THE BRYOLOGIST

A JOURNAL OF BRYOLOGY AND LICHENOLOGY

VOLUME 106 FALL 2003 NUMBER 3

The Bryologist 106(3), pp. 349–364

Phylogenetic Inferences Based on nrDNA Sequences Support Five Morphospecies Within the *Peltigera didactyla* Complex (Lichenized Ascomycota)

BERNARD GOFFINET
Department of Ecology and Evolutionary Biology, 75 North Eagleville Road, University of Connecticut, Storrs CT 06268-3043, U.S.A. e-mail: goffinet@uconn.edu

JOLANTA MIADLIKOWSKA
Department of Biology, Duke University, Box 90338, Durham NC 27708, U.S.A. and Department of Plant Taxonomy and Nature Conservation, Gdansk University, Al. Legionow 9, 80-441 Gdansk, Poland; e-mail: jolantam@duke.edu

TREVOR GOWARD
Herbarium, Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 2B1, Canada. Mailing address: Edgewood Blue, Box 131, Clearwater, British Columbia V0E 1N0, Canada

Abstract. *The* Peltigera didactyla *complex comprises species of section* Peltigera *with laminal and submarginal soredia. Three species (*P. didactyla, P. lambinonii, *and* P. ulcerata*) and one atypical variety (*P. didactyla *var.* extenuata*) are currently recognized within this complex. Phylogenetic inferences of the entire Internal Transcribed Spacer region (ITS) and the 5′ half of the gene encoding the large subunit of the rRNA reveal a robust structure within the complex. Under both the maximum parsimony and the maximum likelihood criterion,* P. didactyla *is resolved as a polyphyletic entity, whereas* P. ulcerata, P. lambinonii, *and* P. didactyla *var.* extenuata *are delimited as monophyletic entities.* Peltigera didactyla *var.* extenuata *appears basal within the group, whereas var.* didactyla *is nested within a clade that also comprises* P. lambinonii *and* P. ulcerata. *The polyphyly of* P. didactyla *is further characterized by the existence of populations that resemble var.* extenuata, *but differ by their brownish upper cortex. These populations, all from the boreal zone of Canada, compose a monophyletic group sister to the* P. didactyla-P. lambinonii *clade. For* P. didactyla *to satisfy a phylogenetic species concept, the var.* extenuata *is reinstated at the species level, and a new species,* P. castanea, *is described. Three populations sampled are characterized by unique sequences that may indicate the presence of additional cryptic taxa within the complex. A key to the accepted species is provided. The presence of* P. lambinonii *in Australia is confirmed and* P. ulcerata *is reported as new for Chile.*

Lichenized fungi of the genus *Peltigera* Willd. form foliose thalli with either a green alga or a cyanobacterium, or both. Although some species are rather small (monophyllous thalli less than one cm in diameter), most species are conspicuous, with thalli reaching 20 or more cm in width. The genus is notorious for its taxonomic challenges. Species are essentially defined by a combination of characters and rarely by distinct unique traits. Taxonomically important anatomical characters are few, and most had been much overlooked until recent critical studies (Holtan-Hartwig 1993; Martínez Moreno 1999; Miadlikowska & Lutzoni 2000). Most morphological characters seem to cover broad ranges of expression. Early workers accommodated many of the extreme forms as new taxa, but as regional monographs progressed, students faced with populations covering much of the morpholog-

0007-2745/03/$1.75/0

ical gradient preferred recognizing few broadly defined species (Thomson 2003) with some of the variation partitioned among infraspecific taxa (Lambinon 1969; Ozenda & Clauzade 1970; Thomson 1950).

As is the case of many lichenized fungi, *Peltigera* is characterized by a diversity of secondary metabolites that accumulate on the outer surface of the hyphae. Critical study of these chemicals, mainly tridepsides or triterpenoids, offered new characters. The presence of some compounds appeared correlated with that of certain morphological characters [e.g., *P. polydactylon* (Neck.) Hoffm. *sensu lato*; White & James 1985], but such correlations between chemotype and morphotype are often more difficult to discern [e.g., *P. aphthosa* (L.) Willd. complex; Holtan-Hartwig 1993].

The lack of extracellular secondary metabolites has been considered a characteristic of the *P. canina* group (Vitikainen 1994). Although the recently described *P. retifoveata* Vitikainen (Vitikainen 1985), a species characterized by the production of several triterpenoids and tridepsides, has recently been excluded from the *P. canina* group (Holtan-Hartwig 1993; Miadlikowska & Lutzoni 2000), this complex remains heterogenous in terms of its chemical composition. Indeed, some populations of the sorediate species *P. didactyla* (With.) Laund. *sensu lato* are known to produce tridepsides, especially if not exclusively, in the soralia. Whereas Swinscow and Krog (1988) regarded the occasional occurrence of these compounds as taxonomically insignificant, Goffinet and Hastings (1995) found a nearly perfect correlation of the presence of these compounds and a discrete combination of morphological characters. These authors recognized paleotropical thalli with dark brown mat-forming rhizines and with gyrophoric acid and methylgyrophorate in the soralia as a new species, *P. lambinonii* Goffinet. In the Northern Hemisphere, the presence of chemicals seemed correlated with broader lobes with abundantly branched whitish rhizines, and a mesophytic habitat. To accommodate this taxon they resurrected var. *extenuata* (Vainio) against the typical and sympatric variety of *P. didactyla*. Their taxonomic concept soon gained wide acceptance (e.g., Berger 1996; Bergsten 1999; Esslinger 1997; Halonen et al. 2000; Martínez et al. 1997; Martínez Moreno 1999; van den Boom 1998), although skepticism prevailed at first (Vitikainen 1994), probably owing to the existence of occasional specimens with the morphology of one taxon but the chemistry of the other. Goffinet and Hastings (1995) had tentatively attributed such oddities to hybridization or thallus fusion, though no formal evidence was presented (see also Miadlikowska et al. 2003).

A recent phylogenetic study of the genus *Peltigera* by Miadlikowska and Lutzoni (2000) based on combined molecular, morphological, and chemical data showed that *P. lambinonii*, *P. didactyla s.str.*, and *P. didactyla* var. *extenuata* form well supported monophyletic groups within section *Peltigera*. More recently based on the combined large subunit (LSU), the entire ITS region of the nuclear ribosomal DNA and coded characters (INAASE and the ITS1 hypervariable region), Miadlikowska et al. (2003) suggested that individuals of var. *extenuata* form a monophyletic group, non-sister to populations assigned to var. *didactyla*. They further found that populations of var. *extenuata* were placed outside of a monophyletic group comprising var. *didactyla*, *P. lambinonii*, and even *P. ulcerata*, a species restricted to temperate climate of tropical and austral regions. All studied morphospecies, phylogenetically defined as monophyletic, were also characterized by unique sequences of a hypervariable ITS1 region (Miadlikowska et al. 2003). These observations led these authors to suggest that var. *extenuata* deserves to be recognized at the species level.

In the present study we test taxonomic and phylogenetic hypotheses within the *P. didactyla* complex, by sampling a 1.4-kb fragment at the 5′ end of the nuclear large subunit rDNA (LSU rDNA) and the entire intergenic transcribed spacer (ITS1, 5.8S, and ITS2). Specifically we tested 1) whether the phylogenetic hypothesis by Miadlikowska et al. (2003) regarding the affinities of *P. didactyla* var. *extenuata* would withstand additional sampling ; 2) whether a morphological species concept as applied to apotypic populations from Canada would also withstand a phylogenetic criterion; and 3) whether the additional sampling within the *P. didactyla* group would affect a species-specific pattern of variation found in the ITS1 hypervariable region (ITS1-HR; Miadlikowska et al. 2003).

Material and Methods

Morphological study.—Two hundred and fourteen collections of *P. didactyla sensu lato* currently held at the Herbarium of the National Nature Museum of Canada and about 25 additional specimens collected by TG in British Columbia (UBC) were examined for their morphological characters.

Chemical analysis.—All examined specimens were tested for the presence of gyrophoric acid using the standard C test. Additional investigation relied on thin layer chromatography following the method developed by Culberson (1972) and White and James (1985), whereby acetone extracts are eluted in solvent G (toluene-ethyl acetate-formic acid in proportion 139:83:8) and C (toluene-acetic acid in proportion 100:15).

Taxon sampling for DNA analysis.—For the phylogenetic analyses we selected 29 individuals from seven *Peltigera* taxa (Table 1). Twenty-six specimens represented three recognized species (*P. didactyla*, *P. lambinonii*, and *P. ulcerata*, including one intraspecific taxon–*P. didactyla*

TABLE 1. Voucher specimen information and GenBank accession numbers for 25 LSU and 29 ITS nrDNA sequences included in this study. * = outgroup specimens; [ ] = specimen identification based on morphological species concept; ( ) = sequences obtained from GenBank (Miadlikowska & Lutzoni 2000; Miadlikowska et al. 2003); all other sequences were generated by this study.

Taxon	Voucher	GenBank accession number	
		LSU nrDNA	ITS nrDNA
Peltigera castanea 1	Canada, BC, *Goward 94-548* (UBC)	AY266020	AY266019
P. castanea 2	Canada, BC, *Goward 01-858* (UBC)	AY266022	AY266021
P. castanea 3	Canada, BC, *Goward 99-41a* (UBC)	AY266024	AY266023
P. castanea 4	Canada, BC, *Goward 95-35* (UBC)	AY266026	AY266025
P. didactyla 1	Poland, *Miadlikowska 5233* (UGDA-L)	(AF286806)	(AY257929)
P. didactyla 2	Canada, BC, *Goward 01-635* (UBC)	AY266028	AY266027
P. didactyla 3	Brazil, *Marcelli, Ahti & Yano 28333* (H)	(AY257932)	(AY257931)
P. didactyla 4	Canada, BC, *Goward 97-129* (UBC)	AY266030	AY266029
P. extenuata 1	Poland, *Faltynowicz & Miadlikowska 5235* (UGDA-L)	(AF286809)	(AY257937)
P. extenuata 2	Poland, *Cieslinski 1296* (KTC)	(AF286808)	(AY257938)
P. extenuata 3	Poland, *Butkus 5236* (UGDA-L)	(AF286810)	(AY257939)
P. extenuata 4	Canada, BC, *Goffinet & Goward 97-289* (UBC)	(AY257941)	(AY257940)
P. extenuata 5	Canada, BC, *Goward 98-56* (UBC)	AY266032	AY266031
P. extenuata 6	Canada, BC, *Goward 01-869* (UBC)	AY266034	AY266033
P. extenuata 7	Canada, BC, *Goward 01-653* (UBC)	AY266036	AY266035
P. lambinonii 1	Australia, *Tibell 12401* (H)	(AF286803)	(AY257933)
P. lambinonii 2	Zaire, *Lambinon 72/Z/I02* (LG)	—	(AY257934)
P. lambinonii 3	Rwanda, *Lambinon 74/788* (LG)	—	(AY257934)
P. lambinonii 4	Rwanda, *Lambinon 72/Rw/500* (LG)	—	AY266037
P. lambinonii 5	Zaire, *Lambinon 71/Z/1111* (HERB. GOFFINET)	—	(AY257936)
P. ulcerata 1	Chile, *Goffinet 7126* (HERB. GOFFINET)	AY266039	AY266038
P. ulcerata 2	Rwanda, *Lambinon 71/Rw/1050* (HERB. GOFFINET)	AY266041	AY266040
P. ulcerata 3	Brazil, *Marcelli, Ahti & Yano 28385* (H)	(AY257958)	(AY257957)
Peltigera sp. 1 [*P. didactyla*]	Mexico, *Goffinet 6302* (HERB. GOFFINET AND DUKE)	AY266043	AY266042
Peltigera sp. 2 [*P. extenuata*]	Canada, Alberta, *Goffinet 3851* (HERB. GOFFINET)	AY266045	AY266044
Peltigera sp. 3 [*P. didactyla*]	Poland, *Lesiak & Czyzewska 9202* (LOD-L)	(AF286804)	(AY257930)
**P. continentalis*	Mongolia, *Huneck 88-151* (H)	(AF286777)	(AY257890)
**P. kristinssonii* 1	Canada, BC, *Goward 81-1718* (UBC)	(AF286779)	(AY257891)
**P. kristinssonii* 2	Canada, BC, *Goward & Findlay 83-506* (UBC)	(AF286778)	(AY257892)

