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Standard Paper

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

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Abstract

Peltigera globulata Miadl. & 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).

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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* Gyeln. and *P. monticola* Vitik. (Vitikainen 1994*a*). *Peltigera monticola*, a southern European species with curled, and often phyllidiate, 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 veining patterns differ' according to the original description by Vitikainen (1994*a*); '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 1994*a*). *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 1994*a*). In the same article, Vitikainen indicated that

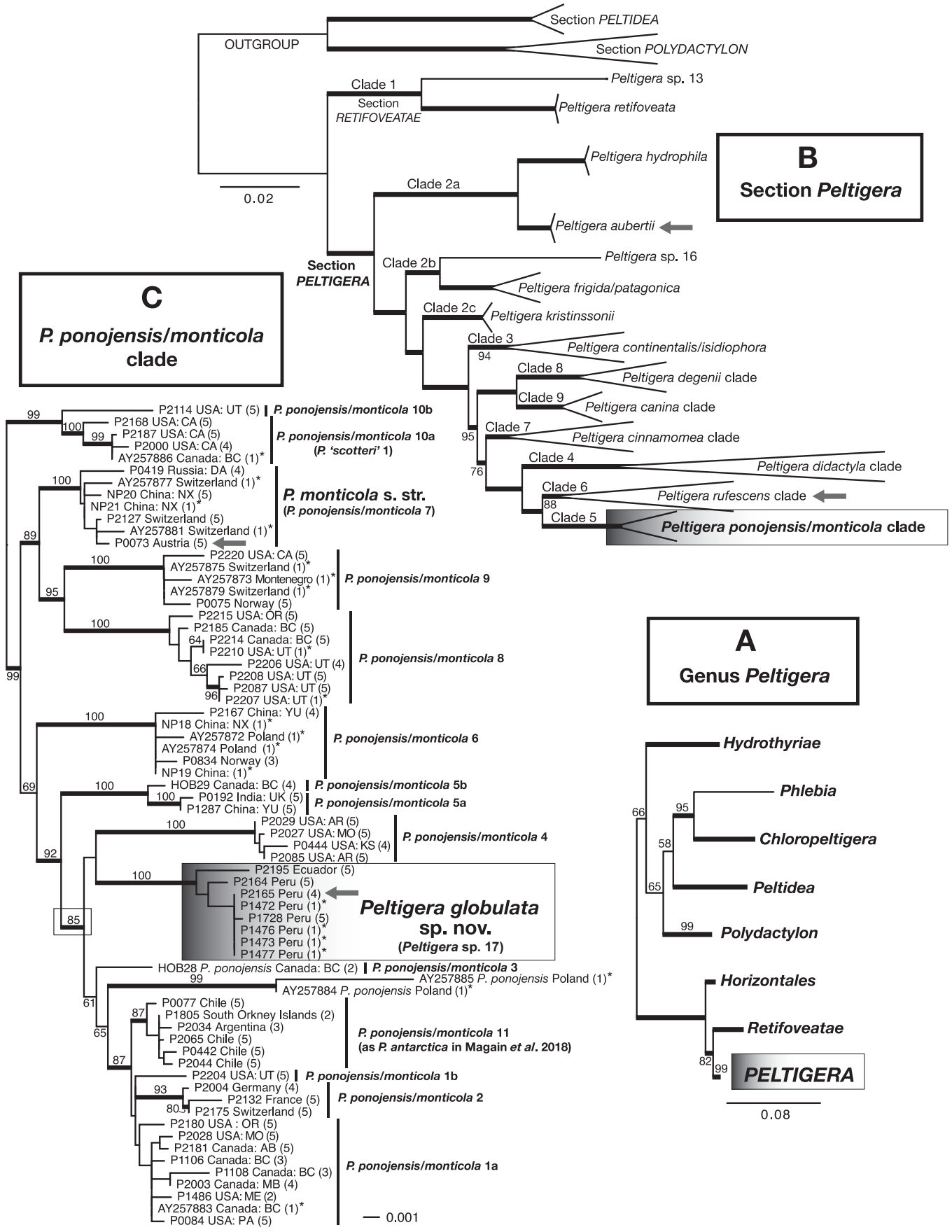

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Figure 1. Phylogenetic placement of *Peltigera globulata* sp. nov. within the genus *Peltigera* (A), section *Peltigera* (B), and *P. ponojensis/monticola* clade (C). For all trees, bootstrap support values (BS) > 50% are shown for each internode when space permits. Thickened branches represent bootstrap support (BS) values ≥ 70%, except for thick branches without support values in A and B, which indicate BS = 100%. Scales represent nucleotide substitutions per site. A, phylogeny of the genus *Peltigera* adapted from fig. 2 of Chagnon *et al.* (2019) depicting the relationships among the eight recognized sections of *Peltigera* established by Miadlikowska & Lutzoni (2000) based on maximum likelihood (ML) analysis of seven loci (ITS, nrLSU, β-tubulin, *RPB1*, COR1b, COR3, COR16). Each section is represented by a single terminal branch. B, phylogeny of sections *Retifoveatae* and *Peltigera* adapted from fig. 1 of Magain *et al.* (2018) depicting relationships within these sections based on ML analysis of five loci (ITS, β-tubulin, COR1b, COR3, COR16). Clades were collapsed using FigTree v. 1.4.3 (Rambaut 2012). Monophyletic groups that include more than two species are labelled 'clade' (e.g. *P. rufescens* clade) and the names of all included species are not listed. The top arrow indicates where the most similar ITS sequences based on BLASTn were found for the type specimen of *P. aubertii*. The other arrow, pointing to the *P. rufescens* clade, shows where the sequences with the highest similarity to sequences of the type material of *P. soredians* and *P. antarctica* were found based on BLASTn of ITS sequences. C, phylogenetic relationships within the *P. ponojensis/monticola* clade (Clade 5 of Magain *et al.* (2018)) as inferred in the present study. The tree was generated with RAxML using a 5-locus dataset (COR16, COR1b, COR3, ITS, β-tubulin; 3970 characters) for 68 taxa including eight representatives of *P. globulata*. The tree was rooted according to the phylogeny shown on fig. 1 of Magain *et al.