1	Estonia	Closed	Ground
431	<i>Peltigera membranacea</i>	TUF58685	LT852899	LS998895			C1	Estonia	Closed	Rock
658	<i>Peltigera membranacea</i>	TUF090870	OR397240 UD-B07674529 J_658_ITS4	OR501993			C1	Norway	Open	Ground
666	<i>Peltigera membranacea</i>	TUF090873	OR397241 UD-B07674534 J_666_ITS4	OR501994			C1	Finland	Closed	Rock
670	<i>Peltigera membranacea</i>	TUF090872	OR397256	OR501995			C1	Finland	Open	Rock
673	<i>Peltigera membranacea</i>	TUF090874		OR501987		A	A3	Finland	Open	Rock
706	<i>Peltigera membranacea</i>	GZU 98-2003	OR397242 UD-B07674550 J_706_ITS4	OR501996			C1	Austria	Closed	Tree
750	<i>Peltigera membranacea</i>	TUF059276	OR397244 UD-B07674559 J_750_ITS4	OR501997			C1	Greenland	Open	Ground
712	<i>Peltigera membranacea</i>	GZU 08-2003(58916)	OR397243 UD-B07674514 J_712_ITS4	OR501992			C1	Austria	Open	Ground

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33	<i>Peltigera neckeri</i>	TUF052867	LT852900	LS998900	OR413276	A	A18	Estonia	Shaded	Ground
35	<i>Peltigera neckeri</i>	TUF052868	LT852901	LS998901	OR413277	A	A9	Estonia	Shaded	Ground
36	<i>Peltigera neckeri</i>	TUF052869	LT852902	LS998902		A	A1	Estonia	Shaded	Ground
156	<i>Peltigera neckeri</i>	TUF052264	LT852903	LS998896		A	A2	Estonia	Open	Ground
166	<i>Peltigera neckeri</i>	TUF052268	LT852904	LS998897		A	A1	Estonia	Open	Ground
181	<i>Peltigera neckeri</i>	TUF052894	LT852905	LS998898		A	A1	Estonia	Open	Ground
182	<i>Peltigera neckeri</i>	TUF052895	LT852906	LS998899		A	A2	Estonia	Open	Ground
439	<i>Peltigera neckeri</i>	TUF055488	LT852907	LS998903		A	A2	Estonia	Open	Ground
441	<i>Peltigera neckeri</i>	TUF055487	LT852908	LS998904		A	A3	Estonia	Open	Ground
460	<i>Peltigera neckeri</i>	TUF052246	LT852909	LS998905	OR413286	A	A3	Estonia	Open	Rock
141	<i>Peltigera "neocanina"</i>	TUF052996	LT852912	LS998908		B	B	Estonia	Closed	Tree
142	<i>Peltigera "neocanina"</i>	TUF052261	LT852913	LS998909		B	B	Estonia	Closed	Tree
418	<i>Peltigera "neocanina"</i>	TUF052223	LT852915	LS998910		B	B	Estonia	Closed	Rock
422	<i>Peltigera "neocanina"</i>	TUF052999	LT852916	LS998911		B	B	Estonia	Closed	Tree
423	<i>Peltigera "neocanina"</i>	TUF053000	LT852917	LS998912		B	B	Estonia	Closed	Tree
429	<i>Peltigera "neocanina"</i>	TUF054214	LT852918	LS998913		B	J	Estonia	Closed	Ground
454	<i>Peltigera "neocanina"</i>	TUF052256	LT852919	LS998914		B	B	Estonia	Shaded	Rock
455	<i>Peltigera "neocanina"</i>	TUF052257	LT852920	LS998915		B	B	Estonia	Closed	Rock
653	<i>Peltigera "neocanina"</i>	TUF090816		OR501945	OR413294	B	B1*	Finland	Shaded	Tree
663	<i>Peltigera "neocanina"</i>	TUF090811		OR501948		B	B	Finland	Closed	Tree
664	<i>Peltigera "neocanina"</i>	TUF090809	OR397204 UD- B07674532 J_664_ITS4	OR501949		B	B	Finland	Closed	Tree
665	<i>Peltigera "neocanina"</i>	TUF090808	OR397205 UD- B07674533 J_665_ITS4	OR501950		B	B	Finland	Closed	Tree
