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Pseudocyphellaria perpetua, a New Lichen from Western North America

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Abstract. Pseudocyphellaria perpetua *McCune & Miadlikowska is described as a new species of lichenized fungus from Oregon, U.S.A. Morphologically similar to some forms of* P. crocata, P. perpetua *is separated from that species by a yellow medulla and predominantly marginal soralia. Comparison of ITS and LSU nrDNA sequences support taxonomic distinctness of these two species. Phylogenetic analyses were conducted on LSU and ITS nrDNA data sets separately and simultaneously using maximum parsimony and maximum likelihood as optimization criteria. All analyses except one (maximum parsimony on LSU nrDNA data alone) confirmed the monophyly of* P. perpetua. *There are two distinct groups within the* P. perpetua *clade represented by specimens sampled from near the type locality in Oregon, and specimens outside of Oregon (eastern Canada, eastern Russia and eastern U.S.A.). The genus* Pseudocyphellaria *is very likely polyphyletic, consisting of at least two highly divergent groups.*

A handful of specimens of *Pseudocyphellaria* from coastal Oregon with predominantly marginal soralia, yellow medulla, and cyanobacteria as the photobiont have puzzled lichenologists for several decades. Variously annotated as *P. aurata* (Ach.) Vainio, *P. crocata* (L.) Vainio, and *P. mougeotiana* (Delise) Vainio, none of those specimens seemed to fit descriptions of these species; especially since the revision of the *P. crocata* group by Galloway (1988, 1992, 1994) and Galloway and Arvidsson (1990). Because these specimens could belong to a new and apparently rare species that is mainly found in coastal old-growth forests, it was listed for ''survey-and-manage'' work in the Northwest Forest Plan (USDA & USDI 1994; as *P. mougeotiana*). McCune and Geiser (1997, p. 253) mentioned this problem in the discussion under *P. crocata*, referring to ''an interesting variant (perhaps another taxon?).''

The goals of this study are 1) to test the monophyletic status of *P. crocata* and the putative new *Pseudocyphellaria* species (*P. perpetua*) using molecular markers, 2) to determine the phylogenetic placement of *P. perpetua* within North American members of the genus *Pseudocyphellaria*, and 3) to propose *P. perpetua* as a new species if the result from the phylogenetic study is in agreement with our conclusions based on morphological evidence. To reach these goals we sequenced a 1.4 kb fragment at the 5′ end of the large subunit nrDNA (LSU nrDNA) and the entire internal transcribed spacer region (ITS1, 5.8S and ITS2 nrDNA) for six *Pseudocyphellaria* taxa from North America and one outgroup species. This first reconstructed phylogeny for this genus provides the foundation for future systematic work on *Pseudocyphellaria*.

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TABLE 1. Voucher specimen information and GenBank accession numbers for 16 LSU and ITS nrDNA sequences included in this study. Vouchers are deposited at OSU unless otherwise indicated.