var. *extenuata*) and one putative species (hereafter referred to as *P. castanea*) from boreal Canada. All taxa are characterized by orbicular laminal or submarginal soralia and were shown to belong to the *P. didactyla* group, section *Peltigera* (Miadlikowska & Lutzoni 2000; Miadlikowska et al. 2003). Two species, *P. continentalis* and *P. kristinssonii*, from the section *Peltigera*, were chosen as outgroup taxa for all phylogenetic analyses, based on phylogenetic reconstructions by Miadlikowska and Lutzoni (2000) and Miadlikowska et al. (2003).

Two species, *P. lambinonii* and *P. ulcerata*, are restricted to tropical regions and the temperate zone of the Southern Hemisphere; these taxa were represented by five and three populations, respectively. The cosmopolitan *P. didactyla* was represented by six specimens morphologically consistent with *P. didactyla* var. *didactyla*, and eight specimens of var. *extenuata*. The collection from Mexico (*Peltigera* sp. 1, Table 1), composed mainly of monophyllous plane to deeply concave thalli, was tentatively assigned to *P. didactyla sensu stricto*, although some morphological features (e.g., glabrous upper surface, submarginal soralia on young lobes, few simple to somewhat fasciculate rhizines restricted to central portion) may be considered atypical for this taxon. One individual of *P. didactyla* var. *didactyla* (namely *Peltigera* sp. 3; Table 1) collected in Poland possesses some atypical morphological features (see discussion) including the presence of tridepsides in the thallus. The specimen of *P. extenuata* 2 is a young thallus with shell-shaped lobes, difficult to distinguish from *P. didactyla* based solely on morphological features. The sample ''*P. extenuata* 1'' has a broadly lobed thallus with thick tomentum on its the upper surface and lacks soralia; however, the lower side of the thallus has pale flocculent rhizines typical typical of var. *extenuata*.

Molecular data.—Genomic DNA was obtained from fresh samples and herbarium specimens. DNA isolation, symmetric PCR amplifications, asymmetric PCR sequencing products purification, and automated sequencing were performed as explained in Miadlikowska and Lutzoni (2000) and Miadlikowska et al. (2002). The LSU and ITS nrDNA sequences were subjected to BLAST searches for the verification of their identity. They were assembled using Sequencher 4.1 (Gene Codes), and aligned with MacClade 4.01 (Maddison & Maddison 2001). Delimitation of the internal spacers and the 5.8S gene was obtained by comparison with complete ITS sequences from closely related taxa within the Peltigerineae (Goffinet & Goward 1998; Goward & Goffinet 2000; Miadlikowska et al. 2003).

Phylogenetic analyses.—Phylogenetic analyses were performed using maximum likelihood (ML) and maximum parsimony (MP) optimization criteria as implemented in PAUP*4.0b4a (Swofford 1998). We obtained LSU sequences for only one of the five samples of *P. lambinonii*, namely *P. lambinonii* 1. Therefore, for the purpose of combinability, phylogenetic analyses were carried out on two ITS data sets: 1) phylogenetic analyses on 29 ITS sequences (ML1 and MP1) including *P. lambinonii* 2–5 and 2) phylogenetic analyses on 25 ITS sequences (ML2 and MP2) without *P. lambinonii* 2–5. Maximum likelihood and maximum parsimony searches were implemented on 25 LSU sequences (ML3 and MP3) and on the combined ITS + LSU data set (ML4 and MP4) for the same 25 individuals.

Using the Hierarchical Likelihood Ratio Test, as implemented in Modeltest 3.04 (Posada & Crandall 1998), K80 two-parameter nucleotide substitution model (Kimura 1980) with equal base frequencies was selected for both the 29 OTUs (ML1) and the 25 OTUs (ML2) ITS data sets (ML1: ti/tv ratio = 3.5326, gamma distribution shape parameter = 0.3804; ML2: ti/tv = 3.4563, gamma distribution shape parameter = 0.4430); the HKY85 (Hasegawa et al. 1985) nucleotide substitution model was selected for the LSU (ML3) data set and the combined ITS + LSU (ML4) data set (ML3: A = 0.2750, C = 0.2085, G = 0.2812, T = 0.2353, ti/tv = 1.4037, gamma distribution shape parameter = 0.016; ML4: A = 0.2694, C = 0.2112, G = 0.2694, T = 0.2500, ti/tv = 2.4208, gamma distribution shape parameter = 0.0096). Maximum likelihood analyses were implemented as heuristic searches with 100 random-addition-sequence replicates, TBR branch swapping, MulTrees option in effect, saving all trees and collapsing branches with maximum branch length equal to zero.

Constant sites were removed from all maximum parsimony analyses. The unambiguously aligned parts of the LSU and ITS nrDNA alignments were subjected to symmetric step matrices constructed as outlined in Miadlikowska et al. (2002). The maximum parsimony analysis of the ITS data set (MP1 and MP2) involved three separate step matrices corresponding to the ITS1, 5.8S, and ITS2 regions. The aligned sites from the LSU nrDNA (MP3) were subjected to one specific step matrix.

Phylogenetic signal from ambiguously aligned portions of the alignments was integrated into maximum parsimony analyses without violating positional homology, using the program INAASE 2.3b (Lutzoni et al. 2000). The weights for substitutions in INAASE were set as following: transitions = 1.0, transversions and indels = 2.0. For maximum parsimony analyses, gaps from the unambiguous portions of the alignments were treated as a fifth character state. All maximum parsimony analyses (MP1–4) were performed as heuristic searches with 1,000 random-addition-sequence replicates, TBR branch swapping, MulTrees option in effect, saving all trees and collapsing branches with maximum branch length equal to zero.

Branch support for MP trees was estimated by bootstrap analyses (BS) (Felsenstein 1985) with full heuristic searches, 1,000 bootstrap replicates and 2 random-addition-sequence per bootstrap replicate. For ML analyses, posterior probability (PP) for each node using the Bayesian Metropolis coupled Markov chain Monte Carlo sampling (B/MC^3) method was calculated as implemented in MrBayes 2.01 (Huelsenbeck 2000). All B/MC^3 analyses were run with four chains simultaneously, each initiated with a random tree and flat prior. One of every 20 trees was sampled for a total of 1,000,000 generations with DNA substitution parameters estimated during the search. The majority rule consensus tree was computed with PAUP* on the last 15,000 trees out of 50,000 sampled with B/MC^3. Bipartitions were considered statistically significant when PP values were $\geq$95%. Congruence between the ITS partition and the LSU partition for 25 OTUs was tested by inspecting BS values >70% on topologies derived from separate MP analyses (Mason-Gamer & Kellog 1996) as explained in Miadlikowska and Lutzoni (2000) and PP values >95% on topologies derived from separate ML analyses as outlined in Kauff and Lutzoni (2002). Conflicts between data partitions would be considered significant only if two different relationships (one monophyletic, the other non-monophyletic) for the same set of taxa were supported by BS $\geq$70% or PP $\geq$ 95%.

Because the placement of one specimen, *P. castanea* 2, was in conflict based on the separate analysis of ITS and LSU data sets (part of the monophyletic *P. castanea* group versus *P. didactyla s.st.* group, respectively) exclusively when using the Bayesian criterion (PP $\geq$ 95%), we were concerned about the reliability of the significant posterior