705	<i>Peltigera "neocanina"</i>	GZU 68-2009	OR397221 UD- B07674521 J_705_ITS4	OR501967		B	B	Italy	Closed	Rock
707	<i>Peltigera "neocanina"</i>	GZU 49-2002	OR397222 UD- B07674519 J_707_ITS4	OR501968	OR413300	B	B	Austria	Closed	Rock
29	<i>Peltigera "neorufescens"</i>	TUF052957	LT852922	LS998925		A	A3	Estonia	Open	Ground
89	<i>Peltigera "neorufescens"</i>	TUF052921	LT852924	LS998935	OR413310	A	A1	Estonia	Open	Ground
90	<i>Peltigera "neorufescens"</i>	TUF052922	LT852925	LS998936	OR413311	A	A17	Estonia	Open	Ground
94	<i>Peltigera "neorufescens"</i>	TUF052924	LT852929	LS998940	OR413312	A	A1	Estonia	Open	Ground
95	<i>Peltigera "neorufescens"</i>	TUF052925	LT852930	LS998941		A	A10	Estonia	Open	Ground

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97	<i>Peltigera "neorufescens"</i>	TUF050025	LT852931	LS998942		A	A1	Estonia	Open	Ground
99	<i>Peltigera "neorufescens"</i>	TUF052969	LT852932	LS998943		A	A8	Estonia	Open	Ground
101	<i>Peltigera "neorufescens"</i>	TUF052971	LT852933	LS998917		A	A1	Estonia	Open	Ground
103	<i>Peltigera "neorufescens"</i>	TUF052927	LT852934	LS998918		A	A1	Estonia	Open	Ground
106	<i>Peltigera "neorufescens"</i>	TUF052974	LT852936	LS998920		A	A1	Estonia	Open	Ground
107	<i>Peltigera "neorufescens"</i>	TUF052975	LT852937	LS998921		A	A1	Estonia	Open	Ground
108	<i>Peltigera "neorufescens"</i>	TUF052976	LT852938	LS998922		A	A3	Estonia	Open	Ground
131	<i>Peltigera "neorufescens"</i>	TUF052933	LT852939	LS998923	OR413268	A	A13	Estonia	Open	Ground
152	<i>Peltigera "neorufescens"</i>	TUF052262.a	LT852940	LS998924		A	A1	Estonia	Open	Ground
355	<i>Peltigera "neorufescens"</i>	private coll. I. Jüriado	LT852943	LS998928	OR413278	A	A6	Estonia	Open	Ground
407	<i>Peltigera "neorufescens"</i>	TUF052949	LT852950	LS998934			A14	Estonia	Open	Ground
46	<i>Peltigera "neorufescens"</i>	TUF052959	LT852951	LS998944		A	A5	Estonia	Open	Ground
461	<i>Peltigera "neorufescens"</i>	TUF052254	LT852952	LS998945	OR413287	A	A15	Estonia	Shaded	Rock
49	<i>Peltigera "neorufescens"</i>	TUF052961	LT852954	LS998955		A	A3	Estonia	Open	Ground
155	<i>Peltigera "neorufescens"</i>	TUF052091	LT852955	LS998946		A	A3	Estonia	Open	Ground
188	<i>Peltigera "neorufescens"</i>	TUF052982	LT852956	LS998947		A	A2	Estonia	Open	Ground
201	<i>Peltigera "neorufescens"</i>	TUF052984	LT852957	LS999030	OR413287		G	Estonia	Open	Ground
102	<i>Peltigera "neorufescens"</i>	TUF052972	LT852959	LS998949		A	A4	Estonia	Open	Ground
105	<i>Peltigera "neorufescens"</i>	TUF052928	LT852960	LS998950	OR413263	A	A4	Estonia	Open	Ground
367	<i>Peltigera "neorufescens"</i>	private coll. I. Jüriado	LT852964	LS998953	OR413279	A	A20	Estonia	Open	Ground
380	<i>Peltigera "neorufescens"</i>	TUF052947	LT852965	LS998956		A	A3	Estonia	Open	Ground
127	<i>Peltigera polydactylon</i>	TUF052989	LT852966	LS998958	OR413266	B	B	Estonia	Closed	Ground
128	<i>Peltigera polydactylon</i>	TUF052990	LT852967	LS998959		B	B	Estonia	Closed	Ground