Taxon	Voucher	GenBank accession no.	
		LSU	ITS
Pseudocyphellaria perpetua 1	U.S.A. Smoky Mts, *McDonald 394* (DUKE)	AF401960	AF401971
P. perpetua 2	RUSSIA, Primorsky Krai, *McCune et al. 24838*	AF401959	AF401972
P. perpetua 3	CANADA, NOVA SCOTIA, *Reeb 7-V-99/1* (DUKE)	AF401958	AF401973
P. perpetua 4	U.S.A. OREGON, *McCune 25752F*	AF401957	AF401974
P. perpetua 5	U.S.A. OREGON, *McCune 25752B*	AF401956	AF401975
P. perpetua 6	U.S.A. OREGON, *McCune 25752A*	AF401955	AF401976
P. perpetua 7	U.S.A. OREGON, *Miadlikowska 13.08.00-5* (DUKE)	AF401954	AF401977
P. crocata (L.) Vainio 1	U.S.A. OREGON, *McCune 25753C*	AF401953	AF401978
P. crocata 2	U.S.A. OREGON, *McCune 25753B*	AF401952	AF401979
P. crocata 3	U.S.A. OREGON, *McCune 25753A*	AF401951	AF401980
P. crocata 4	U.S.A. OREGON, *McCune 23490*	AF401950	AF401981
P. anomala Brodo & Ahti	U.S.A. OREGON, *Miadlikowska & MuCune 11.08.00-2.3* (DUKE)	AF401961	AF401970
P. anthraspis (Ach.) H. Magn.	U.S.A. OREGON, *Miadlikowska & McCune 11.08.00-2.2* (DUKE)	AF401962	AF401969
P. rainierensis Imshaug	U.S.A. OREGON, *Miadlikowska 11.08.00-2.1* (DUKE)	AF401963	AF401968
P. aurata (Ach.) Vainio	U.S.A. FLORIDA, *Rosentreter 6799*	AF401964	AF401967
Lobaria pulmonaria (L.) Hoffm.	CANADA, BRITISH COLUMBIA, *Miadlikowska 26.06.00-1.1* (DUKE)	AF401965	AF401966

MATERIAL AND METHODS

Taxon sampling.—We included five of six published species of *Pseudocyphellaria* present in North America, omitting only *P. mallota* (Tuck.) H. Magn. Except for *P. aurata*, all these species occur and were collected in Oregon. *Pseudocyphellaria perpetua* is an additional potential taxon found in western North America that was included in this study. Seven specimens of *P. perpetua* (four from Oregon) and four specimens of *P. crocata* (exclusively from Oregon) were selected for this study. One representative specimen was selected from each of the remaining four *Pseudocyphellaria* species. *Lobaria pulmonaria* was chosen as the outgroup species for a total of 16 analyzed specimens (Table 1).

Molecular data.—Small sub-terminal thallus fragments from fresh and dried herbarium specimens (the oldest one from 1990) were sampled for DNA isolation with the Purgene Kit (GENTRA Systems), following the manufacturer's protocol for filamentous fungi. DNA concentration was determined by visual comparison with a positive control (λ 100 ladder, concentration 10, 20, 40 ng) on an ethidium-bromide-stained TBE agarose gel. Symmetric polymerase chain reaction (PCR) and asymmetric PCR sequencing of the LSU and ITS nrDNA were performed as outlined in Miadlikowska and Lutzoni (2000) with the following modifications. Two newly designed fungus-specific primers, LIC15R (GGAGGAAAAGAAACCAACAG) and LIC2028 (CGCCAGGGCATCGTTCCTA) were used alternatively with LROR and LR7 (Moncalvo et al. 1993; Vilgalys & Hester 1990) in both symmetric and asymmetric PCR of the LSU nrDNA. The ITS nrDNA amplifications and cycle sequencing reactions were done using ITS1F and ITS4 primers (Gardes & Bruns 1993; White et al. 1990). Two additional primers, 5.8S and 5.8SR (Vilgalys & Hester 1990), were included for the cycle sequencing of the transcribed spacer region. All amplification reactions (50 μl final volume) contained one μl of 100× Bovine Serum Albumin (BSA; BioLabs). Sequencing reactions were performed using Big Dye Terminator Cycle Sequencing kit (ABI PRISM, Perkin-Elmer, Applied Biosystems). The LSU and ITS nrDNA sequence fragments were assembled using Sequencher 3.0 and optimized by eye. Delimitation of the internal spacers and the 5.8S gene was obtained by comparison with complete ITS sequences from closely related taxa within Peltigerineae (Miadlikowska & Lutzoni, per. comm.) and sequences available in GenBank.

