Standard Paper

Towards a nomenclatural clarification of the Peltigera ponojensis/monticola clade including metagenomic sequencing of type material and the introduction of P. globulata Midl. & Magain sp. nov.

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Abstract

Peltigera globulata Midl. & Magain, a new species in the P. ponojensis/monticola species complex of section Peltigera, is formally described. This clade was previously given the interim designation Peltigera sp. 17. It is found in sun-exposed and xeric habitats at high altitudes in Peru and Ecuador. Peltigera globulata can be easily recognized by its irregularly globulated margins covered mostly by thick, white pruina, somewhat resembling the sorediate thallus margins of P. soredians, another South American species from section Peltigera. The hypervariable region of ITS1 (ITS1-HR), which is in general highly variable among species of section Peltigera, does not have diagnostic value for species identification within the P. ponojensis/monticola complex. Nevertheless, no significant level of gene flow was detected among eight lineages representing a clade of putative species (including P. globulata) within this complex. ITS sequences from the holotype specimens of P. monticola Vitik. (collected in 1979) and P. soredians Vitik. (collected in 1981) and lectotype specimens of P. antarctica C. W. Dodge (collected in 1941) and P. aubertii C. W. Dodge (collected in 1952) were successfully obtained through Sanger and Illumina metagenomic sequencing. BLAST results of these sequences revealed that the type specimen of P. monticola falls within the P. monticola/ponojensis 7 clade, which represents P. monticola s. str., and confirmed that the type specimen of P. aubertii falls within a clade identified previously as P. aubertii based on morphology. The ITS sequence from the type specimen of P. soredians, which superficially resembles P. globulata, confirms its placement in the P. rufescens clade. Finally, we discovered that the name P. antarctica was erroneously applied to a lineage in the P. ponojensis/monticola clade. The ITS sequence from the type specimen of P. antarctica represents a lineage within the P. rufescens clade, which is sister to the P. ponojensis/monticola clade.

Keywords: Andean lichens; cyanolichens; new species; species complex; taxonomy

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Introduction

Peltigera Willd. section Peltigera (Fig. 1A & B) includes a high number of undescribed species delimited by Magain et al. (2018). Among the large clades recognized within section Peltigera, the P. ponojensis/monticola clade (Clade 5 in Fig. 1B) has been the most challenging taxonomically. Morphological characters that distinguish putative species are often indistinct, and there is a high level of intraspecific morphological variability. Phylogenetic boundaries between species are in many cases ambiguous, and sampling of specimens is frequently scattered across broad geographic ranges (Magain et al. 2018).

The P. ponojensis/monticola clade, which was labelled ‘A – P. ponojensis group’ in fig. 2 of Miadlikowska et al. (2003), was initially represented by two European species that were relatively easy to recognize: P. ponojensis Geyrn. and P. monticola Vitik. (Vitikainen 1994a). Peltigera monticola, a southern European species, with curled, and often phylloid margins, resembles in its habit and size P. rufescens and P. ponojensis, but the tomentum is less pronounced, the thallus is thinner, and the rhizines and vein patterns differ according to the original description by Vitikainen (1994a); it has slightly pruinose lobe tips with a very sparse tomentum and becomes etomentose and glabrose or somewhat scabrose towards the center of the thallus (Vitikainen 1994a). Peltigera ponojensis, most commonly found in boreal and temperate parts of Europe, resembles P. rufescens (Weiss) Humb. but ‘differs in the paler, often persistently whitish color of its veins and rhizines (when young), which tend to be simple and solitary’ (Vitikainen 1994a). In the same article, Vitikainen indicated that
both species needed further investigation, especially in light of some atypical phenotypes of *P. ponojensis* found in Iceland.