129	<i>Peltigera polydactylon</i>	TUF052991	LT852968	LS998960		B	B	Estonia	Closed	Ground
137	<i>Peltigera polydactylon</i>	TUF052993	LT852969	LS998961		B	B	Estonia	Closed	Tree
140	<i>Peltigera polydactylon</i>	TUF052995	LT852970	LS998957		B	B	Estonia	Closed	Tree
157	<i>Peltigera polydactylon</i>	TUF052221	LT852971	LS998962		B	B	Estonia	Closed	Tree

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158	<i>Peltigera polydactylon</i>	TUF052265	LT852972	LS998963		B	B	Estonia	Closed	Tree
160	<i>Peltigera polydactylon</i>	TUF052267	LT852973	LS998964		B	B	Estonia	Closed	Tree
161	<i>Peltigera polydactylon</i>	TUF052219	LT852974	LS998965		B	B	Estonia	Closed	Tree
165	<i>Peltigera polydactylon</i>	TUF052234	LT852975	LS998966		B	B	Estonia	Closed	Tree
175	<i>Peltigera polydactylon</i>	TUF052277	LT852976	LS998968		B	B	Estonia	Closed	Tree
172	<i>Peltigera polydactylon</i>	TUF052274	LT852977	LS998967		B	B	Estonia	Closed	Tree
447	<i>Peltigera polydactylon</i>	TUF039232	LT852978	LS998969		B	B	Estonia	Closed	Tree
459	<i>Peltigera polydactylon</i>	TUF052245	LT852980	LS998970		B	B	Estonia	Shaded	Tree
475	<i>Peltigera polydactylon</i>	TUF052232	LT853039	LS998971		B	B	Estonia	Closed	Tree
476	<i>Peltigera polydactylon</i>	TUF052222	LT853040	LS998972		B	B	Estonia	Closed	Tree
28	<i>Peltigera ponojensis</i>	TUF052865	LT852981	LS998980		A	A5	Estonia	Open	Ground
39	<i>Peltigera ponojensis</i>	TUF052870	LT852982	LS998981		A	A2	Estonia	Open	Ground
44	<i>Peltigera ponojensis</i>	TUF052872	LT852983	LS998982		A	A1	Estonia	Open	Ground
75	<i>Peltigera ponojensis</i>	TUF052883	LT852984	LS998984	OR413303	A	A1	Estonia	Open	Ground
80	<i>Peltigera ponojensis</i>	TUF052886	LT852986	LS998986	OR413307	A	A16	Estonia	Open	Ground
119	<i>Peltigera ponojensis</i>	TUF052979	LT852988	LS998974	OR413307	A	A11	Estonia	Open	Ground
42	<i>Peltigera ponojensis</i>	TUF052871	LT852989	LS998987		A	A9	Estonia	Open	Ground
60	<i>Peltigera ponojensis</i>	TUF052878	LT852990	LS998989		A	A8	Estonia	Open	Ground
117	<i>Peltigera ponojensis</i>	TUF052977	LT852991	LS998975		A	A1	Estonia	Shaded	Ground
120	<i>Peltigera ponojensis</i>	TUF052980	LT852992	LS998976		A	A1	Estonia	Open	Ground
191	<i>Peltigera ponojensis</i>	TUF052898	LT852993	LS998977		A	A1	Estonia	Open	Ground
204	<i>Peltigera ponojensis</i>	TUF052904	LT852994	LS998978		A	A2	Estonia	Open	Ground
205	<i>Peltigera ponojensis</i>	TUF052985	LT852995	LS998979		A	A13	Estonia	Open	Ground
472	<i>Peltigera ponojensis</i>	TUF052218	LT853055	LS998988	OR413307	A	A22	Estonia	Open	Ground
593	<i>Peltigera ponojensis</i>	TUF053012	LT853056	LS998983		A	A6	Estonia	Open	Ground
718	<i>Peltigera ponojensis</i>	GZU 66-2019	OR397246 UD-B07674552 J_718_ITS	OR501988		A	A1	Greece	Open	Ground
722	<i>Peltigera ponojensis</i>	GZU 90-2008	OR397247 UD-B07674553 J_722_ITS	OR501991		A	A4	Armenia	Open	Ground