Phylogenetic analyses.—Phylogenetic analyses were performed using maximum likelihood and maximum parsimony as optimization criteria, as implemented in PAUP* 4.0b4a (Swofford 1998). The following six analyses were carried out on 16 LSU and 16 ITS nrDNA sequences: maximum likelihood and maximum parsimony analyses on LSU nrDNA data set alone (ML1 and MP1), maximum likelihood and maximum parsimony on ITS nrDNA data set alone (ML2 and MP2), and maximum likelihood and maximum parsimony analyses on LSU and ITS nrDNA combined data set (ML3 and MP3). Constant sites were removed from all analyses.

The unambiguously aligned parts of the LSU and ITS nrDNA alignments were subjected to symmetric step matrices constructed as follows. The 'Show character status/full details/hide excluded characters' options from the ''Data'' menu in PAUP* were selected. From the resulting table, the column 'States' (showing all nucleotide states found at each of the unambiguously aligned and non-constant sites) was saved as a separate plain text file. This file was used as an input file for the program STMatrix 2.1 (written by S. Zoller and available upon request to S.Z. or F.L.), which generates a step matrix (in Nexus format) by calculating the probabilities of reciprocal changes from one state to another (including gaps) and converting them to a cost of changes using the negative natural logarithm of the probability (Felsenstein 1981; Wheeler 1990). The unambiguously aligned portions of the ITS nrDNA data set was subjected to three separate step matrices corresponding to ITS1, 5.8S, and ITS2 regions. The unambiguously aligned sites from the LSU nrDNA were subjected to one specific step matrix.

Signal from ambiguously aligned portions of the align-

ment was integrated into maximum parsimony analyses (MP1–3), without violating positional homology, using the program INAASE 2.3b (Appendix 1; Lutzoni et al. 2000). The weights for transitions and transversions needed for INAASE to generate step matrices were obtained by calculating the average cost of these changes as included in step matrices for unambiguous parts of their respective alignments (i.e., transitions = 1.0, transversions = 3.0 for LSU nrDNA; transitions = 1.0, transversions = 2.4 for ITS). Because of the high level of variation among sequences within ambiguous regions compared to unambiguous regions, costs for indels were set to be equal to cost of transitions. For maximum parsimony analyses, gaps from the unambiguous portions of the alignments were recognized as a fifth character state. All weighted maximum parsimony analyses (MP1–3) 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.

To determine which model of nucleotide substitution with the least number of parameters best fit our data, hierarchical likelihood ratio tests (HLRTs) were performed for LSU and ITS data sets alone and for the LSU and ITS combined data set using the program Modeltest 3.04PPC (Posada & Crandall 1998). Maximum likelihood analyses (ML1–3) were implemented as heuristic searches with 100 random-addition-sequence replicates, TBR branch swapping, Multrees option in effect, saving all trees and collapsing zero-length branches.

Branch support for MP and ML trees was estimated by bootstrap analyses (Felsenstein 1985) with full heuristic searches, 1,000 bootstrap replicates, two random-addition-sequence per bootstrap replicate and by saving all trees. Congruence between data partitions was tested by inspecting bootstrap scores above 70% resulting from the separate MP analyses (Mason-Gamer & Kellog 1996; Miadlikowska & Lutzoni 2000). If bootstrap analyses on two different partitions provided support ≥70% for two different phylogenetic relationships for the same set of taxa, this would be considered as a potential incongruence between two partitions. For the ML analysis on the combined LSU and ITS nrDNA data set (ML3), in addition to bootstrap support, posterior probability for each node using a Bayesian statistical procedure with a Markov-Chain Monte Carlo (B/MCMC) sampling method (Larget & Simon 1999) was calculated as implemented in MrBayes 1.11 (Huelsenbeck; http://brahms.biology.rochester.edu/software.html). One out of every 100 trees was sampled with B/MCMC for 500,000 generations with kappa and DNA substitution parameters estimated during the search. The majority rule consensus tree was computed with PAUP* on the last 4,000 tree topologies out of the 5,000 sampled with B/MCMC. Posterior probability support for bipartitions was considered statistically significant when $p \geq 0.95$.

