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Peltigera chionophila, a New Lichen (Ascomycetes) from the Western Cordillera of North America

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Abstract. *The terricolous lichen* Peltigera chionophila *sp. nov. is described from the western cordillera of North America. It can be distinguished from other members of the* P. aphthosa *group by its even lobe margins, its uniformly corticate apothecial reverses, its well-defined veins that darken gradually toward the thallus centre, and by its strict occurrence in mountainous regions subject to heavy, prolonged snow cover. Comparisons of sequences of the Internal Transcriber Spacer of the nuclear ribosomal DNA repeat support the taxonomic distinctness of this species. A map of its global distribution is provided.*

Five species are currently recognized in the *Peltigera aphthosa* group (*sensu* Holtan-Hartwig 1993). Four of these occur in North America, i.e., *P. aphthosa* (L.) Willd., *P. britannica* (Gyelnik) Holt.-Hartw. & Tonsberg, *P. leucophlebia* (Nyl.) Gyelnik, and *P. malacea* (Ach.) Funk. *Peltigera malacea* is lichenized with a cyanobacterial photobiont (*Nostoc*), while *P. aphthosa, P. britannica,* and *P. leucophlebia* each have two photosynthesizing partners: a primary algal photobiont (*Coccomyxa*), present as a continuous layer beneath the upper cortex; and a secondary cyanobacterial photobiont (*Nostoc*), confined to small, scattered, laminal cephalodia. The fifth member of the *P. aphthosa* group, *P. nigripunctata* Bitter, is closely related to *P. leucophlebia*, but is restricted to southeast Asia (Vitikainen, pers. comm.).

Peltigera aphthosa, P. britannica, and *P. leucophlebia* are often considered ''difficult'' taxa, in which ''intergrading'' specimens are not uncommon. Reflecting this view, the *P. aphthosa* group has been subject to different taxonomic treatments at different times (for further discussion, see Vitikainen 1994). Most modern authors, however, recognize them as distinct species; and in fact, the vast majority of specimens can be reliably distinguished on morphology alone. Important points of separation include degree of venation, cortication of the apothecial reverses, lobe thickness, lobe outline, cephalodial habit, and field ecology (Goward et al. 1995).

In a recent synopsis of the genus *Peltigera* in British Columbia, Goward et al. (1995) recognized a sixth taxon in the *P. aphthosa* group, designated as ''*P.* sp. 1''. Further study has clearly revealed that this taxon warrants recognition as a distinct species. The objectives of this paper are to briefly summarize the results these studies, and to propose the necessary combination.

MATERIALS AND METHODS

Morphological studies.—Using traditional microscopic techniques, we examined approximately 500 North American specimens belonging to the *P. aphthosa* group. This material was made available on loan from ALA, ALTA, CANL, DUKE, OSU, TNFS, UBC, VC, and the personal herbaria of Stuart Harris, Roger Rosentreter, and Rupert Warren.

Chemical analysis.—Extraction of terpenoids and tridepsides of selected specimens was performed in warm hexane or acetone. Extracts were loaded on glass plates coated with silica gel (Merck F256) and chromatographed in solvents A, B′, and C following Culberson and Amman (1979) and Culberson and Johnson (1982), and solvent G as proposed by White and James (1985). Labelling of unidentified triterpenoids follows Vitikainen (1994). The following specimens were studied (UBC unless otherwise indicated): *P. aphthosa* (Goward 98-B–DUKE; Shishkoff 11-22#2–DUKE); *P. britannica* (C. Culberson 3.IX.1969–DUKE; Tonsberg 6521 & 7672–DUKE), *P. chionophila* (Goward 94-947, 95-15, 95-255, 95-519, 95-923, 95-1173, 96-494), *P. leucophlebia* (Eyerdam 1961–DUKE; Goward 98-A–DUKE, 98-C–DUKE, 98-41, 98-43; W. Culberson 22299-DUKE); *P. nigripunctata* Bitter (Park 1517–DUKE).

DNA extraction, amplification, and sequencing.—Lobe tips were removed from the selected specimens for DNA extraction (Table 1). Extraction protocol followed a modification of Doyle and Doyle (1987), as outlined in Goffinet et al. (1998). Amplification and sequencing of the ITS region follow the methods described in Goffinet and Miadlikowska (1999). Editing of the sequences obtained was performed using Sequencher 3.0 (Gene Codes Corporation). Alignment and distance analysis of the sequences was confirmed through comparisons with those obtained using fungus specific primers (Goffinet and Bayer 1997). Delimitation of the individual spacers and the 5.8S gene was determined by alignment against previously

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TABLE 1. Voucher information for collections of *Peltigera aphthosa, P. chionophila, P. leucophlebia,* and *P. malacea* included in the molecular analysis, and their corresponding GenBank accession numbers. Vouchers are deposited at UBC unless otherwise indicated. Sequences obtained by Goffinet and Bayer 1997 are marked by an asterisk.