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1. *P. didactyla* 1	-	3+	5+	4+	19+	19+	43+	43+	44+	43+	43+	43+	43+	55+	24+	25+	24+	25+	28+	28+	28+	28+	28+	42+	46+	46+	49+	85+	83+
2. *P. didactyla* 2	3	-	2	1	16	17+	41	41	42	41	41	41	41	52	22	23	22	21	26	26+	26+	26+	26+	40+	44+	44+	57+	82+	82
3. *P. didactyla* 3	3	0	-	1	16	17+	42	42	43	42	42	42	42	51	21	20	21	20	25	25+	25+	25+	25+	40+	43+	43+	56+	81+	81
4. *P. didactyla* 4	3	0	0	-	16	16+	40	40	41	40	40	40	40	50	21	22	21	20	26	25+	25+	25+	25+	39+	43+	43+	56+	81+	81
5. *Peltigera* sp. 2	10	7	7	7	-	5	43	43	44	43	43	43	43	50	18	17	18	16	21	21+	21+	21+	21+	38+	42+	41+	55+	83+	82
6. *Peltigera* sp. 3	12	9	9	9	16	-	40+	40+	41+	40+	40+	40+	40+	50+	18+	16+	18+	18+	23+	22+	22+	22+	22+	40+	40+	43+	54+	81+	80+
7. *P. extenuata* 1	20	17	17	17	15	15	-	1	1	0	0	0	0	45	39	38	39	37	43	41+	41+	41+	41+	48+	54+	54+	48+	88+	86
8. *P. extenuata* 2	20	17	17	17	15	15	0	-	2	1	1	1	1	45	39	38	39	37	43	41+	41+	41+	41+	51+	55+	55+	48+	88+	86
9. *P. extenuata* 3	21	18	18	18	16	16	1	1	-	1	1	1	1	46	40	39	40	38	41	42+	42+	42+	42+	49+	55+	55+	49+	89+	87
10. *P. extenuata* 4	20	17	17	17	15	15	0	0	1	-	0	0	0	45	39	38	39	37	43	41+	41+	41+	41+	48+	54+	54+	48+	88+	86
11. *P. extenuata* 5	20	17	17	17	15	15	0	0	1	0	-	0	0	45	39	38	39	37	43	41+	41+	41+	41+	48+	54+	54+	48+	88+	86
12. *P. extenuata* 6	20	17	17	17	15	15	0	0	1	0	0	-	0	45	39	38	39	37	43	41+	41+	41+	41+	48+	+54	54+	48+	88+	86
13. *P. extenuata* 7	20	17	17	17	15	15	0	0	1	0	0	0	-	45	39	38	39	37	43	41+	41+	41+	41+	48+	+54	54+	48+	88+	86
14. *Peltigera* sp. 1	25	22	22	22	20	20	14	14	15	14	14	14	14	-	51	49	50	49	55	53+	53+	53+	53+	64+	70+	65+	58+	101+	101
15. *P. castanea* 1	14	11	11	11	7	8	12	12	13	12	12	12	12	20	-	3	0	4	24	23+	23+	23+	23+	34+	39+	40+	51+	84+	82
16. *P. castanea* 2	13	10	10	10	6	7	11	11	12	11	11	11	11	19	1	-	3	3	23	22+	22+	22+	22+	34+	39+	39+	51+	85+	83
17. *P. castanea* 3	14	11	11	11	7	8	12	12	13	12	12	12	12	20	0	1	-	4	24	23+	23+	23+	23+	34+	39+	40+	51+	84+	82
18. *P. castanea* 4	14	11	11	11	7	8	12	12	13	12	12	12	12	20	2	1	2	-	24	23+	23+	23+	23+	34+	39+	39+	49+	82+	80
19. *P. lambinonii* 1	17	14	14	14	10	11	19	19	20	19	19	19	19	24	11	10	11	11	-	4+	4+	4+	4+	45+	48+	48+	57+	79+	78
20. *P. lambinonii* 2	18	15	15	15	11	12	20	20	21	20	20	20	20	24	12	11	12	12	2	-	2+	2+	2+	44+	47+	47+	55+	78+	77+
21. *P. lambinonii* 3	18	15	15	15	11	12	20	20	21	20	20	20	20	24	12	11	12	12	2	0	-	2+	2+	44+	47+	47+	55+	78+	77+
22. *P. lambinonii* 4	18	15	15	15	11	12	20	20	21	20	20	20	20	24	12	11	12	12	2	0	0	-	2+	44+	47+	47+	55+	78+	77+
23. *P. lambinonii* 5	18	15	15	15	11	12	20	20	21	20	20	20	20	24	12	11	12	12	2	0	0	0	-	44+	47+	47+	55+	78+	77+
24. *P. ulcerata* 1	24	21	21	21	17	18	24	24	25	24	24	24	24	30	18	17	18	18	23	24	24	24	24	-	13+	20+	63+	93+	91+
25. *P. ulcerata* 2	28	25	25	25	21	22	28	28	29	28	28	28	28	34	22	21	22	22	27	28	28	28	28	6	-	23+	66+	98+	96+
26. *P. ulcerata* 3	25	22	22	22	18	19	25	25	26	25	25	25	25	29	19	18	19	19	22	23	23	23	23	9	13	-	65+	99+	97+
27. *P. continentalis*	31	28	28	28	25	25	23	23	24	23	23	23	23	25	23	23	23	22	28	29	29	29	29	34	38	35	-	58+	57+
28. *P. kristinssonii* 1	45	42	42	42	38	39	39	39	40	39	39	39	39	42	39	39	39	40	40	41	41	41	41	48	52	47	28	-	3+
29. *P. kristinssonii* 2	44	41	41	41	37	38	38	38	39	38	38	38	38	41	38	38	38	39	39	40	40	40	40	47	51	46	27	1	-

FIGURE 1. Uncorrected pairwise differences among ITS nrDNA sequences included in phylogenetic analyses (MP and ML). Above diagonal: total number of differences including optimal number of changes within ambiguously aligned regions as estimated with INAASE. Indels with multiple consecutive gaps were counted as one change. A "+" sign indicates that the given number of differences can be higher due to the missing sites within ambiguous regions. Below diagonal: character differences among unambiguously aligned regions only. Boxes indicate pairwise sequence differences within recognized taxa, including two newly proposed species, *P. extenuata* and *P. castanea*. Shadow areas indicate pairwise sequence differences among specimens with uncertain taxonomic affiliation (*Peltigera* sp. 1, 2, and 3) and their putatively closely related species (*P. extenuata* and *P. didactyla*, respectively) as revealed from the ML1 (Fig. 3A), MP1 (Fig. 5), ML4 and MP4 (Fig. 4) analyses.

probability of the very short internode (length of internode 0.0005 substitutions per site) supporting the *P. didactyla s.st.* group in the ML3 tree. In order to test if this PP value was overestimated due to chance, we generated ten non-parametric bootstrap data matrices derived from the LSU using Seqboot as implemented in Phylip 3.5c (Felsenstein; http://evolution.genetics.washington.edu/phylip/getme.html). Bayesian analysis with the same search options as for the original LSU data set (outlined above) was completed on each of the pseudoreplicates. Topologies and posterior probabilities for all internodes were recorded and compared. If a PP value of ≤95% was obtained from more than one analysis, we could not reject the null hypothesis that the initial high posterior probablity was due to chance and, therefore we could conclude that the apparent conflict between the two partitions was not significant.

RESULTS

ITS and LSU characters.—The final alignment for the 29 ITS and 25 ITS sequences consisted of 656 sites. Twenty-five ambiguously aligned regions were delimited, resulting in the exclusion of 210 sites giving a total of 446 sites (353 constant and 93 variable) included in the ML1, ML2, and ML4 analyses. For MP1, MP2, and MP4, the 353 constant sites were excluded but the 25 ambiguous regions provided 25 additional INAASE coded characters for a grand total of 118 non-constant characters included in the MP analyses. Out of these, 93 and 86 characters were parsimony informative in the MP1 and MP2 analyses, respectively.

The ITS sequences varied in total length from 560 to 589 nucleotides (ITS1: 208–240; 5.8S: 158–159; ITS2: 188–207) among the sequences included here. The ITS1 and ITS2 sequences were identical in length for all *P. lambinonii* representatives and similar in their range within *P. didactyla s.st.*, except two specimens (namely *Peltigera* sp. 1 and sp. 2; Table 1) with much longer ITS1. All specimens of *P. extenuata* have almost identical lengths of the ITS1 and ITS2 regions.

The number of differences in nucleotide composition of the ITS between taxa is distinctly higher than among specimens from the same taxon. Within all putative species, except one, *P. ulcerata*, there is only slight variation among ITS sequences, including ambiguously aligned parts of the alignment (Fig. 1). A total number of changes for the ITS sequences, including ambiguous regions, ranged from zero to two within *P. extenuata*; zero to four among atypical populations from British Columbia (i.e., *P. castanea*); and from four to five changes within *P. didactyla s.st.* and *P. lambinonii*, respectively. The most variable ITS was found among members of *P. ulcerata*, which differ from each other by a total of 13 to 23+ changes, out of which six to 13 are within the unambiguous parts of the alignment (Fig. 1). *Peltigera extenuata* is separated from *P. didactyla* by 40–44 differences (17–21 within unambiguous portions of the alignment). *Peltigera castanea* shares the least number of changes, 16–51, with *Peltigera* sp. 1 and sp. 2; only six to 20 of them occur in the non-ambiguous parts of the alignment. Among all taxa included in this study, *P. castanea* is most similar to *Peltigera* sp. 1 and sp. 2 (at least 16–18 changes). The most distant species exhibiting the greatest number of total

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. *P. didactyla* 1	-	2	0	1	3	2	10	9	9	9	9	11	9	6	5	0	8	2	5	10	15	10	12	10	10
2. *P. didactyla* 2	1	-	2	2	4	3	11	10	10	10	10	12	10	7	6	1	9	3	8	11	16	12	13	11	11
3. *P. didactyla* 3	0	2	-	2	3	3	11	10	10	10	10	12	10	6	6	1	9	3	8	10	15	12	13	10	11
4. *P. didactyla* 4	0	1	0	-	3	1	11	9	9	9	9	11	9	6	5	1	8	3	7	10	15	11	12	9	9
6. *Peltigera* sp. 2	2	3	2	2	-	1	10	9	9	9	9	8	6	4	3	2	7	2	6	8	14	10	11	9	9
5. *Peltigera* sp. 3	1	2	2	1	2	-	10	8	8	8	8	10	8	5	4	2	7	2	4	9	14	10	11	8	8
7. *P.extenuata* 1	7	9	8	8	7	7	-	3	2	3	2	4	2	11	11	11	14	11	13	16	21	19	14	12	13
8. *P.extenuata* 2	6	7	7	6	6	5	2	-	1	2	1	3	1	11	9	9	12	9	10	15	20	17	12	11	11
9. *P.extenuata* 3	6	7	7	6	6	5	2	0	-	1	0	2	0	11	9	9	12	9	11	15	20	17	12	11	11
10. *P.extenuata* 4	6	7	7	6	6	5	3	1	1	-	0	3	1	11	9	9	12	9	11	15	20	17	12	11	11
11. *P.extenuata* 5	6	7	7	6	6	5	2	0	0	1	-	2	0	11	9	9	12	9	12	15	20	17	12	11	11
12. *P.extenuata* 6	8	9	9	8	8	7	4	2	2	3	2	-	2	13	11	11	14	11	14	17	20	19	14	13	13
13. *P.extenuata* 7	6	7	7	6	6	5	2	0	0	1	0	2	-	11	9	9	11	9	12	15	20	17	12	11	11
14. *Peltigera* sp. 1	4	5	4	4	1	3	9	8	8	8	8	10	8	-	7	6	11	6	8	8	14	12	12	12	12
15. *P. castanea* 1	4	5	5	4	4	3	8	6	6	6	6	8	6	6	-	5	6	3	9	11	17	11	12	10	10
16. *P. castanea* 2	0	1	1	0	2	1	8	6	6	6	6	8	6	4	4	-	8	2	7	10	15	11	12	10	10
17. *P. castanea* 3	7	8	8	7	7	6	11	9	9	9	9	11	8	9	5	7	-	6	12	15	19	14	15	13	13
18. *P. castanea* 4	2	3	3	2	2	1	8	6	6	6	6	8	6	4	2	2	5	-	7	10	15	9	10	8	8
19. *P. lambinonii* 1	2	5	5	4	2	3	9	6	7	8	8	10	8	4	6	4	9	4	-	10	16	15	15	13	12
20. *P. ulcerata* 1	7	8	7	7	6	6	12	11	11	11	11	13	11	7	9	7	12	7	7	-	7	6	16	16	16
21. *P. ulcerata* 2	12	13	12	12	10	11	17	16	16	16	16	16	16	12	14	12	17	12	12	5	-	11	21	21	21
22. *P. ulcerata* 3	9	11	11	10	8	9	16	14	14	14	14	16	14	10	10	10	13	8	12	3	8	-	18	15	17
23. *P. continentalis*	8	9	9	8	6	7	12	10	10	10	10	12	10	7	8	8	11	6	9	10	15	14	-	6	6
24. *P. kristinssonii* 1	7	8	7	7	6	6	10	9	9	9	9	11	9	8	7	7	10	5	8	11	16	12	1	-	0
25. *P. kristinssonii* 2	7	8	8	7	6	6	11	9	9	9	9	11	9	8	7	7	10	5	7	11	16	13	1	0	-

FIGURE 2. Uncorrected pairwise differences among LSU nrDNA sequences included in phylogenetic analyses (MP and ML). Above diagonal: total number of differences including optimal number of changes within ambiguously aligned regions as estimated with INAASE. Indels with multiple consecutive gaps were counted as one change. Below diagonal: character differences among unambiguously aligned regions only. Boxes indicate pairwise sequence differences within recognized taxa, including two newly proposed species, *P. extenuata* and *P. castanea*. Shadow areas indicate pairwise sequence differences among specimens with uncertain taxonomic affiliation (*Peltigera* sp. 1, 2, and 3) and their putatively closely related species (*P. extenuata* and *P. didactyla*, respectively) as revealed from the ML4 and MP4 (Fig. 4) analyses and between the specimen of *P. castanea* 2 and its putatively closely related species (*P. didactyla*) as revealed from the ML3 (Fig. 3B) analysis.

changes (34 to 70+) compared to the remaining ingroup species is *P. ulcerata*.