* (2018). The number of loci included in the data matrix for each specimen is shown in parentheses. Asterisks indicate newly added specimens for which sequences were available in GenBank but were not included in the phylogenetic analyses of Magain *et al.* (2018). Species designation within the *P. ponojensis/monticola* clade follows fig. 1 of Magain *et al.* (2018). *Peltigera monticola* s. str. corresponds to *P. ponojensis/monticola* 7 of Magain *et al.* (2018) based on the high similarity (BLASTn) of the ITS sequence of the holotype (Austria) to the ITS sequence of a specimen of *P. monticola* P0073 (also from Austria) indicated by an arrow. *Peltigera ponojensis/monticola* 11 corresponds to *Peltigera antarctica* in Magain *et al.* (2018). However, based on the ITS sequence of the lectotype material, *P. antarctica* belongs to the *P. rufescens* clade (arrow in panel B). *Peltigera ponojensis/monticola* 10a corresponds to *P.* '*scotteri*' 1 in Miadlikowska *et al.* (2003). The holotype specimen of *P. globulata* (P2165) is indicated by an arrow. Further information about the sequences used in these analyses can be found in Supplementary Material Table S1 (available online).

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. degenii*-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 heterogenous and included morphotypes also identified as *P. rufescens* or *P. degenii* Gyeln., 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. aubertii* 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 00111756) 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

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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 GTRGAMMA 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 with Migration (IM) model implemented in IMa3 v. 1.11 (Hey *et al.* 2018). Population summary statistics and neutrality 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 F_{ST} 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 Monte Carlo (MCMC) 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 (N_e), migration rates ($2N_em$) 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 population phylogeny showing the direction of statistically significant migration events and confidence intervals for effective population sizes and splitting times was generated using the IMfig program (https://github.com/jodyhey/IMa3). All runs were performed on CIPRES using program calls from the IMa3 and IMfig 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 1994*a*) 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. aubertii*, 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 Trimmomatic 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.