Taxon	Voucher	GenBank accession number
P. aphthosa 1*	*Goffinet 3949-b* (hb. Goffinet)	U73492
P. aphthosa 1b‡	*Goffinet 3949-b* (hb. Goffinet)	AF158645
P. aphthosa 2*	*Goward 94-1018*	(= U73492)
P. aphthosa 3*	*Goward 92-319*	(= U73492)
P. britannica 1*	*Goward 92-276*	U73493
P. britannica 2	*Goward 94-946*	(= U73493)
P. britannica 3	*Tønsberg 72672*	AF158646
P. britannica 4	*Goward 98-B*	(= U73493)
P. chionophila 1	*Goward 96-494*	AF158647
P. chionophila 2	*Goward 95-255*	AF158648
P. chionophila 3	*Goward 94-947*	(= AF158648)
P. chionophila 4	*Goward 95-15*	AF158649
P. chionophila 5	*Goward 95-1173*	(= AF158649)
P. leucophlebia 1*	*Goffinet 3939-c* (hb. Goffinet)	U73494
P. leucophlebia 2*	*Goffinet 3747* (hb. Goffinet)	U73495
P. leucophlebia 3*	*Sérusiaux 10955* (hb. Goffinet)	U73496
P. leucophlebia 4	*Goward 98-A*	AF158650
P. leucophlebia 5	*Goward 96-97*	(= U73495)
P. leucophlebia 6	*Goward 98-C*	AF158651
*P. malacea**	*Goffinet 3984* (hb. Goffinet)	U73491

‡ Sequence obtained from same DNA extract as *P. aphthosa* 1, but using automated versus manual sequencing.

studied sequences in the *P. aphthosa* group (Goffinet and Bayer 1997). Representatives of distinct ITS-haplotypes were submitted to GenBank; a copy of the matrix can be obtained from the junior author. Absolute distance of divergence between sequences were used to construct a Neighbor-Joining tree using PAUP 4.0b2 (Swofford 1999), with gaps ignored in pairwise comparisons only.

RESULTS AND DISCUSSION

As earlier revealed in Table 1 of Goward et al. (1995), the chlorophyllous members of the *P. aphthosa* group form at least four distinct morphological units i.e., *P. aphthosa, P. britannica, P. leucophlebia,* and a fourth, undescribed entity. This last taxon can be distinguished as follows 1) from *P. aphthosa* by its distinct veins that darken only gradually toward the thallus center; 2) from *P. britannica* by its uniformly appressed cephalodia; 3) from *P. leucophlebia* by its continuously corticate apothecial reverses; and 4) from all of the above species by its restricted occurrence in sites subject to deep and prolonged snow packs.

Morphological differences are corroborated by divergences in the nucleotide sequences of the ITS region (Table 2). The alignment of ITS sequences used by Goffinet and Bayer (1997) was revised upon insertion of the sequences generated in the course of this study. For some portions of the sequences, automated sequencing yielded a more satisfactory resolution than manual sequencing (Fig. 2), which probably suffered from compressions. Alignment of sequences obtained from the fourth morphological entity required insertions at only two gapped sites (Fig. 2). Populations characteristic of sites with prolonged snow cover share nearly identical sequences, differing by 1, 3, or 4 point mutations (Table 3). When compared with chlorophyllous members of the *P. aphthosa* group, sequences of these populations more closely resemble those of *P. aphthosa* and *P. britannica* than those of *P. leucophlebia* (Fig. 1). In common with the first two species, the fourth taxon further differs from *P. leucophlebia* by the presence of several unambiguous indels (Fig. 2).

TABLE 2. Length variation (in nucleotides) of the ITS1, 5.8S, and ITS2 in *Peltigera aphthosa, P. britannica, P. chionophila, P. leucophlebia,* and *P. malacea* [values in parentheses are based on sequences obtained by manual sequencing; see Goffinet & Bayer (1997)].