The final alignment for the 25 LSU sequences consisted of 1,334 sites. Two ambiguously aligned regions were determined, resulting in the exclusion of 27 sites giving a total of 1,307 sites (1,267 constant and 40 variable) included in the ML3 and ML4 analyses. For MP3 and MP4, the 1,627 constant sites were excluded but the two ambiguous regions provided two additional INAASE coded characters for a grand total of 42 non-constant characters included in MP analyses. Out of these, 21 were parsimony informative.

A pattern of variation similar to that observed among the ITS sequences was present within and among taxa for the LSU sequences. However, the number of changes among species was generally lower compared to the ITS data (Fig. 2). The variation within unambiguous parts of LSU sequences within species ranged from zero to two for *P. didactyla*, zero to four for *P.extenuata*, zero to seven for *P. castanea* and three to eight for *P. ulcerata*. The highest total number of changes, when including ambiguous regions, occurred among *P. ulcerata* (six to 11) and *P. castanea* (two to eight) specimens. Among-species variation was similar for all the taxa including the outgroup species, except *P. ulcerata*. *Peltigera ulcerata* was the most distant species, exhibiting the greatest difference in nucleotide composition with *P. extenuata* (20–21). An unexpectedly high degree of similarity (zero to one total change) was found between sequences of *P. didactyla* and *P. castanea* 2. *Peltigera castanea* 2 was identical with specimen 1 of *P. didactyla* and differs only by a single change from other members of *P. didactyla* (Fig. 2).

Phylogenetic relationships.—When analyzing the 29 OTUs ITS data set alone, a single most likely tree was obtained from the ML1 (ln likelihood = −1216.38158; Fig. 3A), and three most parsimonious trees resulted from the MP1 (tree length = 480.59 steps; Fig. 5). The ML1 and MP1 topologies are identical and both show five *Peltigera* species, including the two putative species, *P. extenuata* and *P. castanea*, as monophyletic and significantly supported (PP > 95% and BS > 97). In both analyses, *P. extenuata* is well separated from *P. didactyla*, forming a sister group to a clade incorporating the rest of the studied taxa. Relationships among taxa within this major clade are well supported only in MP1 due to the phylogenetic signal provided by coded INAASE characters (Figs. 3A & 5). *Peltigera didactyla* and *P. lambinonii* share a common ancestor (BS = 76%) and are the closest relatives to a new species, *P. castanea*. *Peltigera ulcerata* is sister to the *P. didactyla* + *P. lambinonii* + *P. castanea* clade (PP = 100%; BS = 93%). Affiliation of three *Peltigera* individuals, *Peltigera* sp. 1–3, remains uncertain. ML2 and MP2 analyses on the 25 individuals (results not shown) were carried out mainly for combinability purposes, however, the topologies and internode supports were similar to the results obtained from the ML1 and MP1 searches.

Phylogenetic analyses on the LSU nrDNA data set alone, ML3 and MP3, revealed two equally

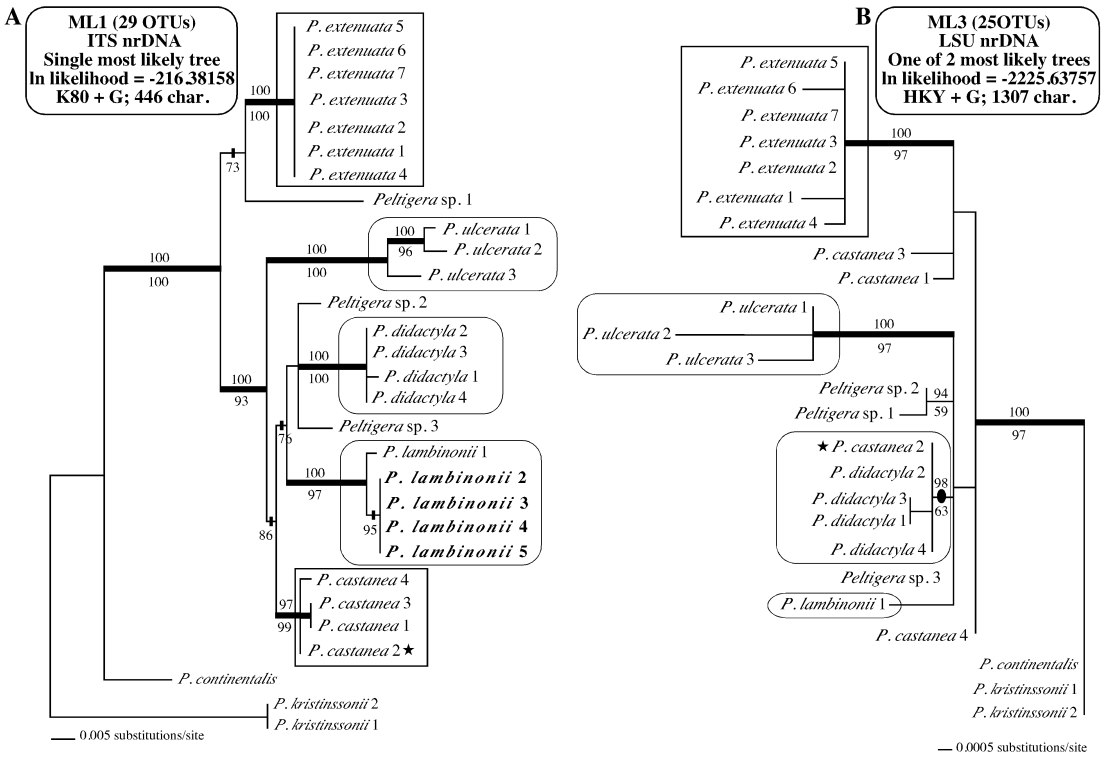

FIGURE 3. Phylogenetic relationships among individuals from three known *Peltigera* species (rounded-cornered boxes), two putative new species, *P. extenuata* (former *P. didactyla* var. *extenuata*) and *P. castanea* (square-cornered boxes), and the outgroup taxa (*P. continentalis* and *P. kristinssonii*) as revealed by maximum likelihood analyses of the ITS (A) and LSU (B) data sets alone. PP values ≥95% are shown above internodes. A star indicates conflicting placement of *P. castanea* 2 as revealed from ML1 and ML3 analyses. — A. ML1 analysis. Sequences of *P. lambinonii* 2–5, which are included only in the ML1 and MP1 analyses, are shown in bold. BS values ≥70% on MP1 are shown below internodes. Thicker lines indicate internodes with PP ≥ 95% on ML1 and BS > 70% on MP1 and MP2. Vertical bars indicate internodes with PP < 95% on ML1 but BS ≥ 70% on MP1 and MP2. — B. ML3 analysis. BS values ≥ 50% are shown below internodes. Thicker lines indicate internodes with PP ≥ 95% on ML3 and BS >70% on MP3. Vertical oval indicate internodes with PP ≥ 95% on ML3 but BS < 70% on MP3.

most likely trees (ln likelihood = −2225.63757; Fig. 3B) and 296 equally most parsimonious trees (tree length = 126.37 steps; results not shown) respectively. Only *P. extenuata* and *P. ulcerata* were reconstructed as monophyletic entities, significantly supported by both PP and BS values. Note that in this analysis, *P. lambinonii* is represented by a single exemplar. *Peltigera castanea* 2 was nested within the *P. didactyla sensu stricto* group in both ML3 and MP3 reconstructions. The monophyly of this composite clade was, however, only well supported by a high posterior probability value (PP = 98% on ML3; Fig. 3B). Relationships among species are mostly not resolved based on the LSU data set alone.

Although the LSU data set alone provided a lower level of resolution and statistical confidence for intra- and interspecific relationships (B/MC[3] and BS support) compared with the separate ITS data set, a conflict was detected only when using the 95% PP value for estimating phylogenetic confidence. Both reconstructions, the placement of *P. castanea* 2 within the monophyletic *P. castanea* group in ML1 (Fig. 3A) and ML2 (results not shown) and within the otherwise monophyletic *P. didactyla* in ML3 (Fig. 3B), were significantly supported (PP > 95%).

Our test showed that the internode leading to the monophyletic *P. didactyla* with *P. castanea* 2 (ML3; Fig. 3B) obtained significant support (PP ≥ 97%) in only three out of the 10 B/MC[3] analyses on the LSU pseudoreplicates. This internode was not present at all in the 50% majority rule consensus trees revealed by the seven remaining pseudoreplicate analyses. Other internodes significantly supported in the ML3 tree (Fig. 3B) were recovered with significant support values (PP ≥ 95%) from each of the 10 LSU pseudoreplicates, except one pseudoreplicate, where *P. extenuata* was delimited as a monophyletic entity excluding specimen 4. All these internodes, except one (sister relationship of *Peltigera* sp. 2 and sp. 1) were also highly sup-