Type specimens sequenced other than for P. globulata. *Peltigera antarctica* C. W. Dodge. **Antarctica:** *Melchior Archipelago*: Omega (Lystad) Island, 1941, *Siple, Frazier & Bailey* 330 (FH 00979245—lectotype, designated by Vitikainen (2002)). GB: OQ955290.

Peltigera aubertii C. W. Dodge. **Kerguelen:** *Grande Terre*: near Molloy point, on basalt escarpments, Butte aux Fougères, muscicola, 1952, *Aubert de la Rue* 55 (FH 00979244—lectotype, designated by Vitikainen (2002)). GB: OQ955289.

Peltigera monticola Vitik. **Austria:** *Tirol*: Stubaier Alpen, Mt Hammerspitze, 2600 m, on calcareous slates, 1973, *Vitikainen* 8884 (H 9500157—holotype). GB: OQ972013.

Peltigera soredians Vitik. **Peru:** *Cuzco*: Paucartambo, Paucartambo-Pillcopata road, just SW of Paso de Tres Cruces, 13°8′S, 71°38′W, 3450 m, 1981, *R. Santesson, A. Tehler & G. Thor* P96:25 (S L63027—holotype). GB: OQ972014.

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

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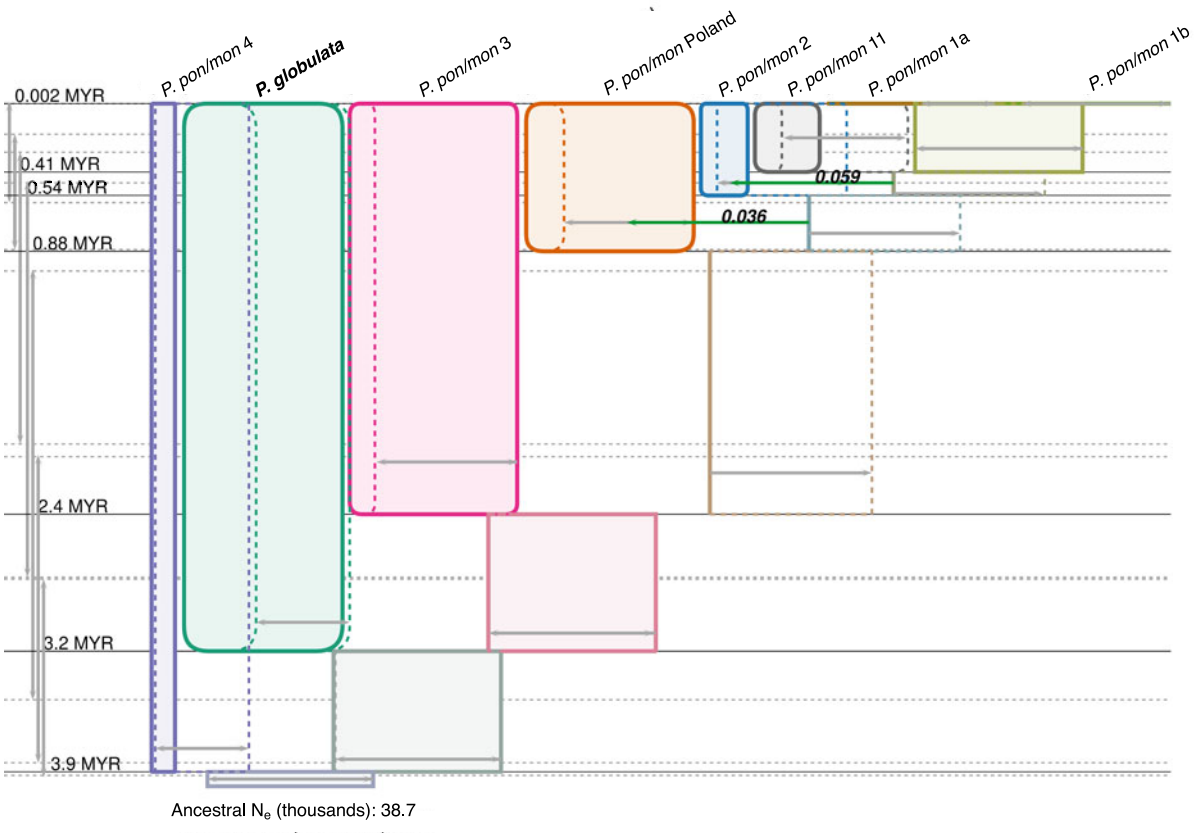