Taxon	ITS1	5.8S	ITS2	Total
P. aphthosa	200	(156–) 159	197	(553–) 556
P. britannica	201	(156–) 159	197	(554–) 557
P. chionophila	206	159	198	563
P. leucophlebia	185	(154,155–) 160	208	(548–) 553
*P. malacea**	192	156	197	544

* Only one population studied.

TABLE 3. Absolute distances for alignable sequences of the ITS region (gaps ignored in pairwise comparisons) between all populations of *Peltigera aphthosa, P. britannica, P. chionophila, P. leucophlebia,* and *P. malacea.*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *P. malacea*	—														
2. *P. aphthosa* 1,2	16	—													
3. *P. aphthosa* 1b	15	1	—												
4. *P. aphthosa* 3	15	0	0	—											
5. *P. britannica* 1,2,4	16	4	3	3	—										
6. *P. britannica* 3	17	5	4	4	1	—									
7. *P. chionophila* 1	18	11	10	10	11	12	—								
8. *P. chionophila* 2,3	17	9	8	8	9	10	4	—							
9. *P. chionophila* 4,5	17	10	9	9	10	11	1	3	—						
10. *P. leucophlebia* 1	98	94	93	93	95	96	97	97	96	—					
11. *P. leucophlebia* 2	100	96	95	95	97	98	99	97	98	5	—				
12. *P. leucophlebia* 3	99	95	94	94	96	97	98	96	97	2	3	—			
13. *P. leucophlebia* 4	101	97	97	96	99	100	101	99	100	6	1	4	—		
14. *P. leucophlebia* 5	100	96	96	95	98	99	100	98	99	5	0	3	1	—	
15. *P. leucophlebia* 6	101	97	97	96	99	100	101	99	100	7	2	5	3	2	—

In contrast with other members of the *P. aphthosa* group, which are chemically highly variable, the fourth taxon varies little in its chemical constituents; it can be assigned to a single chemotype. Nevertheless, its chemical profile is identical to that of chemotype I of *P. aphthosa* (Holtan-Hartwig 1993), and also resembles that of *P. britannica.* In the latter species, however, trace occurrences of phlebic acid A are present (Vitikainen 1994). The fourth taxon is also chemically close to *P. nigripunctata,* though that species lacks phlebic acid B.

From the above findings, we conclude that the fourth taxon should be accorded formal taxonomic recognition as a distinct species.

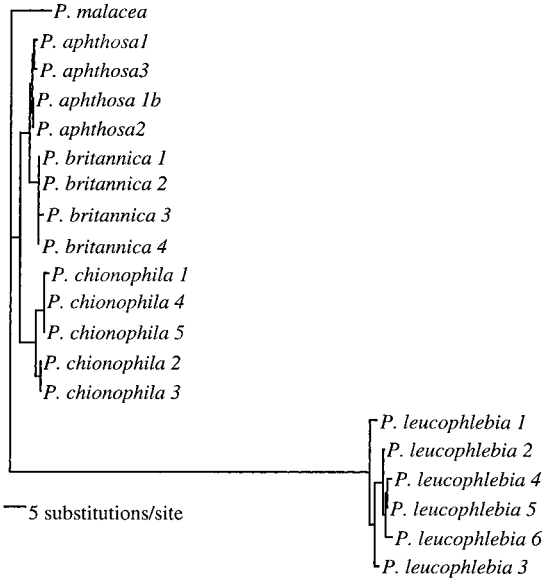

FIGURE 1. Neighbor-joining analysis of the ITS region (ITS1 + 5.8S + ITS2) of populations within the *Peltigera aphthosa* group, using absolute distances when gaps are excluded in pairwise comparisons of sequences.

PELTIGERA CHIONOPHILA Goward & Goffinet, *sp. nov.*

Thallus foliosus, *Peltigerae aphthosae* et *P. leucophlebiae* similis, sed venis conspicuis leniter atrantibus, necnon apotheciis uniformiter corticatis. Apothecia revoluta; sporae bacillari-aciculares, 54–78 × 3.0–4.5 μm, triseptatae. Terricola. Holotypus Goward 95–1173, in UBC.

Type: CANADA. BRITISH COLUMBIA. Clearwater River Basin, Trophy Mountains, 1,800 m, 51°46′N, 119°55′W, muscicolous over siliceous boulder, in open oldgrowth *Abies lasiocarpa-Picea engelmannii* forest, 8 September 1995, *Goward 95-1173* (UBC, Holotype; BM, CANL, H, DUKE).