Nearly ten years later, Miadlikowska *et al.* (2003) demonstrated that both species are monophyletic on the basis of data from ten individuals collected in Europe (mostly Poland). This study also included the closely affiliated *P. scotteri* 1, a putative, undescribed species from Canada with a *P. degennii*-like, etomentose morphology. *Peltigera 'scotteri' 1* was shown to be part of the *P. monticola + P. ponojensis* clade by Miadlikowska & Lutzoni (2000) using the nrLSU + ITS locus combined with morphological characters. Miadlikowska *et al.* (2003) demonstrated the diagnostic utility of the hypervariable region within ITS1 (ITS1-HR) for species recognition within section *Peltigera*, including the *P. ponojensis* group. ITS1-HR sequences showed that *P. monticola* and *P. ponojensis* were commonly found outside of Europe (Magain *et al.* 2018).

With an expanded sampling of 46 specimens mostly from North and South America, and China, Magain *et al.* (2018) reported that the *P. ponojensis* group was a large species complex (Clade 5/*P. ponojensis/monticola* s. lat. in fig. 1 of Magain *et al.* (2018)) containing 15 putative species recognized and validated by multiple analytical methods. Since most of the lineages were morphologically and included morphotypes also identified as *P. rufescens* or *P. degennii* Gylen., no formal taxonomic changes were proposed, and the putative species were left as numbered clades. *Peltigera* sp. 17 stood out because of its unusual globulate margins somewhat resembling *P. soredians* Vitik, when seen in the field. Another monophyletic group that included specimens mostly from Chile was identified as *Peltigera antarctica* C. W. Dodge based on available descriptions and comparisons with identified herbarium collections. One consequence of this expanded sampling was that Magain *et al.* (2018) could no longer confidently identify *P. monticola* s. str. and *P. ponojensis* s. str. based solely on morphology and geography, because typical morphotypes collected in Europe were spread across multiple clades within the *P. ponojensis/monticola* complex. This also raised questions about the identity of the type specimens for these two species, but DNA sequences from their type material were not available at the time.

In order to resolve some of the taxonomic issues within the *P. ponojensis/monticola* species complex, we inferred a phylogeny for this clade based on DNA sequences used by Magain *et al.* (2018) and additional data available in GenBank, for a total of 68 terminal branches (Supplementary Material Table S1, available online). We re-examined the morphology of *Peltigera* sp. 17 to provide a description and a formal name: *P. globulata*. To further validate the presence of multiple species within the *P. ponojensis/monticola* species complex and justify the formal recognition of *P. globulata* as a species new to science, we reassessed species boundaries by estimating levels of gene flow among putative species within the species complex. In order to more confidently link existing species names to putative species-level clades, we sequenced the ITS locus from type specimens of *P. monticola* and *P. antarctica*, two species within the species complex, as well as *P. soredians* and *P. abietii* C. W. Dodge, two other species from section *Peltigera* but outside of the *P. ponojensis/monticola* clade.

### Materials and Methods

#### Specimen examination

Specimens of *Peltigera globulata* were examined using a Leica MZ6 dissecting microscope and a Leica DMLB compound microscope (×400 magnification). Three individuals of *P. globulata* (P2165 and P2164 from Peru, and P2195 from Ecuador; Supplementary Material Table S1, available online) and two representatives of *P. soredians* (P2152: Ecuador, Kalb & Jonitz 39785, DUKE; and P14480: Costa Rica, Clerc & Rojas PC 2013/487, G 00117176) were subjected to thin-layer chromatography (LaGreca, TLC plate #199; 1/27/2021; DUKE) as described in Culberson &Kristinsson (1970), Culberson (1972) and Culberson & Johnson (1982). Small thallus fragments were extracted in hexane, spotted on the pre-coated Merck silica gel 60 F254 glass plates and eluted in solvent systems C (TA in Holtan-Hartwig (1993)) and G (Culberson *et al.* 1981). The chromatograms were developed by spraying with 10% sulphuric acid and heating them at 110 °C for 1 h. Plates were examined under white (normal) light and UV light (350 nm).