Figure 2. Results of the IMa3 analyses generated using the IMfig program (Hey 2019) showing no evidence of significant gene flow between the eight populations representing putative species within the clade containing *Peltigera globulata* (85% bootstrap support in Fig. 1) (*P. pon/mon* = *P. ponojensis/monticola*). The phylogeny is drawn as a hierarchical series of boxes, with ancestor boxes connecting descendent populations and the width of boxes proportional to the estimated effective population size (N_e). The vertical dashed lines to the right and left side of each population box are the 95% confidence intervals for each *Ne* value. Population splitting time (t) values in units of million years ago (MYR) are represented as solid horizontal lines and 95% confidence intervals for splitting times are shown as vertical grey arrows on the left, and parallel dashed lines. Migration arrows (in green) are the estimated migration rate ($2N_em$) values from one population into another over a shared time interval. The two green arrows indicate very low migration rates that are not statistically significant into the clades of *P. ponojensis/monticola* from Poland and *P. ponojensis/monticola* 2.

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

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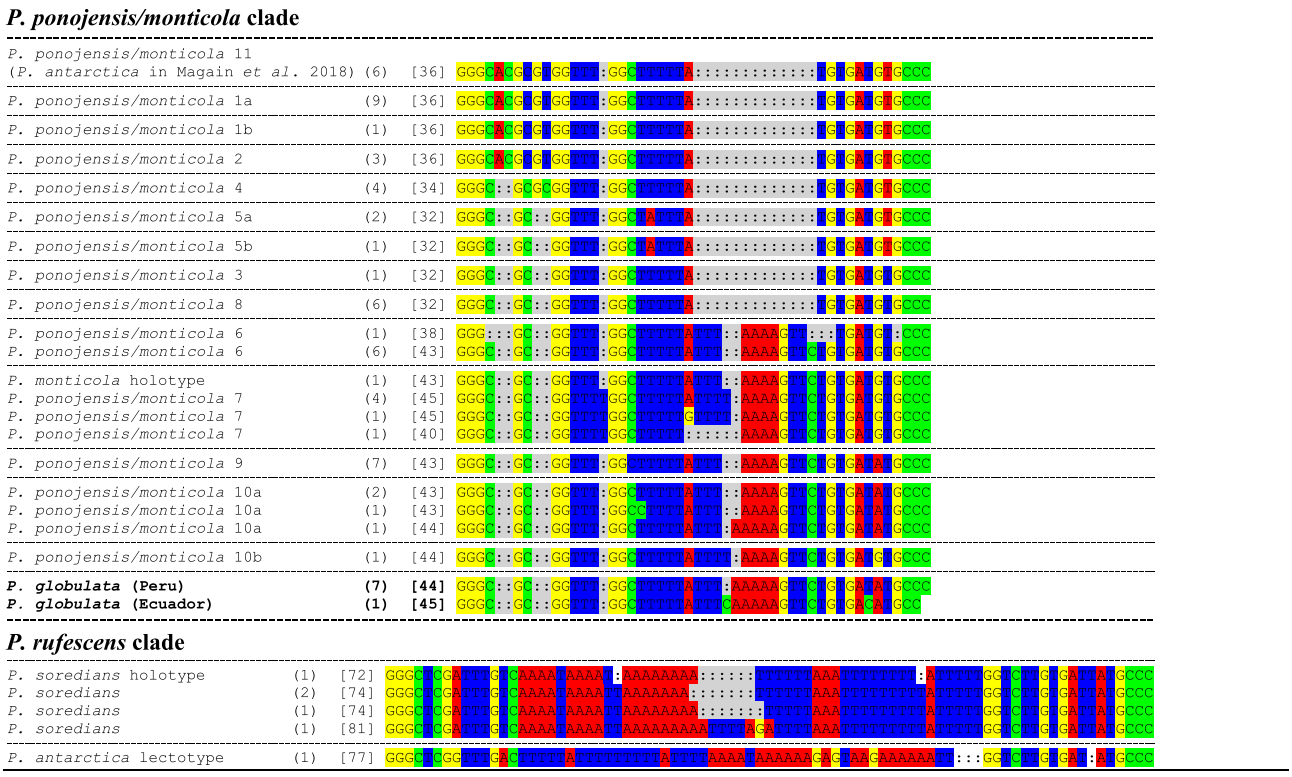