Thallus foliose, loosely attached, large, 10–20(–30) cm across; lobes rather thin, stiff, to 2.5–4.0(–5.5) cm wide, rather short, loosely overlapping, irregularly branching; lobe tips rounded, mostly upturned; lobe margins even to weakly crisped; upper surface pale whitish green, dull, even to weakly billowed, glabrous or with minute glassy hairs near lobe tips, bearing scattered cephalodia, these pale grayish to medium brownish, to 0.5–1.0(–2.0) mm across, appressed throughout; soredia, isidia, and marginal lobules absent; lower surface norcorticate, veined; veins whitish or tan near margins, grading inward to dark brownish (gradation generally gradual), distinct, raised, non-tomentose; interstices whitish, mostly lenticular to ellipsoid; rhizines dark brownish throughout, or occasionally pale near margins, simple and discrete near lobe margins, becoming penicillate and confluent toward thallus center. Cortex 45–60 μm thick; primary photobiont layer 40–50 μm thick, containing *Coccomyxa*; cephalodia appressed throughout, containing *Nostoc*; medulla white, 90–300 μm thick. Cyanomorph not observed. Apothecia frequent, erect, located on narrow, elongate, marginal lobes; disc medium brown, vertically and often also apically reflexed at maturity (i.e., ''saddle-shaped''), to less than one cm