Results and Discussion

DNA sequence variation.—The final alignment for the 16 LSU nrDNA sequences consisted of 1,342 sites. A total of three ambiguously aligned regions were delimited, resulting in the exclusion of 13 nucleotide sites. The presence of one spliceosomal intron site in the sequence of *Lobaria pulmonaria* (Bhattacharya et al. 2000; Zoller et al. 1999) required the exclusion of 74 additional characters. Constant sites (1,194) were excluded for a total of 61 unambiguously aligned sites included in the ML1 analysis. The three ambiguous regions provided three additional (INAASE coded) characters for the MP1 analysis for a grand total of 64 non-constant characters. Of these, 30 were parsimony informative. The ML1 analysis was performed using the HKY substitution model (Hasegawa et al. 1985) with the following parameters: different base frequencies (A = 0.1415, C = 0.3451, G = 0.1635, T = 0.3499), ti/tv ratio = 9.0120 and equal rates for all sites.

LSU sequence differences within species ranged from 0–6 for *P. perpetua*, whereas all sequences of *P. crocata* were identical (Fig. 1). All individuals of *P. perpetua* sampled from Oregon (4–7) had identical LSU nrDNA sequences. The three *P. perpetua* specimens outside of Oregon showed more variation with 2–6 pairwise differences. The LSU sequence of *P. perpetua* 1 collected in the Great Smoky Mountains (Table 1) is identical to sequences from specimens of this species collected in the Pacific Northwest, except for one point mutation within an ambiguous region (Fig. 1). Among-species differences ranged from 3–6 between *P. perpetua* and *P. crocata*, from 20–35 between *P. perpetua* or *P. crocata* and the other four *Pseudocyphellaria* species included in this study, and from 1–46 among the latter four *Pseudocyphellaria* species.

The final alignment for the 16 ITS nrDNA sequences consisted of 503 sites. A total of 16 ambiguously aligned regions were delimited (Appendix 1), resulting in the exclusion of 124 nucleotide sites. Of the remaining 379 sites, 321 were constant for a total of 58 nucleotide sites included in the ML2 analysis. The ambiguous regions provided 16 additional INAASE coded characters (Appendix 1) for a total of 74 characters included in the MP2 analysis. Of these, 47 were parsimony informative. The ITS sequences varied in total length from 466 to 485 nucleotides (ITS1: 160–165; 5.8S: 158; ITS2: 148–162) among the 16 sequences included here, but sequences were identical in length for all specimens within *P. perpetua* and *P. crocata*, as well as between *P. anomala* and *P. anthraspis*. As for LSU nrDNA, ITS sequences were identical among *P. perpetua* specimens from Oregon (*P. perpetua* 4–7; Fig. 2). The ITS sequences from the other three specimens of this taxon collected outside of Oregon (*P. perpetua* 1–3) were much more similar to the sequences from specimens of this species collected in the Pacific Northwest than to any other ITS sequences in this study. The ITS sequences of *P. crocata* were identical, except for one point mutation for *P. crocata* 1. *Pseudocyphellaria perpetua* was separated from *P. crocata* by a total