Figure 3. ITS1 hypervariable region (ITS1-HR; positions 182–335 of the ITS1 alignment) from *Peltigera globulata* in comparison to the other phylogenetic lineages (i.e. putative species) within the *P. ponojensis/monticola* species complex. Also included are the ITS1-HR sequence from the lectotype specimen 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.

fig. 2B, but here we follow the nomenclature of their fig. 1, the primary mycobiont phylogeny. As such, *P. monticola* s. str. has a broad geographical range, with scattered verified records (i.e. sequence data) from southern Europe (Austria and Switzerland), China (Ningxia Province), and Russia (Republic of Dagestan) (Fig. 1C), and possibly North America (Utah; GenBank Accession number MZ243915, with 99% similarity). However, the species is perhaps more common but overlooked (e.g. Miadlikowska (1999) reported it from several localities in Poland based on morphology).

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, *Kihlman* 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* Gyeln. (described from Colorado, USA), which might be conspecific with *P. ponojensis* (Vitikainen 1994*a*), also remains unresolved because we did not sequence the type specimen. North American specimens corresponding to *P. plittii* (*P. ponojensis* morphotype according to Vitikainen (1994*a*)) 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. rufescentiformis* 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. degenii*-like, glabrous morphology (they are often misidentified as *P. degenii*), and a distribution restricted to western North America (confirmed records from British Columbia, California and Utah). Molecular and morphological data