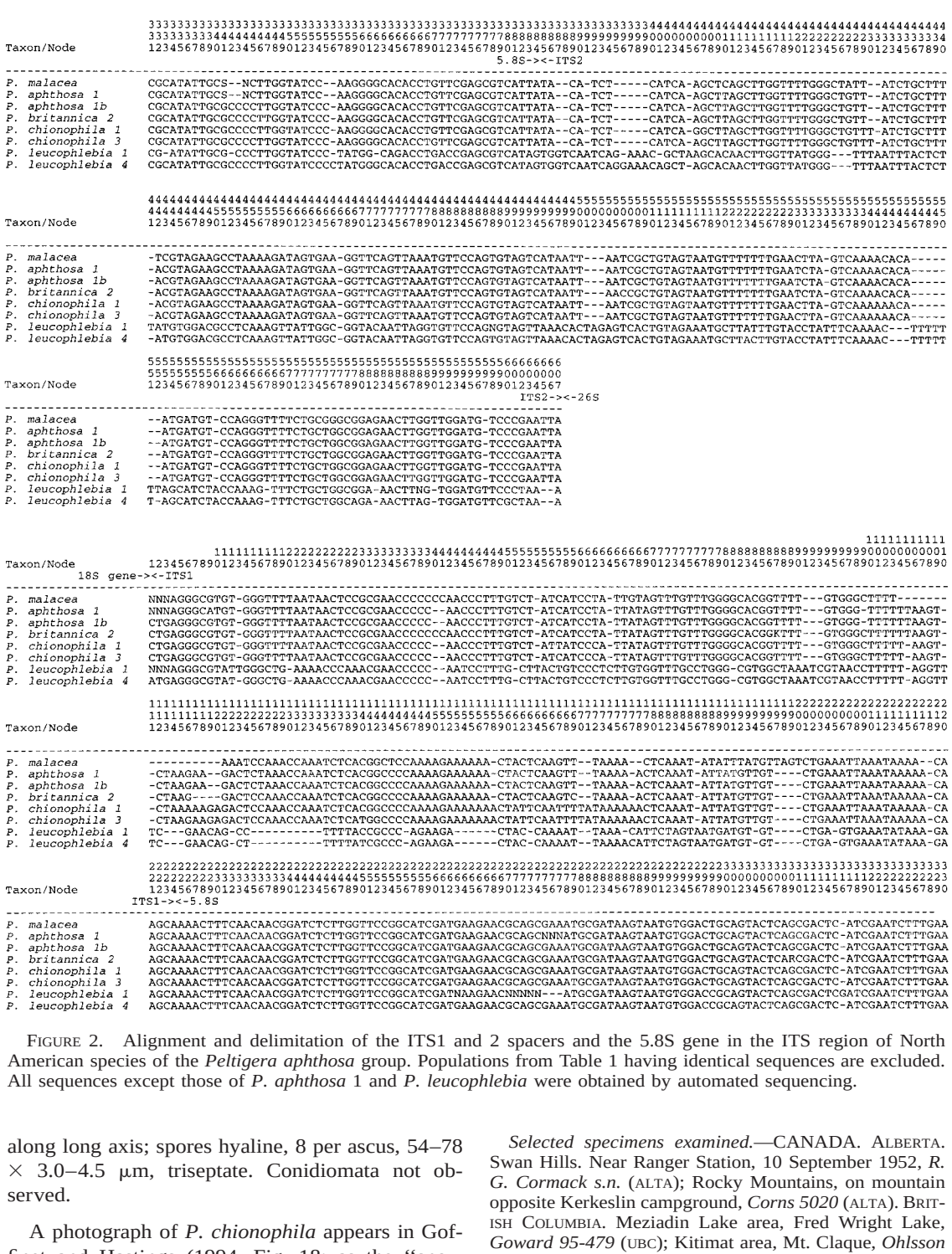

FIGURE 2. Alignment and delimitation of the ITS1 and 2 spacers and the 5.8S gene in the ITS region of North American species of the *Peltigera aphthosa* group. Populations from Table 1 having identical sequences are excluded. All sequences except those of *P. aphthosa* 1 and *P. leucophlebia* were obtained by automated sequencing.

along long axis; spores hyaline, 8 per ascus, 54–78 × 3.0–4.5 μm, triseptate. Conidiomata not observed.

A photograph of *P. chionophila* appears in Goffinet and Hastings (1994, Fig. 18: as the ''snow form'' of *P. aphthosa*).

Chemistry.—Tenuiorin, methyl gyrophorate, phlebic acid B, zeorin (trace amounts), and one unidentified terpenoid corresponding to compound 14 sensu Holtan-Hartwig (1993) or pbr-1 *sensu* Vitikainen (1994).

Selected specimens examined.—CANADA. ALBERTA. Swan Hills. Near Ranger Station, 10 September 1952, *R. G. Cormack s.n.* (ALTA); Rocky Mountains, on mountain opposite Kerkeslin campground, *Corns 5020* (ALTA). BRITISH COLUMBIA. Meziadin Lake area, Fred Wright Lake, *Goward 95-479* (UBC); Kitimat area, Mt. Claque, *Ohlsson 2771* (UBC); Wells Gray Provincial Park, Upper Azure River, *Goward 84-1026* (UBC); Revelstoke National Park, near Eva Lake, *Otto 3052* (UBC). U.S.A. ALASKA. Central Pacific Coast District, Bear Glacier, *Viereck 2111* (ALA); Baranof Island, Harbor Mountain 16 July 1991, *C. Derr. s.n.* (TNFS 1180); Stikine area, Thomas Bay, 31 July 1991, *Wilder s.n.* (TNFS 403). WASHINGTON. Okanogan County, Chopaka Mountain, *Douglas 4497* (ALTA).