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *P. perpetua* 1	-	3	3	1	1	1	1	3	3	3	3	23	23	32	25	27
2. *P. perpetua* 2	3	-	6	4	4	4	4	5	6	6	6	26	26	35	28	30
3. *P. perpetua* 3	2	5	-	2	2	2	2	6	6	6	6	25	25	35	28	30
4. *P. perpetua* 4	0	3	2	-	0	0	0	4	4	4	4	23	23	33	26	28
5. *P. perpetua* 5	0	3	2	0	-	0	0	4	4	4	4	23	23	33	26	28
6. *P. perpetua* 6	0	3	2	0	0	-	0	4	4	4	4	23	23	33	26	28
7. *P. perpetua* 7	0	3	2	0	0	0	-	4	4	4	4	23	23	33	26	28
8. *P. crocata* 1	3	5	5	3	3	3	3	-	0	0	0	20	20	35	25	26
9. *P. crocata* 2	3	6	5	3	3	3	3	0	-	0	0	20	20	35	24	26
10. *P. crocata* 3	3	6	5	3	3	3	3	0	0	-	0	20	20	35	24	26
11. *P. crocata* 4	3	6	5	3	3	3	3	0	0	0	-	20	20	35	24	26
12. *P. anomala*	20	23	22	20	20	20	20	17	17	17	17	-	1	44	40	15
13. *P. anthraspis*	21	24	23	21	21	21	21	18	18	18	18	1	-	44	40	15
14. *P. rainierensis*	27	30	29	27	27	27	27	30	30	30	30	37	38	-	41	42
15. *P. aurata*	21	24	23	21	21	21	21	20	20	20	20	35	34	34	-	46
16. *Lobaria pulmonaria*	22	25	24	22	22	22	22	21	21	21	21	11	10	36	40	-

FIGURE 1. Uncorrected pairwise differences among LSU nrDNA sequences included in the MP1and MP3 analyses. 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 *P. perpetua* and *P. crocata*. A second box within *P. perpetua*, delimits pairwise comparisons among specimens collected in Oregon.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *P. perpetua* 1	-	2	2	8	8	8	8	47	46	46	46	78	78	92	98	95
2. *P. perpetua* 2	1	-	0	7	7	7	7	46	45	45	45	78	78	81	98	94
3. *P. perpetua* 3	1	0	-	6	6	6	6	46	45	45	45	76	76	79	97	92
4. *P. perpetua* 4	2	2	2	-	0	0	0	46	45	45	45	76	76	79	95	83
5. *P. perpetua* 5	2	2	2	0	-	0	0	46	45	45	45	76	76	79	95	83
6. *P. perpetua* 6	2	2	2	0	0	-	0	46	45	45	45	76	76	79	95	83
7. *P. perpetua* 7	2	2	2	0	0	0	-	46	45	45	45	76	76	79	95	83
8. *P. crocata* 1	15	15	15	15	15	15	15	-	1	1	1	61	62	73	91	74
9. *P. crocata* 2	15	15	15	15	15	15	15	0	-	0	0	61	62	74	90	74
10. *P. crocata* 3	15	15	15	15	15	15	15	0	0	-	0	61	62	74	90	74
11. *P. crocata* 4	15	15	15	15	15	15	15	0	0	0	-	61	62	74	90	74
12. *P. anomala*	22	22	22	20	20	20	20	17	17	17	17	-	0	75	79	43
13. *P. anthraspis*	22	22	22	20	20	20	20	17	17	17	17	0	-	75	78	43
14. *P. rainierensis*	29	29	29	27	27	27	27	25	25	25	25	27	27	-	76	88
15. *P. aurata*	38	39	39	38	38	38	38	35	35	35	35	31	31	30	-	92
16. *Lobaria pulmonaria*	26	26	26	24	24	24	24	21	21	21	21	12	12	32	38	-

FIGURE 2. Uncorrected pairwise differences among ITS nrDNA sequences included in the MP2 and MP3 analyses. 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 *P. perpetua* and *P. crocata*. A second box within *P. perpetua* delimits pairwise comparisons among specimens collected in Oregon.

of 45–47 differences, only 15 of which were within unambiguous portions of the alignment. For the ML2 analysis, the K80 (Kimura 1980) model of substitution was selected by Modeltest and implemented with the following parameters: equal base frequencies, ti/tv ratio = 3.1388 and equal rates for all sites.