741	<i>Peltigera ponojensis</i>	Oslo L64281	OR397248 UD-B07674557 J_741_ITS5	OR501989		A	A1	Norway	Open	Ground
505	<i>Peltigera ponojensis</i>	private coll. I. Jüriado	OR397253	OR501938		A	A10	Finland	Open	Ground
45	<i>Peltigera praetextata</i>	TUF052988	LT852996	LS998996		B	B	Estonia	Closed	Rock

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135	<i>Peltigera praetextata</i>	TUF052992	LT852997	LS998990		B	B	Estonia	Closed	Tree
138	<i>Peltigera praetextata</i>	TUF052994	LT852998	LS998991		B	B	Estonia	Closed	Tree
144	<i>Peltigera praetextata</i>	TUF052997	LT852999	LS998992		B	B	Estonia	Closed	Tree
145	<i>Peltigera praetextata</i>	TUF0853000	LT853000	LS998993		B	B	Estonia	Closed	Tree
159	<i>Peltigera praetextata</i>	TUF052266	LT853001	LS998994		B	B	Estonia	Closed	Tree
176	<i>Peltigera praetextata</i>	TUF052278	LT853002	LS998995		A	A1	Estonia	Closed	Tree
452	<i>Peltigera praetextata</i>	TUF042658	LT853003	LS998997	OR413283		J	Estonia	Closed	Rock
474	<i>Peltigera praetextata</i>	TUF052233	LT853050	LS998998		B	B	Estonia	Closed	Tree
481	<i>Peltigera praetextata</i>	TUF052247	LT853051	LS998999		A	A1	Estonia	Shaded	Ground
483	<i>Peltigera praetextata</i>	TUF052288	LT853052	LS999000		B	B	Estonia	Closed	Tree
487	<i>Peltigera praetextata</i>	TUF052290	LT853053	LS999001		A	A2	Estonia	Closed	Tree
488	<i>Peltigera praetextata</i>	TUF052291	LT853054	LS999002		B	B	Estonia	Closed	Tree
652	<i>Peltigera praetextata</i>	TUF090820	OR397202 UD- B07674525 J_652_ ITS	OR501944		B	B	Finland	Closed	Rock
654	<i>Peltigera praetextata</i>	TUF090813		OR501946		A	A1	Finland	Shaded	Rock
677	<i>Peltigera praetextata</i>	TUF029984	OR397207 UD- B07674539 J_677_ ITS	OR501953		B	B	Estonia	Closed	Tree
775	<i>Peltigera praetextata</i>	TUF053872	OR397235 UD- B07674563 J_775_ ITS	OR501983		B	B	Japan	Closed	Tree
797	<i>Peltigera praetextata</i>	TUF053928	OR397238 UD- B07674566 J_797_ ITS	OR501986		B	B	Japan	Closed	Tree
1	<i>Peltigera rufescens</i>	TUF052859	LT853004	LS999003		B	B	Estonia	Open	Ground
2	<i>Peltigera rufescens</i>	TUF052860	LT853005	LS999015		A	A1	Estonia	Open	Ground
16	<i>Peltigera rufescens</i>	TUF052861	LT853006	LS999007		A	A2	Estonia	Shaded	Ground
17	<i>Peltigera rufescens</i>	TUF052862	LT853007	LS999008		A	A2	Estonia	Shaded	Ground
18	<i>Peltigera rufescens</i>	TUF052863	LT853008	LS999009		A	A2	Estonia	Shaded	Ground
19	<i>Peltigera rufescens</i>	TUF052864	LT853009	LS999010		A	A2	Estonia	Shaded	Ground
30	<i>Peltigera rufescens</i>	TUF052866	LT853010	LS999018		A	A2	Estonia	Shaded	Ground
52	<i>Peltigera rufescens</i>	TUF052874	LT853011	LS999021	OR413291	A	A5	Estonia	Open	Ground
53	<i>Peltigera rufescens</i>	TUF052875	LT853012	LS999022		A	A5	Estonia	Open	Ground
54	<i>Peltigera rufescens</i>	TUF052876	LT853013	LS999023		A	A1	Estonia	Open	Ground
58	<i>Peltigera rufescens</i>	TUF052877	LT853014	LS999024	OR413292	A	A8	Estonia	Open	Ground