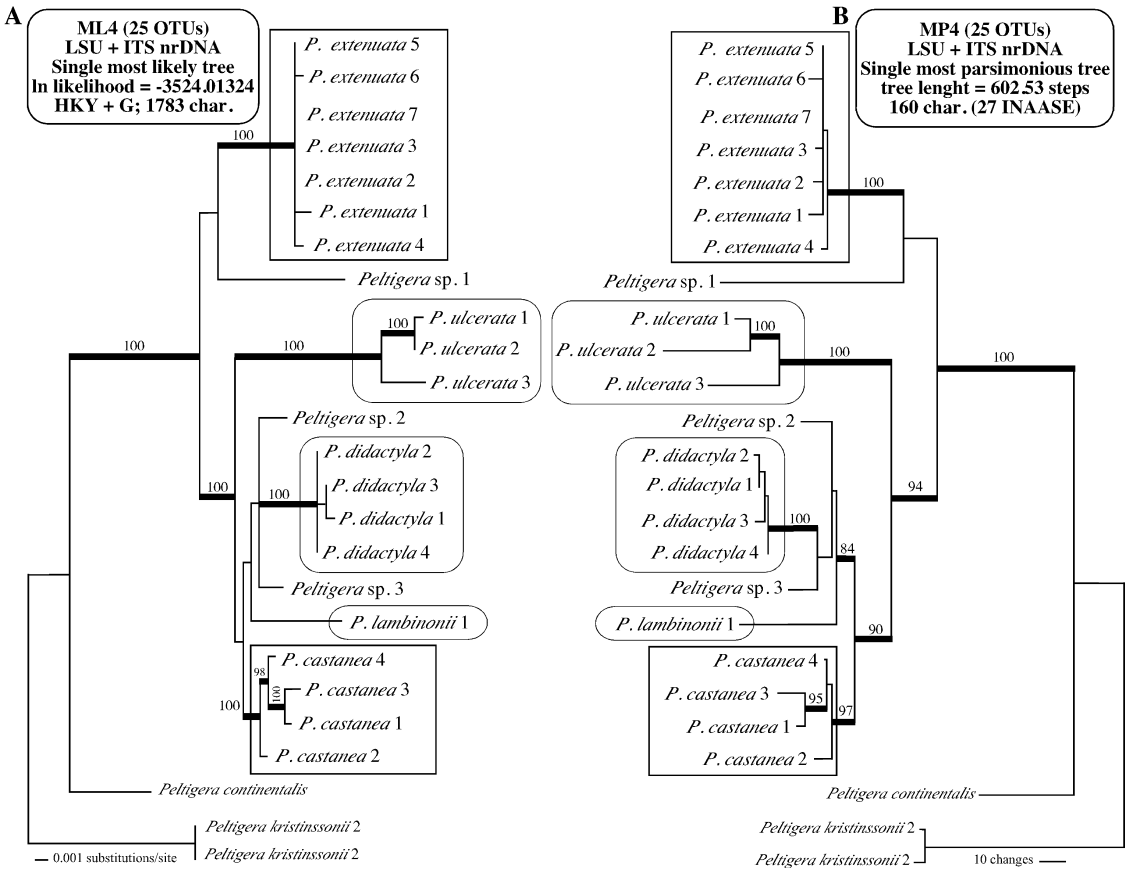

FIGURE 4. Phylogenetic relationships among 25 individuals from three known *Peltigera* species (round-cornered boxes), two putative species *P. extenuata* (former *P. didactyla* var *extenuata*) and *P. castanea* (square-cornered boxes), and the outgroup taxa (*P. continentalis* and *P. kristinssonii*) as revealed by maximum likelihood analysis (A) and maximum parsimony analysis (B) on the combined ITS and LSU nrDNA data set. — A. ML4 analyses. PP values ≥ 95% are shown above internodes (thicker lines). — B. MP4 analyses]CI (excluding uninformative characters) = 0.8637, RI = 0.9271]). BS values ≥70% are shown above internodes (thicker lines).

ported by ML and MP bootstrap values (BS > 70%). This simple test indicated that the high PP value obtained with the LSU data set for the conflicting internode (*P. didactyla* with *P. castanea* 2) was due to chance. Because the ITS and LSU data partitions were congruent based on the bootstrap 70% criterion we combined the 25 sequences and analyzed them simultaneously with maximum likelihood (ML4) and maximum parsimony (MP4).

The single most likely tree resulting from ML4 analysis (ln likelihood = −3524.01324; Fig. 4A) was identical to the single most parsimonious tree obtained from the MP4 analysis [tree length = 602.53 steps, CI (excluding uninformative characters) = 0.8637, RI = 0.9271; Fig. 4B] and the most parsimonious tree obtained from the MP2 analysis (results not shown). All tested species, including *P. extenuata* and *P. castanea*, are monophyletic and significantly supported (PP = 100% and BS ≥ 97%). The relationships among species are better resolved and more robust when using a parsimony criterion that allowed us to incorporate INAASE coded characters (Fig. 4B). A non-sister relationship between *P. didactyla* var. *didactyla* and *P. didactyla* var. *extenuata* was revealed from both combined analyses. *Peltigera castanea* shares a common ancestor with *P. didactyla sensu stricto* and *P. lambinonii* group (BS = 84%), and represents a well defined species within the *P. didactyla* group. *Peltigera ulcerata* is closely related to the *P. didactyla sensu stricto* + *P. lambinonii* + *P. castanea* monophyletic group. Phylogenetic placement of *P. lambinonii*, which is represented in the combined data set by only a single sequence, is the same as in the ML1 (Fig. 3A) and MP1 (Fig. 5) trees. There are three specimens, named *Peltigera* sp. 1–3, with uncertain taxonomic status and phylogenetic affiliations.

Sites between position 92 and 147 of the ITS alignment were extremely variable in length and

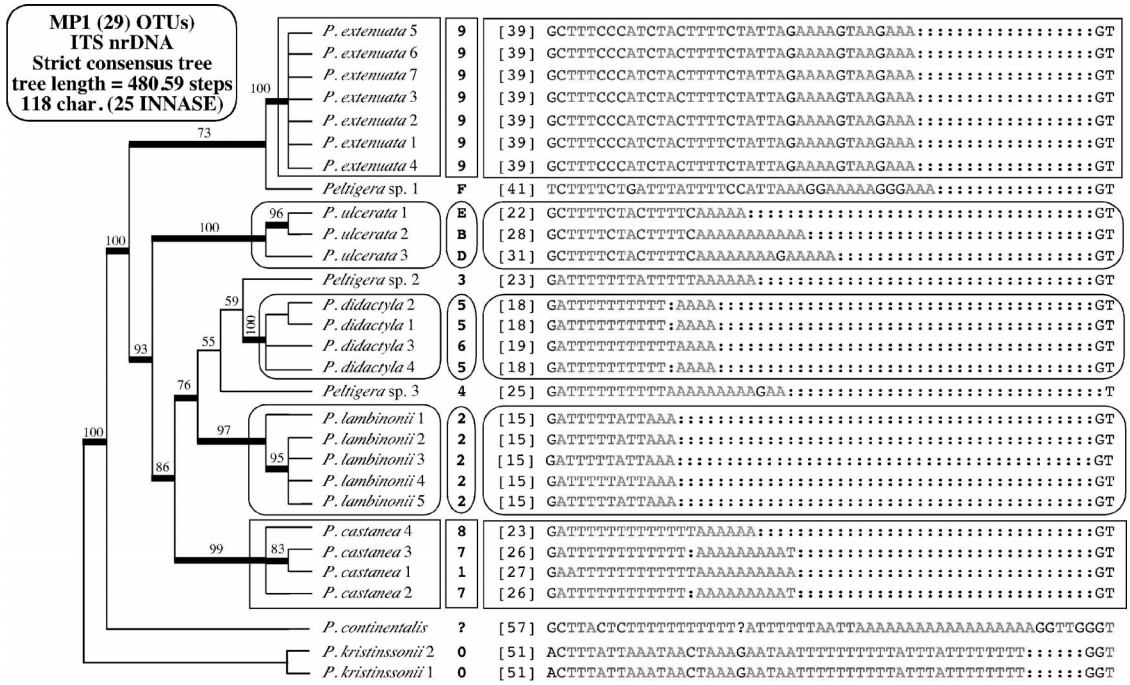

FIGURE 5. Comparison between species delimitation based on the phylogenetic relationships among 29 individuals as revealed from the analysis on ITS data set alone (MP1; revealed also from ML1, ML4, and MP4 analyses) and sequence variation within the ITS1 hypervariable region (*sensu* Miadlikowska et al. 2003) included in the MP analysis as one out of 25 INAASE coded characters. Strict consensus tree of three maximum parsimonious trees (CI [excluding uninformative characters] = 0.8973, RI = 0.9517). BS values > 50% are shown above each internode. Thicker branches indicate BS > 70%. Sequence from the ITS1 hypervariable region is provided for each individual following a character state resulting from the INAASE coding. The number in square brackets indicates the length of the sequence (number of nucleotides). Boxes delimit *Peltigera* species recognized here and their ITS1 marker sequences; square-cornered boxes represent the newly delimited species, *P. extenuata* and *P. castanea*. Sequences of the ITS1 hypervariable region are aligned within each species.

nucleotide composition across species, but relatively conserved and unique within each delimited species (Fig. 5). Variation in length of the ITS1 hypervariable region (sensu Miadlikowska et al. 2003) among the recognized members of the *P. didactyla* group ranged from 15 (*P. lambinonii*) to 41 (*Peltigera* sp. 1) nucleotides, whereas this region was much longer, 51–57 nucleotides, in the outgroup taxa. This hypervariable ITS1 region was identical for all specimens of *P. extenuata* and *P. lambinonii* and almost identical (single nucleotide insertion) for individuals from *P. didactyla s.st. Peltigera ulcerata*, and *P. castanea* show more variation among the sequences of this region. The sequences of *Peltigera* sp. 1, 2, and 3 are different from their respective closely related species (*P. extenuata* and *P. didactyla s.st.*) and each of them is unique.

DISCUSSION

Analysis of the variation observed among sequences of the ITS and the LSU loci of the rDNA tandem repeat reveals a distinct genetic structure within the *P. didactyla* complex. Phylogenetic inferences suggest: 1) that most individuals assigned to *P. didactyla* var. *extenuata* form a monophyletic group distinct from *P. didactyla* var. *didactyla*; 2) that most populations of the latter compose a distinct monophyletic group; 3) that var. *didactyla* is more closely related to *P. lambinonii* and other sorediate taxa than to var. *extenuata;* 4) that apotypic populations from (oro-)boreal zones of Canada share a common recent ancestor; and 5) that they are sister to a clade comprising *P. didactyla*, *P. ulcerata*, and *P. lambinonii*. Variation among sequences of the ITS1 hypervariable region confirmed each of the five monophyletic morphotaxa, and revealed the genetic distinctness of samples *Peltigera* sp. 1–3 (Fig. 4). In summary, *P. didactyla sensu* Vitikainen (1994) is polyphyletic due to the nested position of *P. ulcerata* and *P. lambinonii*. For taxonomic units to represent natural groupings, and hence satisfy the monophyletic criterion, the concept of *P. didactyla* must be either broadened to accommodate *P. ulcerata* and *P. lambinonii*, or narrowed to allow for retention of the latter species.

In the latter case, var. *extenuata* and the material from boreal parts of Canada must be accorded species rank.

The level of divergence between var. *didactyla* and other putative taxa is similar to, if not greater than those observed among other closely related species of *Peltigera*, e.g., within the *P. aphthosa* complex (Goffinet & Bayer 1997; Goward & Goffinet 2000), among members of the section Horizontales (Goffinet & Miadlikowska 1999), or among members of the *P. canina* group (Miadlikowska et al. 2003). The incongruence between our initial identification and the phylogenetic affinities of some populations (*Peltigera* sp. 1 to 3 in Figs. 3–4) may be seen as evidence that falsifies the hypothesis of multiple species composing this morphological complex. However, given their genetic divergence from other population clusters, we interpret these populations as representatives of additional species as yet undescribed (see below).