Phylogenetic relationships.—Four equally most likely trees were obtained from the ML1 analysis (Fig. 3A), and two equally most parsimonious trees resulted from the MP1 analysis (Fig. 3B); CI (excluding uninformative characters) = 0.8951, RI = 0.8678. The ML1 and MP1 topologies (Fig. 3A, B) were similar. Together, *P. perpetua* and *P. crocata* formed a monophyletic group, but with bootstrap support (BS) < 50% for ML1, and = 72% for MP1. Only the ML1 analysis of the LSU nrDNA revealed *P. perpetua* as being monophyletic (BS = 54%). In both analyses, *P. aurata* was the closest relative to the *crocata-perpetua* clade, forming a paraphyletic clade with *P. rainierensis* with complementary support from the ML1 and MP1 analyses (BS = 65% and 80% for ML1; 89% and 55% for MP1). Specimens of *Pseudocyphellaria anomala* and *P. anthraspis* were shown to be monophyletic by both the ML1 and MP1 analyses (BS < 50% and = 91%, respectively).

A single most optimal tree (when maximum branch length equal to zero are collapsed) was revealed from each phylogenetic analysis of the ITS region using maximum likelihood (ML2) and maximum parsimony (MP2); CI (excluding uninformative characters) = 0.8866, RI = 0.9236 (Fig. 3C, D). Phylogenetic relationships among species were highly supported by bootstrap analyses with BS mostly greater than 90%. *Pseudocyphellaria perpetua* and *P. crocata* formed two distinct monophyletic entities with bootstrap values of 98% and 91%, respectively, on ML2, and 100% on MP2. Specimens of *P. perpetua* collected outside of Oregon (1–3) formed a monophyletic group with BS = 75% (ML2) and 96% (MP2). Specimens of *P. perpetua* collected near the type locality in Oregon were also shown as monophyletic entity, but with MP2 only. Both analyses revealed *P. crocata* as monophyletic, although its relationship to *P. perpetua* differed depending on the analysis. According to the ML2 analysis, *P. crocata* was sister to the *P. rainierensis-P. aurata* clade (BS = 57%), whereas the MP2 analysis showed *P. crocata* sharing a most recent common ancestor with *P. perpetua* (BS = 92%). Contrary to LSU nrDNA analyses, *P. rainierensis* and *P. aurata* are sister species based on ITS nrDNA data. This monophyletic relationship was highly supported (BS = 93% for ML2 and 84% for MP2). *Pseudocyphellaria anomala* and *P. anthraspis* were consistently shown to be monophyletic and outside the remaining *Pseudocyphellaria* species included in this study.

Although topologies derived from LSU and ITS nrDNA were different when analyzed separately, conflict (using the 70% bootstrap support criterion) was detected only when using maximum parsimony as the optimization character. To confirm that the source of conflict between partitions resulted from an analytical artifact rather than data sampled from two different gene trees, we removed the INAASE coded characters from the data set subjected to the MP2 analysis. Without these coded characters the data sets subjected to the MP and ML analyses were identical. Under these conditions the conflict persisted using maximum parsimony as the optimization criterion. We concluded that the apparent conflict was analytically based and was not due to two bipartitions sampling different phylogenies because there was no conflict when using ML. On this basis we combined the LSU with the ITS nrDNA data sets and analyzed them simultaneously with maximum likelihood (ML3) and maximum parsimony (MP3).

One most-parsimonious tree (tree length = 465.72 steps, CI [excluding uninformative characters] = 0.8713, RI = 0.9095) resulted from this combined analysis (MP3 result not shown). Phylogenetic relationships based on the MP3 analysis were most similar to the ITS data set subjected to MP2 analysis (Fig. 3D). The maximum likelihood analysis of the combined LSU and ITS nrDNA data set (ML3) was implemented using the HKY model of nucleotide substitution (Hasegawa et al. 1985; ti/tv ratio = 5.0596) with unequal base frequencies (A = 0.1725, C = 0.3209, G = 0.2042, T = 0.3024), and equal rates for all sites. The single most likely tree resulting from ML3 (Fig. 4), reflects phylogenies obtained from maximum parsimony search on the combined data set (MP3) (not shown) and on the ITS nrDNA data set alone (MP2; Fig 3D).

