Peltigera hydrophila (Lecanoromycetes, Ascomycota), a new semi-aquatic cyanolichen species from Chile

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Abstract. Peltigera hydrophila, a new species from Chile tentatively distinguished based on phylogenetic evidence but not yet named, is formally described here. Morphological differences (e.g., non-tomentose thallus) and habitat preferences (semi-aquatic) corroborate molecular and phylogenetic distinctiveness of this early diverging lineage in section Peltigera. Due to overlapping ecological ranges, P. hydrophila shares some morphological traits with aquatic species from the phylogenetically unrelated section Hydrothyriace.

Key words: cyanolichen, cyanobiont, Nostoc, mycobiont, symbiosis, taxonomy

Introduction

The most recent multi-locus phylogenetic revision of sections Peltigera and Retifoveatae of the genus Peltigera (Fig. 1) suggested the presence of 88 species, of which 50 were new to science (Magain et al. 2018). Forty-nine of the newly delimited species are part of section Peltigera, which includes species with tomentose thalli (with some exceptions, e.g., P. frigida and P. degenetii) and lacking secondary metabolites detectable by thin layer chromatography (Miadlikowska & Lutzoni 2000; Magain et al. 2018). Most of these newly delimited putative species are restricted to a single biogeographic region, and hence few are widespread. In section Peltigera, specificity of Peltigera species (mycobionts) in their association with Nostoc phylogroups (cyanobionts) ranges from strict specialists (associating with only one Nostoc phylogroup) to broad generalists (up to eight Nostoc phylogroups), with widespread Peltigera species recruiting a broader selection of Nostoc phylogroups than species with limited distributions (Magain et al. 2018). One potentially new species, P. sp. 13, was found to belong to section Retifoveatae (Fig. 1B).

Two species – Peltigera sp. 14 and P. sp. 16 – were found to be part of the two earliest diverged lineages (clades 2a and 2b, respectively) within section Peltigera (Fig. 1B). Both species seem to be morphologically distinct and geographically restricted, however multiple collections from different localities are available only for P. sp. 14. Together with its sister species P. auberti, known from the Holantarctic Kingdom (Martínez et al. 2003), P. sp. 14, which is restricted to Chile, represents the first divergence event within section Peltigera (Fig. 1B). This species was recognized as potentially new to science by Miadlikowska et al. (2014; corresponding to ‘Peltigera sp. nov.’) in the phylogenetic context of the Lecanoromycetes. Peltigera sp. 14 is one of the rare cases in the genus Peltigera where both symbionts are specialists and associate almost exclusively with each other (Magain et al. 2017a, b, 2018; Miadlikowska et al. 2018; Pardo-De la Hoz et al. 2018; Chagnon et al. 2019). The mycobiont forms thalli with Nostoc phylogroup XXIII, which was also found in a single specimen of its closest relative, P. auberti, a species also found in Chile that otherwise associates with phylogroup XXII (Magain et al. 2018). Peltigera sp. 14 is the second water-associated lineage in the genus Peltigera. However, this species has a broader ecological amplitude than the distantly related North American species of section Hydrothyriace (Fig. 1A), as it can also grow in moist terrestrial habitats (e.g., on mosses subjected to waterfall splashes) compared to the strictly aquatic P. aquatica, P. hydrothyriace, and P. gowardii of section Hydrothyriace. Here, we formally describe P. hydrophila to accommodate Peltigera sp. 14, the non-tomentose, semi-aquatic new species in section Peltigera.
Materials and methods

Specimens were examined under a Leica MZ6 dissecting microscope. A total of 20 ascospores from three apothecia were measured using a Leica DMLB compound microscope. For the descriptions and use of terminology we followed Vitikainen (1994: 5–17). Thin layer chromatography (TLC) was performed on four specimens, using solvents A, B’ and C (Culberson & Ammann 1979; Culberson & Johnson 1982; solvent C is the same as solvent ‘TA’ of Holtan-Hartwig 1993). GenBank accession numbers for sequences from specimens P2039, P2062 and P1534 are provided in Magain et al. (2018), whereas the accession numbers for the remaining specimens are part of the voucher information provided below.