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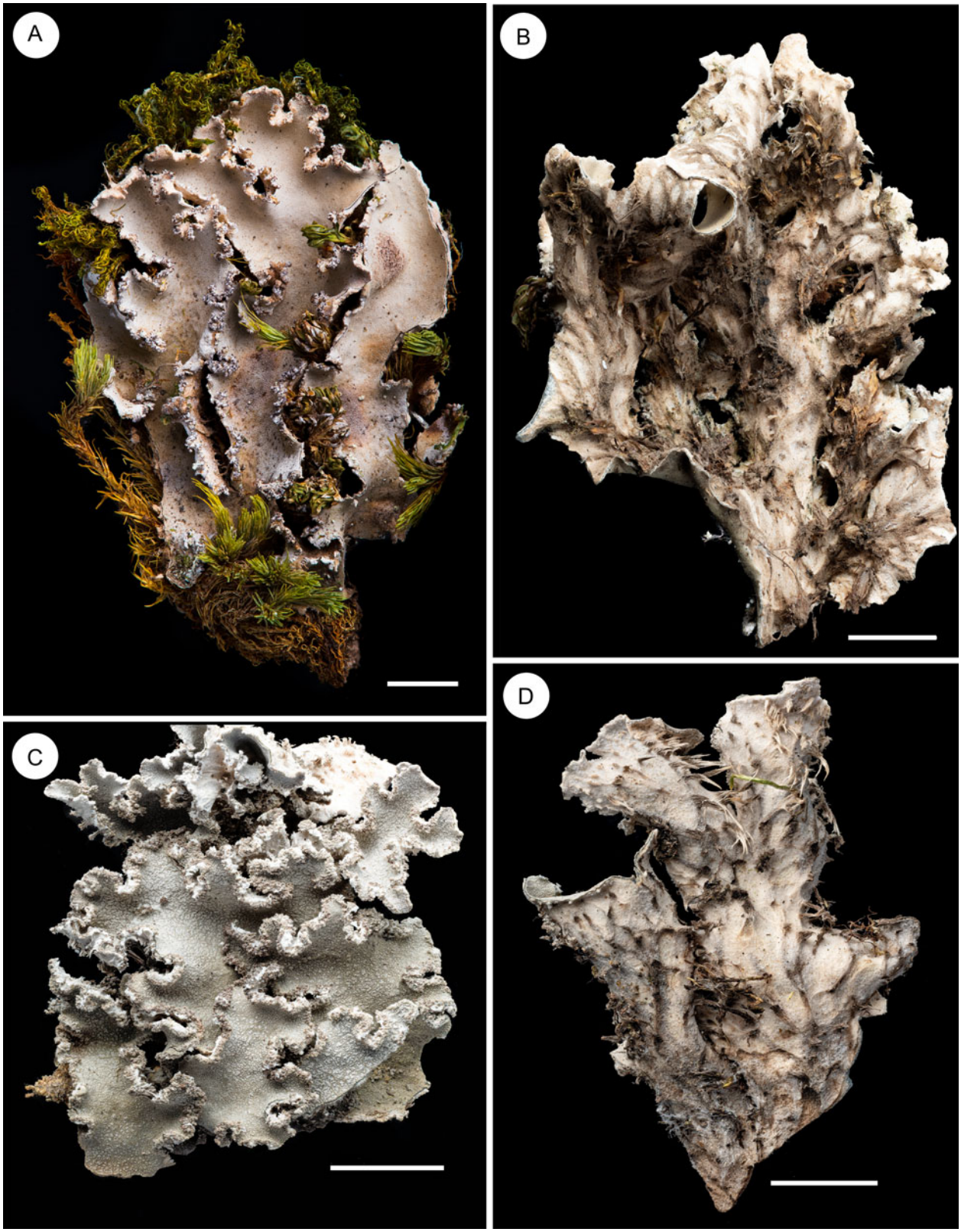

Figure 4. *Peltigera globulata* (P2165). A, thallus habit with globulate lobe margin, upper side. B, under side of the thallus. *Peltigera soredians* (P14480). C, thallus habit with sorediate lobe margin, upper side. D, under side of the thallus. Scales = 5 mm.