#### Phylogenetic analyses

To assemble the data matrix for this study, we started with the 5-locus dataset (COR16, COR1b, COR3, ITS and β-tubulin) for 48 individuals that Magain *et al.* (2018) used to infer the phylogeny and delimit species in the *P. ponojensis/monticola* species complex (i.e. Clade 5, fig. 2B in Magain *et al.* (2018)). We added 20 ITS sequences that were not included in the phylogenetic analyses of Magain *et al.* (2018) (Supplementary Material Table S1). We...
adjusted the alignments manually with Mesquite v. 3.51 (Maddison & Maddison 2018). The final data matrix consisted of 68 individuals and 3970 characters (available on FigShare: 10.6084/m9.figshare.c.6636131). We divided the dataset into eight subsets (COR16, COR1b, COR3, ITS, β-tubulin 1st, 2nd and 3rd codon positions and introns) to determine the best partition scheme using PartitionFinder2 v. 2.1.1 (Lanfear et al. 2017). We applied the corrected Akaike information criterion (AICc) and the greedy algorithm (Lanfear et al. 2012). Maximum likelihood (ML) phylogenetic searches were implemented with RAxML v. 8.2.12 (Stamatakis 2006; Stamatakis et al. 2008) using the CIPRES Science Gateway v. 3.3 (Miller et al. 2015), with the GTBAGamma model applied to each of the eight initially specified partitions. Bootstrap support values were obtained from 1000 pseudoreplicates.

Gene flow analyses
We investigated gene flow among populations representing putative species within a well-supported clade (85% bootstrap support; see small box for this specific internode in Fig. 1C) that encompassed eight lineages, including P. globulata, based on the same five loci mentioned above and using an Isolation et al. (2013) and 3970 characters (available on FigShare:10.6084/m9.figshare.c.6636131). The eight predefined tests were performed on full-length sequence alignments using SITES v. 1.1 (Hey & Wakeley 1997). The eight predefined populations (Fig. 1C) corresponded to species delimited by Magain et al. (2018). The two specimens of P. ponojensis from Poland were assigned to a single population. Pairwise FST values > 0.5 supporting the strong genetic differentiation between predefined populations and the results from Tajima’s D, Fu and Li’s D, and Fu and Li’s D* neutrality tests demonstrating that all five loci did not deviate significantly from neutrality and are therefore suitable for coalescent analysis are included in Supplementary Material Table S2 (available online). We first estimated the best rooted population phylogeny by calculating the posterior probability distributions of topologies and hyperprior distributions for population rate parameters under a finite sites model. To ensure proper Markov chain mixing, we used a burn-in of 1 000 000 iterations prior to sampling and 256 heated chains with geometric heating (-ha0.97 and -hb0.80), according to the IMa3 documentation (Hey 2019). Estimates of effective population sizes (Nₑ), migration rates (2Nm) and population splitting times (t) were based on MCMC simulations of 50 000 sampled genealogies per locus using a fixed population topology. Population parameter estimation on demographic scales was based on a mutation rate of 1 × 10⁻⁹ per base per generation (Edwards & Rhodes 2021) and a generation time of 17 years (Richardson et al. 2013). Final parameter estimation was based on convergence of parameter distributions from at least two runs each with high swapping rates (> 0.9) between successive chains and high effective sample sizes (ESS > 10 000). Visualization of the direction of statistically significant migration events and confidence intervals for effective population sizes and splitting times was generated using the IMA3 program (https://github.com/jodyhey/IMA3). All runs were performed on CIPRES using program calls from the IMa3 and IMA3 workflow (https://tools.cifr.ncsu.edu/ima3) implemented in the DeCIFR toolkit (https://decifr.cifr.ncsu.edu/).