To determine the potential distribution of P. hydrophila, we used ecological niche modelling (ENM) techniques for the estimation of the habitat suitability for this species. The ENM was conducted using the maximum entropy method implemented in MaxEnt version 3.4.1 (Phillips et al. 2017, 2020) based on all known localities for P. hydrophila and all nineteen bioclimatic variables available in Worldclim 2.1 at 30 arc seconds (Phillips & Dudik 2008; Fick & Hijmans 2017). We chose to use MaxEnt because it has been shown to work well for modeling species with very few known records (Etich et al. 2006; Pearson et al. 2006; de Siqueira et al. 2009). Locality data were thinned with a 5 km radius rule to minimize spatial autocorrelation using the R package spThin (Aiello-Lammens et al. 2015), and background extent was limited by a point buffer of 15 degrees. Model tuning included a variety of models reconstructed with different feature classes [i.e., linear (L), linear – quadratic (LQ), linear – quadratic – hinge (LQH), and hinge (H)] and different regularization multipliers (1, 2, 3, and 4) implemented in dismo (Hijmans et al. 2017) and ENMeval packages (Muscarella et al. 2014). Preparation runs were performed using the package Wallace version 1.0.6.2 to optimize the run settings (Kass et al. 2018). The area under the ROC curve (AUC) for each model created was calculated to estimate the credibility of the analysis. AUC values were calculated automatically and the higher AUC was selected as a reliable indicator of performance (Phillips et al. 2009; Peterson et al. 2011). Raster files were prepared in R (R Core Team 2020) and all maps were edited in QGIS version 3.12.0 (QGIS Development Team 2020).
**Taxonomy**

*Peltigera hydrophila* W. R. Buck, Miadl. & Magain, sp. nov. (Figs 2–4)

MycoBank MB 834180

Diagnosis: Unique in its glabrous thallus upper surface, which becomes deep bluish-violet when wet; possessing somewhat phyllidiate and indented thallus margins; apothecia round, flat to convex, reddish-brown to dark brown. Occurring in very humid to aquatic habitats. Differs from other species in section *Peltigera*, including its sister species *P. aubertii*, by its unique ITS1 hyper-variable region (ITS1-HR in Miadlikowska et al. 2003; positions 182–335 of the ITS1 alignment in Magain et al. 2018) (Fig. 5).

Type: Chile, Región de Magallanes y Antártica Chilena, Prov. Antártica Chilena, Parque Nacional Cabo de Hornos, Isla Hermite, ENE arm of Seno Courtenay, head of the fjord, 54°34′33″S, 71°10′36″W, floodplain forest with *Nothofagus betuloides*, *N. antarctica*, *Drimys winteri*, shrubs, pools and liverwort carpet (“Enchanted Forest”), on small granitic stones in river, occasionally submerged, January 11, 2013, B. Shaw 17848 (DUKE 0401861 – holotype; NY – isotype; DNA extraction #P2039; GB ITS: MH758218).

**Description.** Thallus very small (to 4 cm in diameter), irregular and often partitioned, thin and fragile or rigid and brittle, tightly appressed to mosses and other substrates; lobes narrow, usually about 5 mm or less wide, occasionally up to 1 cm, often with laminal cracks, lobe margins lacerate and often phyllidiate, lobe tips crenulate and curled, indented. Upper surface glabrous, smooth and matte, sometimes bulged along veins; small laminal patches of pruina (white crystals of calcium oxalate) often present, pale to dark gray with a bluish hint, or brownish and becoming dark and almost greenish-black in aquatic specimens when dry, deep bluish-violet when wet, often maculate. Lower surface smooth, compact, neither fluffy nor arachnoid, ochraceous or whitish, lobe tips turning brown towards the thallus center. Veins smooth, compact, flat to slightly elevated and narrow and well defined in shape and color, to convex (ropy), usually pale (beige or ochre) towards lobe tips (sometimes brown from the tips especially under the fertile lobes), becoming brown and diffuse towards the thallus center, arranged in a parallel way along the lobes, weakly branched. Rhizines simple, smooth, composed of parallel conglutinated hyphae, not fluffy, often short, straight and pointed, to 1–2 mm long, arranged in short confluent rows along the veins, pale ochre towards the lobe tips and turning brown toward the center, or much longer and brown across the thallus. Apothecia always present, sometimes numerous, round or almost round, flat to convex, vertical to almost horizontal, reddish-brown to dark brown or black in some specimens, to 4 mm in diameter, developing on marginal parts of lobes, which are not always elongate, sometimes brown underneath. Ascospores *Peltigera*-type, mostly 3-septate, 37.5–55.0 × 3.5–6 μm.