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

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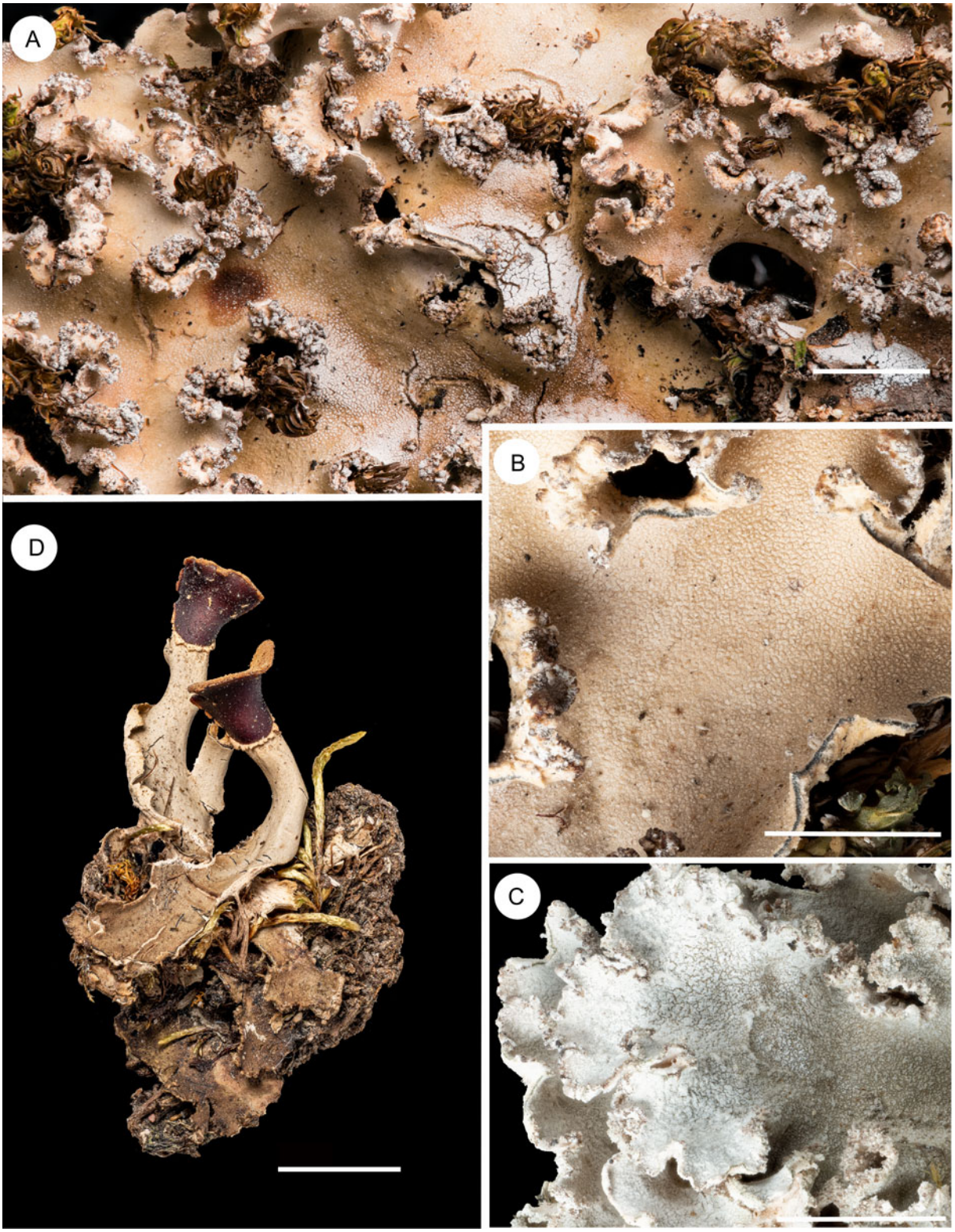

Figure 5. *Peltigera globulata*. A, lobes with patches of white pruina on the thallus surface and globulated margin (P2165–holotype). B, appressed tomentum resembling scabrose-like upper thallus surface, beige in colour when dry (P1477). C, *Peltigera soredians* (P14480), thickly tomentose upper thallus surface, pale grey to whitish when dry. D, *Peltigera globulata* (P2195) fertile lobes with saddle-shaped apothecia. Scales = 5 mm.

matts of mosses in the Ozark region of the USA (Arkansas, Kansas, Missouri).

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 (1994*a*) reported

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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 tomentum and white, flaky pruina 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 Lucia, 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 pruina. 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 pruina, 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* phylogroup XXVIb (the most common photobiont for *P. globulata* from Peru; shared with *P. ponojensis/monticola* 6 and *P. laciniata*), phylogroup 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, coarse, whitish grey soredia, and a greyish thallus colour when dry because of the persistent, thick whitish appressed tomentum similar to *P. laciniata* (G. Merr.) Gyeln. (for a detailed description, see Vitikainen (1994*b*)). 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 Lucia, along Arequipa-Juliaca 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 [DNA extraction: P1476] (DUKE 0357964); *ibid.*, 2012, on thick layer of mosses and plant debris along the road, *J. Miadlikowska* & *E. Rivas Plata* 22.05.2012 [DNA extraction: P1477] (DUKE 0357964); along Arequipa-Juliaca road, 12 km past Lagunillas, 15°38′56″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°4′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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