Sequencing type specimens
We extracted DNA and used PCR and Sanger sequencing to obtain the ITS region from the holotype of P. monticola (Vitikainen 1994a) following Magain et al. (2018). A similar attempt on the type of Peltigera ponojensis was unsuccessful. For the holotype of P. soredians, we were unable to amplify the entire ITS region, and instead separately amplified and sequenced the two spacers with primer pairs ITS1F-ITS2 and ITS3-ITS4 (Gardes & Bruns 1993; White et al. 1990). For lectotypes of P. antarctica and P. auribertii, DNA was extracted using the ClearYield™ kit from BioLink Laboratories (Washington, DC, USA) following the manufacturer’s instructions. Libraries (150 bp paired end) were prepared with the KAPA HyperPrep kit (Roche Sequencing Solutions, Pleasanton, CA, USA) following the manufacturer’s instructions and sequenced on an Illumina NovaSeq 6000 S Prime flow cell. The library preparation and sequencing were completed at the Duke Sequencing and Genomic Technologies core facility. We trimmed low-quality read ends (< Q20) using Trimomatic v. 0.39 (Bolger et al. 2014) and assembled the metagenomes using the –meta option in SPAdes v. 3.14.1 (Bankevich et al. 2012; Nurk et al. 2017) with kmer sizes 45, 65 and 85 bp. We then conducted a BLASTn search on the assembled metagenomic contigs using a 5.8S sequence from Peltigera pulverulenta (Taylor) Nyl. (GenBank OM349079). Finally, from each metagenome assembly, we extracted the contig that contained the BLAST hit to the 5.8S region and ran ITSx (Bengtsson-Palme et al. 2013) to delimit and assemble the ITS1, 5.8S and ITS2 regions. The species-level identity of the ITS sequences of the type specimens was established using BLASTn with the NCBI nucleotide database.


Results and Discussion
Our phylogeny of the P. ponojensis/monticola clade (Fig. 1C) largely agrees with the phylogenetic trees presented by Magain et al. (2018). Eighteen newly added ITS sequences cluster with the species delimited previously by Magain et al. (2018), whereas two individuals of P. ponojensis from Poland might represent a new lineage within the species complex. Peltigera globulata (Peltigera sp. 17 in Magain et al. 2018) forms a monophyletic group (BS = 100%) within a broader well-supported clade (BS = 85%). However, its precise phylogenetic placement within that clade remains uncertain (sister relationship with
P. ponojensis/monticola 4 received bootstrap support of 48%; Fig. 1C). With a few exceptions, the relationships among lineages within the large clade where P. globulata is placed (BS = 85% in Fig. 1C) are not well supported (see also Veas-Mattheos et al. 2023). The multiple IMa3 runs converged on the same topology (Fig. 2), which differs from the ML tree (Fig. 1C). However, the relationships among the lineages where P. globulata is placed are in general poorly supported and therefore unsettled. Overall, no significant gene flow was detected among the eight putative species. Low levels of gene flow were detected for the P. ponojensis clade from Poland and P. ponojensis/monticola 2 (Fig. 2). Among the eight populations (i.e. putative species) considered, the ancestral population of P. ponojensis/monticola 4 and P. globulata was inferred to be the oldest within this clade (Fig. 2). Based on the IMa3 results, the putative species delimited by Magain et al. (2018) across the entire P. ponojensis/monticola clade probably represent genetically isolated populations, and therefore could be recognized at the species level. However, many of these phylogenetic lineages were not well sampled and are in need of further investigation to better understand phenotypic and molecular variation across their geographical ranges. Peltigera globulata is an exception because of its unique and easily recognizable morphology (i.e. globulated margins) and narrow geographical distribution (i.e. currently reported only from Peru and Ecuador).

Most of the c. 50 delimited species in section Peltigera (Magain et al. 2018), even if morphologically cryptic, can be recognized using ITS1-HR (Miadlikowska et al. 2003; Magain et al. 2018). Unfortunately, this hypervariable region has low diagnostic value within the P. ponojensis/monticola species complex. Two main ITS1-HR patterns were detected within this species complex, characterized by a 13-base pair indel (Fig. 3). However, taxa with one or the other main sequence type do not form monophyletic groups, which could be the result of incomplete lineage sorting. Moreover, within the two main sequence types, similar or identical ITS1-HR sequences were detected across multiple taxa (see also Veas-Mattheos et al. 2023). For example, the same ITS1-HR sequence (44 nucleotides long) is shared between P. globulata and P. ponojensis/monticola 10a (Fig. 3; see also supplementary figure S2 of Magain et al. 2018).