**Photobiont.** *Nostoc* phylogroup XXIII (shared with one specimen of *P. aubertii* from Chile; Magain et al. 2018; GB rbcLX: MH770527, MH770528, MH770529), which gives the thallus a deep bluish-violet color when wet.
Figure 4. *Peltigera hydrophila*. A – fertile lobes (Reserva Nacional Mocho-Choshuenco, Chile, E. Terlova & L. Lewis s.n.); B – under surface of the fertile lobes (Reserva Nacional Mocho-Choshuenco, Chile, E. Terlova & L. Lewis s.n.); C – part of the thallus of the type specimen (Isla Hermite, Chile, B. Shaw 17848); D – under surface of the thallus of the type specimen (Isla Hermite, Chile, B. Shaw 17848). Photo credit: T. Barlow.
### Figure 5
Sequences of the ITS1 hypervariable region (ITS1-HR; positions 182–335 of the ITS1 alignment; Clade 2a, Magain et al. 2018) from *P. hydrophila* and its sister species *P. aubertii*. In square brackets, the number of individuals represented by each sequence type is shown before the slash and the length of the sequence is provided after the slash.

| Species          | Reps | Sequence Type | Length |
|------------------|------|---------------|--------|
| *P. hydrophila*  | 4/52 | GGGGAGACTATGGC| 193    |
| *P. hydrophila*  | 1/52 | GGGGAGACTATGGC| 193    |
| *P. hydrophila*  | 1/51 | GGGGAGACTATGGC| 193    |
| *P. aubertii*    | 2/50 | GGGGAGACTATGGC| 193    |
| *P. aubertii*    | 4/49 | GGGGAGACTATGGC| 193    |

### Figure 6
Currently known localities for *Peltigera hydrophila* (orange circles). A logistic projection (color gradient) shows the most suitable areas for the potential presence of this species.
No secondary metabolites were detected with TLC.

The name acknowledges the water-loving nature of this species.

On mossy rocks in semi-aquatic habitats (e.g., near waterfalls or along streams) or submerged, as well as on mosses and rocks in humid Nothofagus forests or shrubby and herbaceous vegetation in temperate areas of southern Chile.

This species is currently known from seven collections in Chile, extending from Región de Los Ríos and Región de Los Lagos to the southernmost tip of Región de Magallanes within most of the range of the temperate rainforest (Fig. 6).

Because of its semi-aquatic habitat, *P. hydrophila* resembles in some features, e.g., the placement and shape (flat to convex) of the apothecia and the compact underside of the thallus and veins, the morphology of species from section *Hydrothyriae*, which are always submerged in streams in North America (Miadlikowska et al. 2014). *Peltigera aubertii* (type material from Kerguelen Island; Vitikainen 2002) and *P. frigida* (type material from Tierra del Fuego; Vitikainen 2002), two other species from section *Peltigera* that also can have reddish-brown, round, and flat, apothecia, are relatively common in Argentina and Chile (Vitikainen 2002; Martínez et al. 2003; Quilhot et al. 2012; Nelson & Wheeler 2016), and are partially sympatric with *P. hydrophila*. However, the distribution of *P. frigida* does not extend north of Región de Los Ríos in Chile (Quilhot et al. 2012). The apothecia of *P. aubertii* and *P. frigida* (unlike *P. hydrophila*) are not convex, being mostly ‘horizontal’ in *P. frigida* (Fig. 7A), and vertical, finger-shaped, and often exceeding 4 mm in diameter in *P. aubertii* (Fig. 7C). Most importantly, the upper thallus surface of *P. hydrophila* is never tomentose like *P. aubertii* (Fig. 7B) nor glossy like *P. frigida* (Fig. 7A) (Vitikainen 2002). The ascospores are not discriminating among these three species – mostly 3-septate, similar in shape and size (*P. frigida*: 40–44 × 3.5–6.0 μm according to Vitikainen 2002; *P. aubertii*: 37.5–50 × 3.7–6.25 μm from this study). The only specimen of *P. aubertii* that shares Nostoc phylogroup XXIII with *P. hydrophila* consists of a few fertile lobes in rather poor condition and did not yield the same bluish-violet color of the wet thallus as observed in *P. hydrophila*. Four of the collections of *P. hydrophila* were found on mossy rocks occasionally submerged or under running water (i.e., small waterfalls or streams), but the remaining three were growing in very moist but non-aquatic conditions.