All internodes, except one, were almost equally well supported by bootstrap values derived from ML3 and MP3 (see circled BS values on Fig. 4). Bootstrap support was generally higher with maximum parsimony than with maximum likelihood because of the additional 19 INAASE characters. The phylogenetic signal from the 19 ambiguously aligned regions can currently be accommodated only within maximum parsimony framework. When these INAASE characters were removed from the MP3 analysis, the bootstrap value for the internode supporting the sister relationship of *P. perpetua* with *P. crocata* (see circled internode in Fig. 4) dropped from 99 to <50%, the latter value being closer to the low support (51%) derived from the ML3 analysis. Posterior probability values reached a signifi-

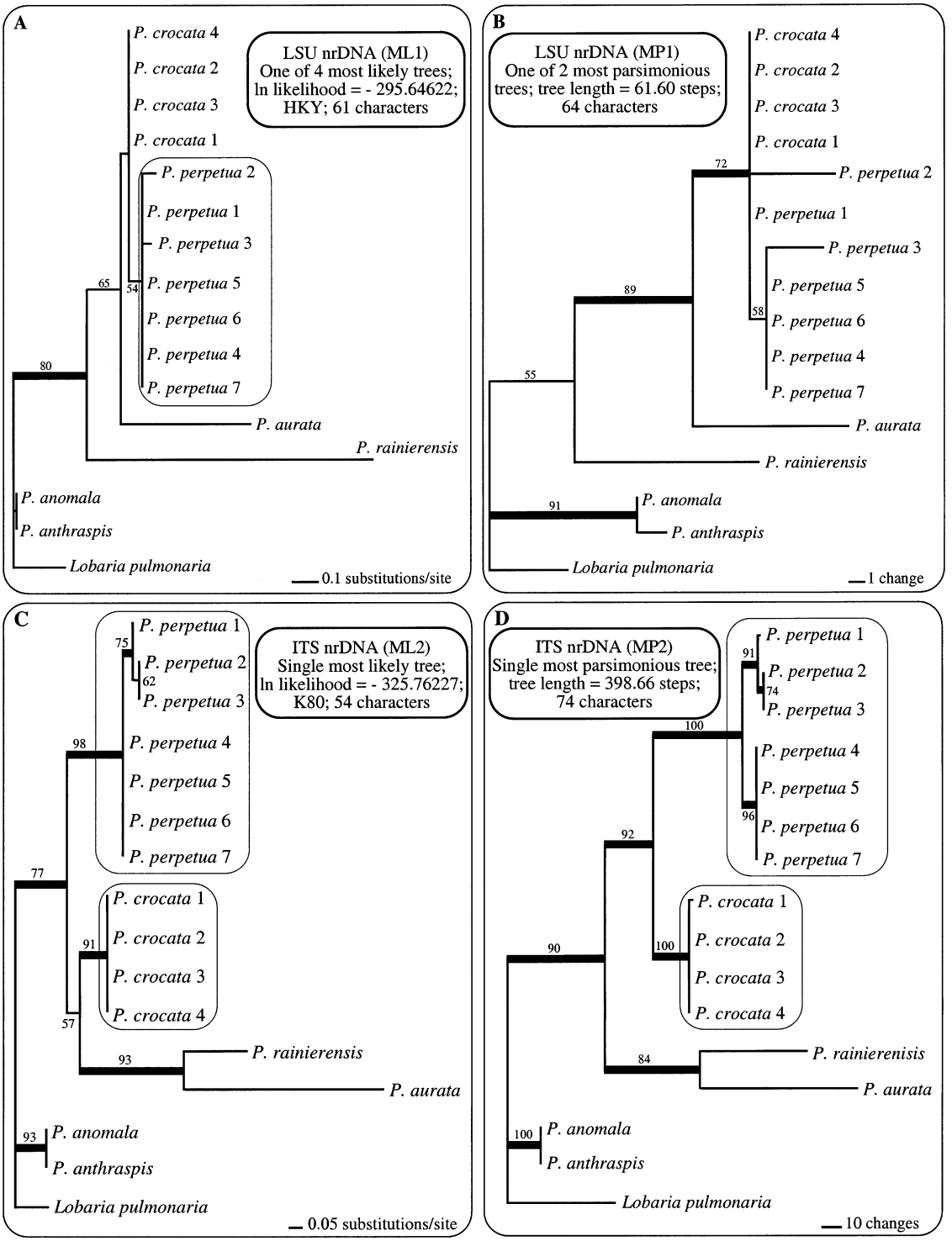

FIGURE 3. Phylograms representing phylogenetic relationships among 16 individuals from five known *Pseudocyphellaria* species, the putative species *P. perpetua* and the outgroup taxon (*Lobaria pulmonaria*) as revealed by maximum likelihood (A and C) and maximum parsimony (B and D) analyses of the LSU (A and B) and ITS regions of the nrDNA (C and D). Bootstrap support >50% shown above branches. Boostrap support >70% represented by thicker branches. — A. One of four equally most likely trees (part of two islands hit 100 times out of 100 random-addition-sequence) based on the LSU nrDNA alone. — B. One of two equally most parsimonious trees (part of one

cant level of $p > 0.95$ for three out of six recognized bipartitions (*P. perpetua*, *P. anomala* + *P. anthraspis*, and *Pseudocyphellaria* without *P. anomala* + *P. anthraspis*) (Fig. 4). The two major lineages within *Pseudocyphellaria*, separated by the longest internode, represent two distinct non-sister groups (putative genera), as revealed from our analysis of the nuclear LSU rDNA, but for an extensive set of taxa from the Peltigerineae (results not shown). The *Pseudocyphellaria* lineage