Based on BLAST results (100% query cover; 99.66% similarity with P0073 from Austria, see Fig. 1C) of the ITS sequence from the Austrian holotype of P. monticola, we confirm that P. ponojensis/monticola 7 represents P. monticola s. str. as hypothesized by Magain et al. (2018). Note that Magain et al. (2018) inadvertently swapped the labels for P. ponojensis/monticola 7 and 9 in their
P. ponojensis/monticola clade

<table>
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<th>Species</th>
<th>Number of individuals</th>
<th>Number of putative species</th>
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<tr>
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<tr>
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<td>7</td>
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<td>7</td>
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P. rufescens clade

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<td>P. antarctica lectotype</td>
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Figure 3. ITS1 hypervariable region (ITS1-HR; positions 182–335 of the ITS1 alignment) from Peltigera globulata in comparison to the other phylogenetic lineages (i.e. putative species) within the P. ponojensis/monticola species complex. Also included are the ITS1-HR sequence from the lectotype specimen of P. antarctica and sequences for P. soredians, a morphologically similar, co-occurring sorediate species from the P. rufescens group. The number of individuals represented by each ITS1-HR sequence type within each species or putative species is shown in parentheses, whereas the numbers in square brackets represent the length of each ITS1-HR sequence type.

It remains uncertain which clade within the P. ponojensis/monticola complex represents P. ponojensis s. str. because our attempt to sequence the holotype (collected in Murmansk, Russia, in 1889, Kilhman 258, H!) was unsuccessful. There are four potential candidate clades with the P. ponojensis morphotype that have been collected in Europe (P. ponojensis/monticola 9, 6, 2, and the new lineage from Poland). Peltigera ponojensis/monticola 2 was suggested by Magain et al. (2018). The phylogenetic identity of P. plittii Gylen. (described from Colorado, USA), which might be conspecific with P. ponojensis (Vitikainen 1994a), also remains unresolved because we did not sequence the type specimen. North American specimens corresponding to P. plittii (P. ponojensis morphotype according to Vitikainen 1994a) were found to be affiliated with multiple putative species (see North American specimens in Fig. 1C).

Based on morphological description and the placement of specimens identified as P. antarctica (Dodge 1968; described from mosses in Antarctica), Magain et al. (2018) erroneously applied this name to a Neantarctic clade containing individuals predominantly from Chile corresponding to Peltigera sp. 23 in Veas-Mattheos et al. (2023) and P. ponojensis/monticola 11 in Fig. 1C. The ITS sequence of the lectotype specimen of P. antarctica blasts with 100% cover and 99% similarity to two accessions (OP602838 and OP602833) collected in the high Andean steppes of the Región de Aysén, Reserva Nacional Coyhaique in Chile, which were considered to potentially represent a new species (Peltigera sp. 24 in Veas-Mattheos et al. 2023) sister to P. rufescensiformis in P. rufescens clade, part of section Peltigera, but outside of the P. ponojensis/monticola clade (Fig. 1B). Based on the ITS sequence from lectotype material of P. aubertii, with 100% cover and similarity to specimen P2037 (MH758230) from Chile, we confirm that the name was correctly applied in Magain et al. (2018), based on morphology, to a clade sister to the semi-aquatic P. hydrophila W. R. Buck et al. (Fig. 1B). Both species have Holantarctic distributions and represent a lineage sister to the rest of section Peltigera (Magain et al. 2018). The ITS1 and ITS2 sequences from the holotype of P. soredians with 99% coverage was found to be 99% similar to P2151, a specimen collected in Ecuador (MH758348), which confirms that Magain et al. (2018) correctly applied this name to a lineage in the P. rufescens clade.