The range of *P. hydrophila* extends along the temperate macroclimate of southern Chile, including the wet coast lines of the antiboreal macroclimate (Luebert & Pliscoff 2017). Due to the continuity of the temperate macroclimate along continental Chile in the Aisén region,
it is expected that the large gap in its current geographical distribution (Fig. 6) results from the lack of collections, but it is also possible that the species has been overlooked or misidentified in the past (Quilhot et al. 2012). All models for the current distribution of *P. hydrophila* had AUCs > 0.90 after tuning. The chosen model complexity was LQH_1, with a resulting AUC value of 0.995. According to this result (Fig. 6), the area suitability for *P. hydrophila* (above 0.9) covers 16182.2 km², whereas at its minimum training presence (understood as the lowest predicted suitability value for a known occurrence point), it covers a total of 36840.7 km². The predicted distribution ranges from Región del Biobío to Región de Magallanes in Chile, with a few extensions across the Andes to Argentina, in the low valleys that allows the entrance of the Valdivian rain forest to the eastern slopes of the Andes Cordillera. In Región de Aisén the model suggests that *P. hydrophila* could be present in highly oceanic areas with high precipitation in the temperate hyperoceanic macroclimate (Luebert & Pliscoff 2017), with another high probability of occurrence in the boreal hyperoceanic macroclimate following the outermost islands of southern Chile and Argentina, including Tierra del Fuego and Staten Island (Isla de los Estados). Additionally, our model suggests that *P. hydrophila* could be present in the Falkland Islands (Islas Malvinas; Fig. 6), where a recent checklist (Fryday et al. 2019) indicates the presence of four *Peltigera* species, one of them being *P. auberti*. This distribution is not uncommon among temperate South American endemics (e.g., *Pseudocyphellaria vaccina* (= *Podostictina endochrysa*) and *Polychidium polychidioides*).

Another species representing one of the earliest divergences within section *Peltigera*, *P. sp. 16* (*P. ‘xerica’*) – sister to a clade consisting of *P. frigida* and *P. patagonica* – is the only representative from North America in clade 2b (USA, NE Oregon, Hart Mountain National Antelope Refuge, N of Hotsprings campground, 42°30′10″N, 110°41′25″W, alt. 1822 m, on soil among rhyolite outcrops in *Artemisia* steppe, June 2012, B. McCune 31966, McCune pers. herb., DNA extraction #P2186) (Fig. 1B; see also Magain et al. 2018). Morphologically, this putative species resembles the tomentose morphotype of *P. ponojensis*, but develops patches of cortex on fertile lobes under the relatively large apothecia, which become lacerated (Figs 8 and 9). The only specimen known of this taxon was collected from a very dry, high desert habitat.

*Peltigera* sp. 13 (*P. ‘inopinata’*), from China, is the second species known in section *Retifoveatae* (Fig. 1B) (Yunnan Province, Lijiang Prefecture, Lijiang Co. S of Lijiang, Jihue village, Laojunshan Mountain, at the border with Jianchuan Co., montane forest dominated by *Abies* and further up by *Rhododendron*, along trail from parking lot to the peak, at the base of tree, 26°39′13′′N, 99°46′35′′E, alt. ~3451 m, July 16, 2010, B. Goffinet 9974...
with L. Wang, S. L. Guo & S. Y. Huang s.n. (CONN), DNA extraction #P2225). Unlike its sister species P. retifoveata, it does not produce secondary compounds detectable by TLC and has a small, pitted, crenulate thallus with non-squarrosely branched rhizines (Fig. 10). For both P. ‘xerica’ and P. ‘inopinata’, we are awaiting further collections before formally describing them.

**Specimens examined.** CHILE. Región de Magallanes y Antártica Chilena, Antártica Chilena Province, Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, N shore of Isla Hoste, Caleta Colono, 55°06′22′′S, 69°49′06′′W, bryophyte-dominated rocky area in front of glacier, on wet horizontal rock among bryophytes, January 11, 2014, W. R. Buck 62274 (NY), DNA extraction #P1534; Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, E coast of Isla Hermite, Caleta Saint Martin, 55°51′35′′S, 67°34′23′′W, wet Nothofagus antarctica-Drimys forest adjacent granitic dome, submerged in small waterfall, January 19, 2005. W. R. Buck 47968 (NY), AFTOL-1838, DNA extraction #P1534; Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, Isla Grande de la Tierra del Fuego, immediately W of Glaciar Italia along the N side of the Brazo Noroeste of the Beagle Channel, 54°55′21′′S, 69°13′34′′W, subhydrous and herbaceous vegetation adjacent small waterfall on steep E-facing slope and adjacent granitic dome, submerged in small waterfall, January 19, 2005. W. R. Buck 62634 (NY), DNA extraction #P6152 (GB ITS: MN886242); Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, Isla Grande de la Tierra del Fuego, immediately E of Seno Puga, SE corner just E of large waterfall, 54°41′43′′S, 67°42′22′′W, alt. 1450 ft, on mineral soil, January 23, 2008, T. Wheeler & P. Nelson 5191 (CONC), DNA extraction #P2062. Región de Los Ríos, Reserva Nacional Mocho-Choshuenco, 39°56′2′′S, 72°6′21′′W, alt. 1172 m, primary Valdivian temperate Nothofagus forest, on mossy vertical boulder behind a small waterfall, March 14, 2017, E. Terlova & L. Lewis s.n., Chile-Reg. #5191.

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