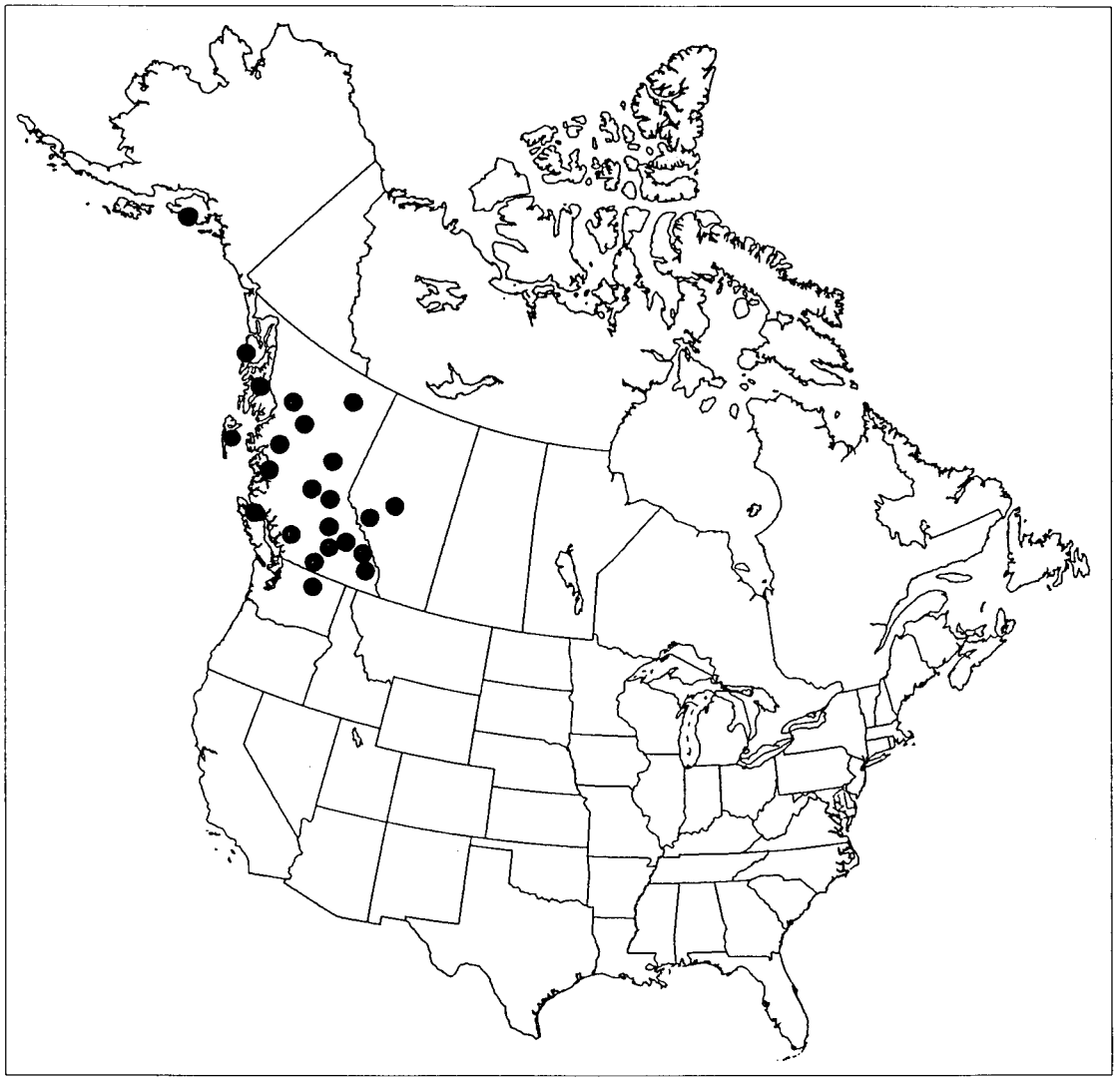

FIGURE 3. The world distribution of *Peltigera chionophila.*

The name *chionophila* derives from the Greek ''chion'' (snow) and ''philos'' (loving), in reference to the habitat ecology of the species.

Relationships.—In its apothecial features as well as in the habit of its lobe margins, *P. chionophila* is morphologically similar to *P. aphthosa* and *P. britannica.* At the same time, its production of distinct veins that darken only gradually toward the thallus center is reminiscent of *P. leucophlebia.* Comparisons of ITS sequences support a relationship with the first two species, but not with the last species (Fig. 1). Indeed, *P. chionophila* shares with *P. aphthosa* and *P. britannica* many of the indels that distinguish these species from *P. leucophlebia* (Fig. 2). At the same time, however, the degree of molecular differentiation inferred from its ITS sequences is greater than that reported between *P. aphthosa* and *P. britannica* (Goffinet and Bayer 1997; see also Table 3), and is comparable to that observed between species of the *P. neckeri* group (Goffinet & Miadlikowska 1999).

When well developed and fertile, *P. chionophila* is readily distinguished from related species on the basis of several morphological and chemical characters (see Table 1 in Goward et al. 1995). Sterile specimens can be more difficult to identify, and are often confused especially with *P. leucophlebia.* That species, however, has distinctly crisped lobe margins and a much broader ecological amplitude (Goward et al. 1995).

Distribution and ecology.—Peltigera chionophila appears to be restricted to northwestern North

America, where it occurs from coastal Alaska southward through British Columbia to northern Washington (Fig. 3). Throughout its range, it inhabits mossy sites in open to somewhat sheltered forests. Unlike other members of the *P. aphthosa* group, it is entirely restricted to mountainous regions, especially at elevations above about 1,000 m, where snow often persists late into the year. Indeed, it has been observed emerging from receding snowbanks in late June or, in one case, early July (Goward, pers. obs.). Such a pronounced tolerance for prolonged snow cover is unusual among macrolichens (Goward & Ahti 1992), but is obviously shared at least by *Cladonia ecmocyna* Leighton, *s.lat.*, with which *P. chionophila* often co-occurs. Within the *P. aphthosa* group, only *P. aphthosa s.str.* regularly inhabits similar sites; and in a few instances that species has been found growing intermixed with *P. chionophila.* When occurring in snowy sites, however, *P. aphthosa* is typically confined to elevated substrates (e.g., mossy logs and boulders), whereas *P. chionophila* is generally restricted to shallow depressions.

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