78	<i>Peltigera rufescens</i>	TUF052884	LT853015	LS999025		A	A1	Estonia	Open	Ground

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79	<i>Peltigera rufescens</i>	TUF052885	LT853016	LS999026		A	A1	Estonia	Open	Ground
81	<i>Peltigera rufescens</i>	TUF052887	LT853017	LS999027		A	A1	Estonia	Open	Ground
82	<i>Peltigera rufescens</i>	TUF052888	LT853018	LS999028		A	A2	Estonia	Open	Ground
98	<i>Peltigera rufescens</i>	TUF052890	LT853019	LS999029	OR413313	A	A3	Estonia	Open	Ground
109	<i>Peltigera rufescens</i>	TUF052891	LT853020	LS999004		A	A2	Estonia	Shaded	Ground
110	<i>Peltigera rufescens</i>	TUF052892	LT853021	LS999005		A	A2	Estonia	Shaded	Ground
116	<i>Peltigera rufescens</i>	TUF052893	LT853022	LS999006		A	A2	Estonia	Shaded	Ground
190	<i>Peltigera rufescens</i>	TUF052897	LT853023	LS999011		A	A1	Estonia	Open	Ground
196	<i>Peltigera rufescens</i>	TUF052900	LT853024	LS999012		A	A1	Estonia	Shaded	Ground
197	<i>Peltigera rufescens</i>	TUF052901	LT853025	LS999013		A	A1	Estonia	Shaded	Ground
198	<i>Peltigera rufescens</i>	TUF052902	LT853026	LS999014		A	A2	Estonia	Shaded	Ground
203	<i>Peltigera rufescens</i>	TUF052903	LT853027	LS999016		A	A2	Estonia	Open	Ground
211	<i>Peltigera rufescens</i>	TUF052905	LT853028	LS999017		A	A2	Estonia	Shaded	Ground
462	<i>Peltigera rufescens</i>	TUF052252	LT853029	LS999019		A	A4	Estonia	Shaded	Rock
471	<i>Peltigera rufescens</i>	TUF052226	LT853030	LS999020	OR413288	A	A1	Estonia	Open	Rock
496	<i>Peltigera rufescens</i>	TUF090821		OR501931		A	A2	Finland	Open	Rock
497	<i>Peltigera rufescens</i>	TUF090805		OR501932		A	A13	Finland	Open	Rock
498	<i>Peltigera rufescens</i>	TUF090807		OR501933		A	A3	Finland	Open	Ground
500	<i>Peltigera rufescens</i>	TUF090823		OR501935		A	A2	Finland	Open	Ground
504	<i>Peltigera rufescens</i>	TUF090814		OR501937		A	A10	Finland	Open	Rock
506	<i>Peltigera rufescens</i>	TUF090815	OR397198 UD- B07674522 I_506_ ITS	OR501939		A	A5	Finland	Open	Ground
646	<i>Peltigera rufescens</i>	TUF090802	OR397201 UD- B07674527 I_646_ ITS4	OR501998		A	D	Finland	Open	Ground
721	<i>Peltigera rufescens</i>	GZU 66-2014		OR501975		A	A9	Greece	Open	Ground
746	<i>Peltigera rufescens</i>	Oslo L147757	OR397245 UD- B07674558 I_746_ ITS4	OR501990		A	A4	Norway	Open	Ground

Appendix 2. Maximum-likelihood (ML) tree of the *rbclX* region of lichen symbiotic *Nostoc*. Thickness of the branches indicates the support values of the phylogenetic analyses, including bootstrap (BS) in ML analysis and posterior probability (PP) in Bayesian analysis. The sequences generated for this study are shown in black, the sequences downloaded from GenBank are shown in grey. The corresponding *trnL* variant (letters A-K) (Jüriado et al., 2019) and the phylogroup (roman numerals) according to Magain et al. (2017a, 2018) are indicated in the parentheses after the specimens.