The monophyly of *P. extenuata*, *P. castanea*, *P. lambinonii*, *P. ulcerata*, and *P. didactyla sensu stricto* is well supported by ITS data alone or in combination with the LSU sequences, regardless of the optimality criterion used. The data also offer maximum support for the sister group relationships between *P. extenuata* and the remaining ingroup taxa, but relationships among the latter are ambiguous (Figs. 3–4) . When analyzed alone, sequences of the LSU locus provide similar support for *P. extenuata*, *P. ulcerata*, and *P. didactyla*, but no support for the monophyly of *P. castanea*. It should be noted, however, that topological incongruence between the two character sources do not seem significant as alternative relationships are not each characterized by high support values. This is not surprising considering that the LSU gene is considered to accumulate mutations at a lower rate than the non-coding intergenic spacer region, hence little homoplasy is needed to break down a monophyletic group. The only exception to this pattern concerns the affinities of the sample *P. castanea* 2, which is resolved as part of the *P. didactyla sensu stricto* clade based on LSU characters (Fig. 3). This close relationship between *P. castanea* 2 and members of *P. didactyla sensu stricto* was also indicated by the high similarity of their LSU sequences (Fig. 1). One possible explanation for this relationship could involve an incomplete lineage sorting for *P. castanea* and *P. didactyla* detected for the LSU locus (Kroken & Taylor 2001) and not seen in the ITS-based phylogeny where the two putative taxa were well delimited and supported (Fig. 3A). We also considered recombination as the potential factor for this grouping (e.g., Posada 2000; Guttman 1997), however the occurrence of interspecific hybridization has not been conclusively demonstrated in lichen-forming fungi in general and within the *P. canina* species complex in particular (e.g., Miadlikowska et al. 2003). Because our test was designed to evaluate the level of confidence obtained from the Bayesian analysis on the separate LSU data set, which revealed considerable uncertainty about monophyletic delimitation of *P. didactyla s.st.* including *P. castanea* 2 (non-monophyly resulted from Bayesian analysis on seven out of 10 LSU pseudoreplicates), we concluded that the PP value for this conflicting node was due to chance (Fig. 3B). Furthermore this is the only internode in the ML3 tree that obtained significant PP support but low (<70%) BS values resulting from both maximum likelihood and maximum parsimony analyses (Fig. 3B). Our results are in agreement with recent simulation studies showing that in some cases the posterior probability is unreasonably high on very short but wrong branches (Alfaro et al. 2003; Suzuki et al. 2002). Suzuki et al. (2002) suggested that, in such cases, bootstrap probabilities are more suitable for assessing the reliability of phylogenetic trees than posterior probabilities.

Broadening the concept of *P. didactyla* would require abandoning *P. lambinonii* and *P. ulcerata*, which are morphologically clearly distinct (Goffinet & Hastings 1995). Finally, the populations composing the two most basal clades within the complex can be defined by morphological characters, even if the differences may appear subtle at best, at least to the untrained eye.

The lack of support for branches defining the relationships among the clades within the *P. didactyla* complex (Fig. 3A; except for the sister-group relationship of *P. extenuata* to the rest of the ingroup) may suggest that we are dealing with a single taxon, with highly partitioned genetic diversity. Although this is clearly a possibility, and actually the null hypothesis, we argue that the pattern in molecular differentiation overlaps with trends in morphological changes. It should be noted that the lack of support characterizes analyses from which characters of ambiguous homology have been excluded (Fig. 3A–B, and Fig. 4A), but that the integration of these characters, following the methods developed by Lutzoni et al. (2000), results in additional phylogenetic signal and increased support values for these branches (Figs. 1 & 4B) without affecting the topology (results not shown). Finally, the lack of strong support for most cladogenic events within the complex may simply reflect rapid speciation events, with much of the molecular differentiation occurring subsequently. Our data certainly provide strong support for the monophyly of the various ''terminal'' clades, and the number of uncorrected pairwise differences (patristic distance) compare well with those reported among other spe-

cies in *Peltigera* (Miadlikowska et al. 2003). We predict that additional characters from other loci will resolve and provide strong support for relationships among these species.

Based on these arguments, we propose to reinstate *P. didactyla* var. *extenuata* at the species rank and recognize the specimens from Canada as a distinct species. Hence, we recognize five species within the *Peltigera didactyla* complex.

1. PELTIGERA EXTENUATA (Vainio) Lojka, Lichenoth. Univ., Fasc. V, no. 222. 1886.

Peltigera canina var. *extenuata* Vainio, Medeland. Soc. Fauna Fl. Fennica 2: 49. 1878. TYPE: FINLAND. Tavastia australis, Asikkala, Kaitas, *Silén & Norrlin* (H, lectotype). *Peltigera canina* var. *extenuata* Norrlin, Notiser Sällsk. Fauna Fl. Fennica Förhandl. 11: 178. 1870. (*nomen nudum*).

Peltigera canina [subsp.] *extenuata* (Vainio) Vainio, Meddel. Soc. Fauna Fl. Fennica 6: 129. 1881.

Peltigera didactyla (With.) Laund. var. *extenuata* (Vainio) Goffinet & Hastings, Lichenologist 27: 48. 1995.

Note.—In his exsiccati, Lojka was the first to recognize Vainio's var. *extenuata* at the species level, but did not provide a rationale for his new combination. However, neither his nor Vainio's concepts were followed by later lichenologists. The variety was recently resurrected by Goffinet and Hastings (1995).

Chemistry.—Methyl gyrophorate and gyrophoric acid are often present in the soralia.

Differentiation.—*Peltigera extenuata* typically forms spreading thalli with a gray to light brown, dull, minutely roughened, laminally sorediate upper cortex that is tomentose especially toward the lobe tip, and a lower surface with white to pale veins and flocculent rhizines. The species rarely bears apothecia or pycnidia. Goffinet and Hastings (1995) considered the presence of tridepsides in the soralia to be diagnostic of *P. extenuata* with regard to *P. didactyla*. Our more recent studies of this species have revealed that these tridepsides are not constant among populations; hence, rather than invoking hybridization to account for this polymorphism (see above), it may be more parsimonious to consider the presence of tridepsides in this species as variable. A similar hypothesis is proposed for *P. lambinonii* and *P. didactyla*. A description along with photographs of *P. extenuata* are provided in Goffinet and Hastings (1995).

Ecology.—The species grows in rather mesic forests, usually over or among terricolous or epixylic mosses. Unlike *P. didactyla*, *P. extenuata* rarely grows directly over soil.

Distribution.—*Peltigera extenuata* was reported by Goffinet and Hastings (1995) from North America, Central Europe and China. The species (as *P. didactyla* var. *extenuata*) has subsequently been reported from scattered localities throughout Europe (Berger 1996; Bergsten 1999; Esslinger 1997; Halonen et al. 2000; Martinez et al. 1997; Martinez Moreno 1999; Miadlikowska 1999; van den Boom 1998). Emmanuël Sérusiaux (LG; pers. comm.) collected the species in Papua New Guinea.

2. PELTIGERA ULCERATA Müll. Arg., Flora 63: 261. 1880. TYPE: ''Prope Apiahy Brasilae merid. Crescit: Puiggari n. 1023'' (G, not seen).

Chemistry.—Methyl gyrophorate and gyrophoric acid are present in the soralia. According to Vitikainen (pers. comm.) the type was too small to check for a C reaction. None of the specimens we have examined lack the above tridepsides, and we are not aware of any mention of chemical polymorphism within this species.

Differentiation.—This species is easily identified by submarginal soralia and glabrous upper cortex. Fertile populations occur throughout the range. A formal description can be found in Galloway (1985) and Swinscow and Krog (1988); the latter authors also provide an illustration of the species.

Ecology.—In Africa, *P. ulcerata* grows on ''soil or over mosses, on earth banks, rocks and fallen tree trunks in montane forests and the ericaceous zone. . . '' (Swinscow & Krog 1988) whereas in New Zealand it occurs on soil or among mosses above the tree line (Galloway 1985).

Distribution.—*Peltigera ulcerata* is widespread in temperate regions of the Southern Hemisphere (Galloway 1985; Swinscow & Krog 1988). The species was hitherto not known from Chile (Galloway & Quilhot 1998), and is here reported as new to this country from the Province of Osorno (W of Parque Nacional Puyehue, along road to Antillanca, about 1, 2 km E of junction with Rd 215; seepy road bank, dominated by mosses, below bamboos and tree ferns, elev. 380 m, March 20, 2000, *Goffinet 7126 & Cox* (herb. B. Goffinet).

3. PELTIGERA CASTANEA Goward, Goffinet, & Miadlikowska, *sp. nov.* FIG. 6

Thallus foliosus, Peltigerae extenuatae similis sed pagina superna nitida et castanea differt. Apothecia ignota. Pycnidia frequentes. Habitat in supibus muscosis.

TYPE: CANADA: BRITISH COLUMBIA. Clearwater River Basin. Philip Creek, Battle Mountain Road, elev. 1,500 m, 51°52′ N, 119°55′ W, on mossy boulder, in open forest on south-facing hillside, 1 October 2001, *Goward 01–858* (UBC, holotype; CANL, H, isotypes).

Thallus foliose, loosely appressed, small, to 6–8 cm across; lobes stiff, fragile, to 1.0–1.5 cm wide, not much longer than wide, strongly concave or sometimes plane, loosely overlapping, irregularly branched; lobe tips rounded, upturned; lobe mar-

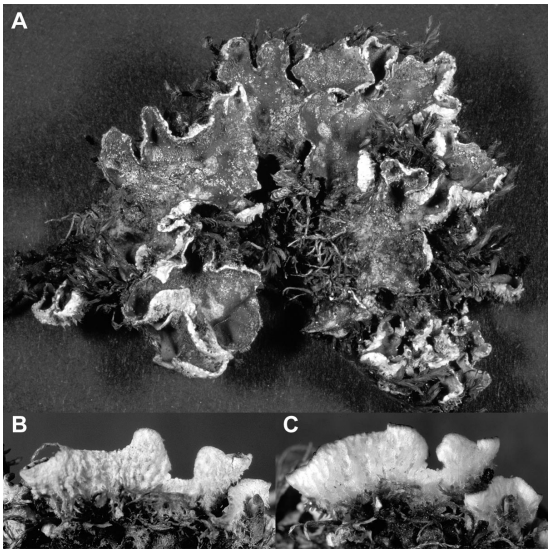

FIGURE 6. *Peltigera castanea* (holotype, UBC). — A. Habit (×1.3). — B. Lower surface when dry (×2). — C. Lower surface when wet (×2).

gins even; upper surface dark chestnut brown (pale bluish gray where sheltered), more or less shiny, glabrous, except tomentose near lobe tips, sorediate; soredia granular, brown, loose, in rounded to irregular soralia to 2–3 mm across; lower surface densely veined; veins white, grading inwards to brown or black; rhizines copious, tufted and usually strongly hedgerow-forming near lobe tips, but becoming flocculent toward thallus center, concolorous with veins. Cortex 40–60 μm thick; photobiont layer 50–20 μm thick, containing *Nostoc*, medulla white, 80–120 μm thick. Apothecia unknown. Pycnidia frequent, marginal, pycnoconidia ellipsoid, 6–7 × 4–5 μm.