In addition to P. globulata, formal descriptions for P. ponojensis/monticola 10 (a and b) and 4 are in preparation. Specimens of P. ponojensis/monticola 10a and 10b have a unique P. degeni-like, glabrous morphology (they are often misidentified as P. degeni), and a distribution restricted to western North America (confirmed records from British Columbia, California and Utah). Molecular and morphological data
support their formal recognition as one species using the name *P. 'scotteri'* as proposed by Trevor Goward (Miadlikowska & Lutzoni 2000; Miadlikowska et al. 2003). *Peltigera ponojensis/monticola* 4 represents another well-defined morphotype with an etomentose thallus resembling *P. degenii* but with smaller and rather roundish and isolated lobes, growing on thick
The remaining putative species within the *P. ponojensis/monticola* species complex should be described later. Additional sampling is required to gain a better understanding of the variation in geographically widespread clades. For example, we still lack any sequence data from the *P. ponojensis/monticola* complex in Iceland, for which Vitikainen (1994a) reported matts of mosses in the Ozark region of the USA (Arkansas, Kansas, Missouri).

morphologically unusual specimens of *P. ponojensis*. Sequencing type material using a metagenomic approach has proved to be extremely helpful in resolving nomenclatural issues within the genus *Peltigera* (Magain et al. 2023), especially for old historical herbarium specimens that could not be sequenced using PCR and Sanger sequencing. Using this approach, existing species names can be applied with confidence to specific lineages (e.g. Leavitt et al. 2019), and therefore phylogenetic lineages that lack conclusive phenotypic characteristics (including chemistry and distribution) can, when appropriate, be described as novel species.

**Taxonomy**

*Peltigera globulata* Miadl. & Magain sp. nov.

MycoBank No.: MB 848772

Thallus margins disintegrating into irregular globules that are often covered with erect tumentum and white, flaky pruinina and therefore somewhat resembling the sorediate margins of *P. soredians*. Upper thallus surface pale to dark brown when dry, partly scabrid, partly tomentose and pruinose but never entirely grey in colour and thickly tomentose across the lobes as in *P. soredians*. Differs from *P. soredians* by the nucleotide sequence at positions 182–335 of the ITS1 hypervariable region (Fig. 3).

Type: Peru, Puno, Lampa, Santa Lucía, along Arequipa-Juliaca road, 12 km past Lagunillas, 15°38′58″S, 70°43′47″W, 4325 m, on thick layer of mosses along the road, 22 May 2012, F. Lutzoni s. n. [DNA extraction: P2165] (DUKE 0401811—holotype).

(Figs 4 & 5)

Thallus up to 7 cm diam., but often smaller, lobes narrow, elongated, 0.5–1.5 cm wide, with distinctly upturned, wavy (irregularly flexuose) margins. Margins uneven, partly split into globules, becoming flat or irregular in shape, often darker than thallus and brownish in colour, but covered with tomentum and whitish pruinina. Upper thallus beige to pale brown when dry, the surface structure varies: partly tomentose (short and less appressed toward the lobe tips), partly scabrid, partly glabrous, and partly covered with irregular powdery or coarse granular and flaky white pruinina, sometimes forming distinct white patches. Underside ochraceous pale with weakly defined, loosely angular and irregularly rigid venation; veins only slightly darker than interspaces, becoming brownish toward the thallus centre; interspaces in older parts of the thallus are often covered with whitish, loose and fluffy nets of hyphae; rhizines short, pale and almost simple and straight, or divided into multiple parallel hyphal bundles at the base in young marginal parts of the thallus, becoming pale brown or darker in colour, longer, fasciculate and fibrillose in shape, and more sparse towards thallus centre (difficult to separate from the substratum because often intermixed with thick mats of mosses). *Photobiont Nostoc* phyllogroup XXVIb (the most common photobiont for *P. globulata* from Peru; shared with *P. ponojensis*/*monticola* 6 and *P. laciniiata*), phyllogroup XXXIX (found in a single collection from Ecuador; shared with other species from section *Peltigera*) and two unique haplotypes (Supplementary Material Table S1, available online; Magain et al. 2018). Apothecia saddle-shaped, on narrow, extended lobes (only two were present on a single specimen). Because spore shape and size have very limited diagnostic value for the identification of *Peltigera* species, the apothecia were not cross-sectioned.