Etymology.—The name *castanea* describes the dark, chestnut-colored upper surface characteristic for this species.

Chemistry.—No compounds were detected by TLC.

Differentiation.—*Peltigera castanea* forms spreading lobes, with a smooth, shiny, chestnut brown, mostly non-tomentose upper cortex, and darkening veins and flocculent rhizines that are often tufted and hedgerow forming (Fig. 6). Apothecia are unknown, but marginal pycnidia are common. *Peltigera castanea* closely resembles *P. extenuata*, but that species has less concave lobes and a dull, abundantly tomentose, minutely scabrid, bluish gray or (where exposed) purplish brown upper cortex usually covered in dense tomentum. The veins and rhizines also usually remain pale throughout.

Fertile specimens of *P. didactyla* are readily recognized by the occurrence of a thick tomentum over the upper surface (in addition of course to the presence of apothecia). Sterile material must be carefully distinguished by the more pouch-like habit and the mostly discrete, non-flocculent rhizines. Like *P. castanea*, *P. didactyla* can be somewhat shiny, but in that case the cortex has a predominantly purplish brown cast quite different from the castaneous hue of *P. castanea*.

Ecology.—*P. castanea* occurs in (oro)boreal forests and alpine heaths, where it grows in open sites, possibly exclusively on south facing outcrops. Most of the existing specimens were collected from rather xerophytic moss mats.

Distribution.—Currently known only from Canada.

Paratypes.—CANADA. ALBERTA. Banff National Park, Snow Creek Valley, *Beder s.n.* (CANL 22128). BRITISH COLUMBIA. Atlin Lake: Warm Bay Springs, *Goward 82–599* (UBC); Philip Creek, *Goward 02–2173* (UBC), Wildhorse Creek, *Goward 01–1064* (UBC). ONTARIO. Penn Island, *Kershaw s.n.* (CANL 36013). YUKON. Kluane National Park: Mount Maxwell, *Scotter 20165* (CANL). Fish Creek, Lupine tundra, *Cannings s.n.* (UBC).

4. PELTIGERA LAMBINONII Goffinet, The Lichenologist 27: 45, 1995. TYPE: ZAÏRE: KIVU, Top of Mt. Biega, heath with *Erica bequaertii* and *Philippia*, 2,740 m, 6 January 1972, *Lambinon 72/Z/102* (LG, holotype; herb. B. Goffinet, isotype).

Chemistry.—Methyl gyrophorate and gyrophoric acid are typically, albeit not always present in the soralia. So far we have only seen the specimens examined by Goffinet and Hastings (1995). As in the case of *P. extenuata*, these authors argued that chemical variation in this species may be due to hybridization with sympatric *P. didactyla*. However, it would be more parsimonious to consider the species chemically polymorphic until such time as the hybridization hypothesis can be critically tested.

Differentiation.—Except for the soralia, *P. lambinonii* is reminiscent of *P. rufescens*, both species having elongate, rather concave lobes with a (typically) densely tomentose upper surface. The lower surface bears dark (rarely pale) brown, branched rhizines that form a dense mat. Apothecia are common on the tips of erect lobes. The species is illustrated and described in Goffinet and Hastings (1995).

Ecology.—Based on the African material, *P. lambinonii* is known to ''grow over soil and humus, among mosses in open sites, in the upper tropical, montane forest between 1,700 and 2,740 m'' (Goffinet & Hastings 1995). In Australia, Tibell collected this species on the ground in a low *Eucalyptus* forest with *Banksia asplenifolia*.

Distribution.—Goffinet and Hastings (1995) described this species as an endemic of the East-Cen-

tral African mountains. Miadlikowska and Lutzoni (2000) included *P. lambinonii* in their analysis based on a collection from Australia by Leif Tibell. Our analysis confirms the conspecificity of the Australian and the African material, indicating that this species has a broader distribution in the paleotropics than formerly suspected. *Peltigera lambinonii* is here reported as new to Australia (AUSTRALIA. NEW SOUTH WALES. New England N.P., Point Lookout, 30°30′ S, 153°25′ E; elev. 1,550 m, April 18, 1981, *Tibell 12401* [H, UPS]).

5. PELTIGERA DIDACTYLA (With.) J. R. Laundon, Lichenologist 16: 217. 1984.

Lichen didactylus With., Bot. Arr. Veg. Gr. Brit. 1, 2: 718. 1776. See Vitikainen (1994) for exhaustive list of synonyms. TYPE: Dillenius, Hist. Musc. tab. 28, fig. 108. 1742. (OXF, holotype, epitype [typotype]; see Laundon 1984).

Vitikainen (1994) provided a formal description of this species. Although the original concept of this taxon is here revised, *P. didactyla* remains a taxon of great morphological and chemical variability (see below).

Chemistry.—Tridepsides are typically absent; but if present, as in several specimens from across the range, they are restricted to the soralia. We consider this chemical variation to reflect phenotypic polymorphism rather than evidence of past hybridization events.

Differentiation.—Two distinct phases of thallus development can be recognized for *P. didactyla*: one asexual, the other sexual. In its asexual phase, this species, when very young, forms weakly concave ''saucers'' that soon develop into deep, monophyllous ''pouches.'' Many specimens of *P. didactyla* appear to remain in this asexual state indefinitely; they eventually form shiny, more or less elongate lobes with cupped tips. In extreme cases the lobes measure as much as 10–15 mm long and 4–8 mm wide. Such thalli invariably bear soredia, which thus provide the only obvious means of reproduction. The upper cortex is mostly brownish, often dark chestnut or purplish brown, smooth, and somewhat shiny. Prior to syngamy, tomentum is lacking in most specimens, or is at most sparse and restricted to the vicinity of the lobe margins. Characteristic for this species is the presence, in the distal portions of the lobes, of scattered, pale, slender rhizines that usually taper to a pointed tip. Thalli with abundant short and branched rhizines occur throughout the range, but can be distinguished from other tomentose species by the shiny upper cortex.

Roughly half of the 122 specimens of *P. didactyla* observed in our study were fertile. The transition to the sexual phase seems to be marked by the development of a dense tomentose nap over the distal portion of the upper surface. Often one observes an abrupt transition from the glabrous basal portion of the lobes to the distinctly tomentose distal portions. The presence of scattered apothecial initials along the lobe tips is another signal that syngamy has occurred, as is the development of one or more thickened ''anchor'' rhizines at the base of such lobes. Soredia do not form on these fertile lobes, though they can persist indefinitely on their basal portions. Eventually the fertile lobes become erect, and the apothecia develop to their mature size, usually about 3–4(–7) mm in diameter (Vitikainen 1994). Characteristically, the apothecia are folded lengthwise, and hence appear much longer than wide. Fertile specimens of the somewhat similar *P. rufescens* have somewhat larger apothecia (5–8 mm in diameter (Martínez Moreno 1999), and to 10 mm wide according to Goffinet and Hastings 1994) that are less conspicuously folded. Pycnidia are also often present along the lobe margins.

Ecology.—*Peltigera didactyla* is a species of early succession, being found most often over soil or thin, mostly acrocarpous moss mats.

Distribution.—Despite the much narrower concept of *P. didactyla* adopted here, this species retains a cosmopolitan distribution, being known from all continents, including Antarctica (Øvstedal & Lewis Smith 2001; Vitikainen 1994).

KEY TO THE SPECIES IN THE P. DIDACTYLA COMPLEX

1. Thallus attached by a single holdfast; upper surface glabrous; triterpenoids present in medulla, tridepsides lacking ..... *P. vainioi*
1. Thallus attached by scattered rhizines; upper surface glabrous or tomentose; triterpenoids absent, tridepsides present or absent 2
2. Upper cortex glabrous throughout 3
2. Upper cortex tomentose at least along margin 4
3. Upper cortex shiny; soralia at least partly elongate, situated along lobes margins; soralia containing gyrophoric acid and methyl gyrophorate ..... *P. ulcerata*
3. Upper cortex dull; soralia mostly in rounded patches, situated toward thallus center, or at least not marginal; secondary substances typically absent ..... *P. didactyla* (young sterile forms)
4. Rhizines dark to pale brown, typically abundantly branched and forming a dense mat; upper surface often tomentose toward center ..... *P. lambinonii*
4. Rhizines whitish, to brown or black toward center, simple to flocculent, but not forming dense mat, upper surface with tomentum restricted to vicinity of lobe margin, rarely extending inward (except on fertile lobes) 5
5. Apothecia and/or apothecial initials present 6
5. Apothecia and apothecial initials absent 7
6. Apothecia common, located at the tips of upright lobes; secondary substances typically absent ..... *P. didactyla*
6. Apothecia rare, located along lobe margins;

soralia containing gyrophoric acid and methyl gyrophorate ..... *P. extenuata*

7. Lobes monophyllous, soon strongly concave and pouch-like; outermost rhizines mostly tapered to a point; on soil or thin moss ..... *P. didactyla*
7. Lobes usually polyphyllous, weakly to strongly concave, but not usually pouch-like; outermost rhizines flaring and/or flocculent; mostly on thick moss 8
   8. Upper surface smooth, more or less shiny, castaneous brown; lower surface darkening toward thallus center; rhizines often tufted hedgerow-forming toward lobe tips; pycnidia frequent along lobe margin ..... *P. castanea*
   8. Upper surface minutely roughened, dull, dove-gray to purplish brown; lower surface mostly pale; rhizines more or less flocculent throughout; pycnidia rare ..... *P. extenuata*

Aberrant samples.—As mentioned above, three of the samples included in this study had nucleotide sequences that were either incongruent with our initial identification or diverged sufficiently from those of other taxa to warrant a cautious note regarding their taxonomic status. The collection from Veracruz (Mexico; *Peltigera* sp. 1) grew on soil along a road in the botanical garden of Xalapa. The sample was tentatively identified as *P. didactyla*, based on the narrow strongly concave and upright lobes, the lack of highly branched rhizines, and the absence of tridepsides (confirmed by TLC). The lack of cortical hairs and the mainly submarginal position of the soralia would point to *P. ulcerata*, but that species has two tridepsides in its soralia, and these compounds are lacking here. The specimen differs, however, from both taxa, in that the lobes bear few rhizines, which are mostly produced toward the center of the thallus. *Peltigera vainioi* Gyelnik, a sorediate taxon from South America, is characterized by a single holdfast, but differs from the Mexican specimen by the presence of triterpenoids (Marcano et al. 1997). The sequences, which we confirmed twice, are most similar to those of *P. extenuata* (Figs. 1–4), but do nevertheless differ from the latter in the same order of magnitude as those of *P. castanea* and *P. didactyla* (Figs. 1–2). We have not yet seen other specimens that are morphologically similar or that have been collected from this area, and taxonomic interpretation of this variation should await further study.