*Pycnidia* immersed in marginal globules but too old to make detailed observations.

**Chemistry.** No lichen secondary products were detected by TLC.

**Etymology.** The name refers to the irregularly globulated margins of thalli, a signature morphological feature of this species.

**Ecology.** Found on thick layers of mosses and plants on the ground and boulders or directly on the ground; mostly along road banks in rocky, extremely xeric, exposed areas of the high Andes (elev. 3400–4325 m).

**Distribution.** Known from South America only; collected from three localities in Peru (Puno) and a single locality in Ecuador.

**Notes.** *Peltigera globulata* resembles the overall thallus size and habit of *P. soredians*. Its globulate and thickly pruinose margins can be mistaken for sorediate margins of *P. soredians*, when examined with the naked eye. However, *P. soredians* differs by the presence of granulose, white, whitish grey soredia, and a greyish thallus colour when dry because of the persistent, thick whitish appressed tomentum similar to *P. laciniiata* (G. Merr.) Gyeln. (for a detailed description, see Vitikainen (1994b)).

Specimens of *P. soredians* were also observed that had a beige thallus colour when dry, and a less pronounced tomentum, which gives an areolate appearance to the thallus surface. In most specimens we examined, the overall underside of the thallus of *P. soredians*, in comparison with *P. globulata*, was paler in colour, the interspaces were more shallow and less defined, and the veins were covered with more dense rows of rhizines. Both species occur along road banks in the high Andes of Peru and Ecuador; however, currently *P. soredians* has a much broader ecology and distribution in Central and South America.

**Additional specimens examined (paratypes).** Ecuador: Pichincha: Pasochoa, Reserva de Vida Silvestre Pasochoa, trail Palma de Cera, 0°25′52″S, 78°30′45″W, 3400 m, open secondary forest, along the trail on ground covered by mosses, 2013, C. Truong 3976 [with apothecia, DNA extraction: P2195, TLC] (DUKE 0401864).—Peru: Puno: Lampa, Santa Lucía, along Arequipa-Julíaca road, 12 km past Lagunillas, 15°38′58″S, 70°43′47″W, 4325 m, on a thick layer of mosses along the road, 22 v 2012, J. Miadlikowska s. n. [DNA extraction: P1472] (DUKE 0401811 p.p.); ibid., on a thick layer of mosses and plant debris along the road, 2012, F. Lutzoni 05.22.2012-1 [DNA extraction: P1473] (DUKE 0357994); ibid., on soil along the road, 2012, J. Miadlikowska 05.24.2012-1 [DNA extraction: P1476] (DUKE 0357964); ibid., on thick layer of mosses and plant debris along the road, 2012, J. Miadlikowska & E. Rivas Plata 22.05.2012 [DNA extraction: P1477] (DUKE 0357964); along Arequipa-Julíaca road, 12 km past Lagunillas, 15°38′36″S, 70°43′51″W, 4317 m, on thick layer of mosses along the road, 2012, F. Lutzoni 05.22.2012-8 [DNA extraction: P1728] (DUKE 0401804); Azángaro, Santiago de Pupuja, along Juliaca-Azángaro road, 3 km past el poblado Mataro Chico, 15°43′37″S, 70°10′51″W, 3865 m, S exposure, on soil and mosses on boulders, 2012, J. Miadlikowska 05.24.2012 [DNA extraction: P2164] (DUKE 0357990).

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**Competing Interests.** The authors declare none.

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