In 1994, Goffinet collected a sorediate species of *Peltigera* growing appressed over a thin layer of mosses over a decaying tree trunk in the North Saskatchewan River valley in Edmonton (Alberta, Canada). The thallus is brownish-gray and lobed with upturned margins. The surface is undulate, with numerous soralia, and tomentum that is mostly lacking, although thin and extensive on one lobe. The medulla is thin, but the veins are distinct and some are strongly ropy, giving the lower surface a foveolate appearance. The rhizines are simple and flaring upon contact with the substrate. The lower surface is white throughout. The thallus was void of any secondary chemicals detectable by TLC. The nucleotide sequences of this population (*Peltigera* sp. 2) are similar to those of *P. didactyla* but deviate from the consistency observed among the other samples of this species. This population grew near other sorediate thalli (*Goffinet 3853,* herb. Goffinet) composed of smaller, mostly erect lobes and numerous simple rhizines. The morphology fits our concept of *P. didactyla*, a hypothesis we confirmed based on the ITS sequence. Thus, the specimen (*Goffinet 3851*) differs morphologically and ''genetically'' from typical and sympatric *P. didactyla*. It is possible that this specimen represents a distinct haplotype of *P. didactyla* not worthy of a taxonomic recognition, but the level of divergence between this sample and typical *P. didactyla* samples is similar to that observed between other species. Here too, additional observations are needed to elucidate the significance of the above morphological differences.

The population from Poland was collected from soil (sparse mosses) on the edge of a *Pinus* forest in an oligotrophic habitat. The specimen resembles *P. didactyla* in its monophyllous thalli with abundant apothecia. The upper surface bears laminal soralia that are rather sparsely distributed and sometimes fused. On many lobes the tomentum extends toward the center; it is extremely compact and whitish or the same color as the cortex, and almost unnoticeable due to the dull cortex. On other lobes, the tomentum is sparse to even lacking. The veins are well defined, often convex and broad, and have the same beige color as the thallus. The rhizines are simple with pointed ends or divided but never flocculent or penicillate. They are abundant toward the center but sparse along the margin where they are grouped along the veins in short rows. This specimen has gyrophoric acid and methyl gyrophorate. As for the preceding sample, the combination of characters is atypical for *P. didactyla*, but whether this should warrant formal taxonomic recognition as may be extrapolated from the observed sequence divergence, is not clear at this point.

Acknowledgments

This study was made possible through financial support from the University of Connecticut and from the National Science Foundation (DEB-0089633) to BG. Harry Sipman (B) and Orvo Vitikainen (H) offered help in locating the type of *P. ulcerata*. Many thanks to Kenneth G. Wright and François Lutzoni for critical reading of the manuscript. Thanks to Pak Yau Wong, CANL and Olivia Lee, UBC, for forwarding specimens for study. Finally, we are grateful to David Wagner (UCONN) for taking the picture

of *P. castanea* and to Virge Kask for assembling the plates. The sequences were generated in F. Lutzoni's lab, Departement of Biology, Duke University and in B. Goffinet's lab at the University of Connecticut.

Literature Cited

Alfaro, M. E., S. Zoller & F. Lutzoni. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Molecular Biology and Evolution 20: 255–266.

Berger, F. 1996. Neue und seltene Flechten und lichenicole Pilze aus Oberösterreich, Österreich II. Herzogia 12: 45–84.

Bergsten, J. 1999. En för Sverige ny filtlav, *Peltigera monticola*, och några andra noteringar om släktet *Peltigera*. Svensk Botanisk Tidskrift 93: 279–285.

Culberson, C. F. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. Journal of Chromatography 46: 85–93.

Esslinger, T. L. 1997. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. North Dakota State University: http://www.ndsu.nodak.edu/instruct/esslinge/chcklst/chcklst7.htm (First Posted 1 December 1997, Most Recent Update 27 August 2001), Fargo, ND.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Galloway, D. J. 1985. Flora of New Zealand Lichens. P. D. Hasselberg, Government Printer, Wellington.

——— & W. Quilhot. 1998. Checklist of Chilean lichen-forming and lichenicolous fungi [Lista patron de los liquenes y hongos liquenicolas de Chile]. Gayana Botanica 55 (2): 111–185.

Goffinet, B. & R. I. Hastings. 1994. The lichen genus *Peltigera* (lichenized Ascomycetes) in Alberta. Provincial Museum of Alberta Natural History Occasional Paper 21: 1–54.

——— & ———. 1995. Two new sorediate taxa of *Peltigera*. Lichenologist 27: 43–58.

——— & R. J. Bayer. 1997. Characterization of mycobionts of photomorph pairs in the Peltigerineae (lichenized Ascomycetes) based on ITS sequences of specifically amplified fungal ribosomal DNA. Fungal Genetics and Biology 21: 228–237.

——— & T. Goward. 1998. Is *Nephroma silvae-veteris* the cyanomorph of *Lobaria oregana*? Insights from molecular, chemical and morphological characters, pp. 41–52. *In* M. G. Glenn, R. C. Harris & M. S. Cole (eds.), Lichenographia Thomsoniana: North American Lichenology in Honor of John W. Thomson. Mycotaxon Ltd, Ithaca, NY.

——— & J. Miadlikowska. 1999. *Peltigera phyllidiosa* (Peltigeraceae, Ascomycotina), a new species from the Southern Appalachians corroborated by ITS sequences. Lichenologist 31: 247–256.

Goward, T. & B. Goffinet. 2000. *Peltigera chionophila*, a new lichen (Ascomycetes) from the western cordillera of North America. The Bryologist 103: 493–498.

Guttman, D. S. 1997. Recombination and clonality in natural populations of *Escherichia coli*. Tree 12: 16–22.

Halonen, P., M. Kukwa, J. Motiejunaite, P. Lohmus & L. Martin. 2000. Notes on lichens and lichenicolous fungi found during the XIV Symposium of Baltic Mycologists and Lichenologists in Järvselja, Estonia [Samblike ja lihhenikoolsete seente leidudest XIV Balti Mükoloogide ja Lihhenoloogide Sümpoosiumil Järvseljal, Eestis]. Folia Cryptogamica Estonica 36: 17–21.

Hasegawa, M., H. Kishino & T. Yano. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160–174.

Holtan-Hartwig, J. 1993. The lichen genus *Peltigera*, exclusive of the *P. canina* group, in Norway. Sommerfeltia 15: 1–77.

Huelsenbeck, J. P. 2000. ''MrBayes: Bayesian inference of phylogeny'', Distributed by the author (http://morphbank.ebc.uu.se/mrbayes/), University of Rochester.

Kauff, F. & F. Lutzoni. 2002. Phylogeny of Gyalectales and Ostropales (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. Molecular Phylogenetics and Evolution 25: 138–156.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Kroken, S. & J. W. Taylor. 2001. A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. Mycologia 93: 38–53.

Lambinon, J. 1969. Les Lichens. Les Naturalistes Belges, Bruxelles, Belgium.

Laundon, J. R. 1984. The typification of Withering's neglected lichens. Lichenologist 16: 211–239.

Lutzoni, F., P. Wagner, V. Reeb & S. Zoller. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Systematic Biology 49: 628–651.

Maddison, W. P. & D. R. Maddison. 2001. MacClade: analysis of phylogeny and character evolution, version 4.01. Sinauer, Sunderland, MA.

Marcano, V., A. Morale s Méndez, S. Mohali & E. Palacios-Prü. 1997. Morphological and chemical observations on *Peltigera vainioi* Gyelnik (Lichenized Ascomycetes, Peltigeraceae) from South America. Lichens 1: 1–10.

Martínez Moreno, M. I. 1999. Taxonomía del Género *Peltigera* Willd. (Ascomycetes Liquenizados) en la Península Ibérica y Estudio de sus Hongos Liquenícolas. Ruizia 15: 1–200.

Martínez, I., A. R. Burgaz & O. Vitikainen. 1997. Studies on the genus *Peltigera* in the Iberian Peninsula. II. Nova Hedwigia 64: 367–391.

Mason-Gamer, R. & E. Kellogg. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Systematic Biology 45: 524–545.

Miadlikowska, J. 1999. Rodzaj *Peltigera* (Peltigerales, Ascomycota) na tle jego wspolczesnej systematyki. Ph.D. dissertation, Gdansk University, Gdansk, Poland.

——— & F. Lutzoni. 2000. Phylogenetic revision of the genus *Peltigera* (lichen-forming Ascomycota) based on morphological, chemical, and large subunit nuclear ribosomal DNA data. International Journal of Plant Sciences 161: 925–958.

———, B. McCune & F. Lutzoni. 2002. *Pseudocyphellaria perpetua*, a new lichen from western North America. The Bryologist 105: 1–10.

———, F. Lutzoni, T. Goward, S. Zoller & D. Posada.

2003. Gap-rich regions from ITS and rDNA large subunit are incorporated into phylogenetic analyses to resolve the *Peltigera canina* species complex. Mycologia (in press).

ØVSTEDAL, D. O. & R. I. LEWIS SMITH. 2001. Lichens of Antarctica and South Georgia. Cambridge University Press, Cambridge, UK.

OZENDA, P. & G. CLAUZADE. 1970. Les Lichens: Étude Biologique et Flore Illustrée. Masson & Cie, Paris, France.

POSADA, D. 2000. How does recombination affect phylogeny estimation. Tree 15: 489–490.

——— & K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics Applications Note 14: 817–818.

SWINSCOW, T. D. V. & H. KROG. 1988. Macrolichens of East Africa. British Museum of Natural History, London, U.K.

SWOFFORD, D. L. 1998. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods), Version 4. Sinauer, Sunderland, MA.

SUZUKI, Y., G. V. GLAZKO & M. NEI. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proceedings of the National Academy of Sciences 99: 16138–16143.

THOMSON, J. W. 1950. The species of *Peltigera* of North America north of Mexico. American Midland Naturalist 44: 1–68.

———. 2003. Lichens of Wisconsin. Wisconsin State Herbarium, Madison.

VAN DEN BOOM, P. 1998. Enkele interessante vondsten van lichenen en lichenicole fungi in Nederland II. Buxbaumiella 47: 40–43.

VITIKAINEN, O. 1985. Three new species of *Peltigera*. Annales Botanici Fennici 22: 291–298.

———. 1994. Taxonomic revision of *Peltigera* (lichenized Ascomycotina) in Europe. Acta Botanica Fennica 152: 1–196.

WHITE, J. F. & P. W. JAMES. 1985. A new guide to microchemical techniques for the identification of lichen substances. Bulletin of the British Lichenological Society 57 (suppl.): 1–41.

ms. received Feb. 19, 2003; accepted April 15, 2003.