that includes *P. crocata* is sister to a lineage of *Sticta* that includes *S. fuliginosa*, *S. limbata*, and *S. weighelii* s.l. The *Pseudocyphelaria anomala* + *P. anthraspis* lineage is sister to a monophyletic group consisting of the *Pseudocyphelaria* + *Sticta* group mentioned above, with a subset of the genus *Lobaria*, suggesting that *Lobaria* is also non-monophyletic.

Pseudocyphellaria perpetua is clearly monophyletic and distinct from *P. crocata*. The monophyly of both taxa was supported by bootstrap values ≥90%. *Pseudocyphellaria perpetua* had 100% bootstrap support from the ML3 and MP3 analyses, as well as a posterior probability of 1.00 with the B/MCMC method. The monophyly of our sample of *P. crocata* is supported by bootstrap values of 100% with MP3 and 90% with ML3. Because the B/MCMC approach is maximum likelihood based, and currently cannot accommodate phylogenetic signal found in the 19 ambiguously aligned regions, this could explain why the monophyly of *P. crocata* was not found to be significant using B/MCMC. Based on these results we propose the recognition of *Pseudocyphellaria perpetua* as a new species of lichen-forming ascomycete. Morphological and chemical characters corroborate delimitation of this new species based on molecular data.

PSEUDOCYPHELLARIA PERPETUA McCune & Miadlikowska, *sp. nov*. Front Cover

Thallus foliosus, laxe adnatus, corticola, ad 10 cm diametro, griseus ad brunneus, lobis sublinearibus, 2–10 mm latis, sorediatus; medulla flavescens; subtus eburneus ad brunneus, dense tomentosus, pseudocyphellae flavida. Apothecia ignota. Cortex K-, KC-, P-, UV+ sublazulinus; medulla K+ flavida, C+ aurantiaca, KC+ aurantiaca, P+ aurantiaca, UV- vel rubiginosa.

TYPE: U.S.A. OREGON. Lane Co., Gwynn Creek trail, within 3 km of Pacific Ocean, Siuslaw National Forest, 44°16.3′ N 124°06′ W, 40–110 m, old-growth and mixed-age *Picea sitchensis* forest, with *Pseudotsuga menziesii* and *Tsuga heterophylla*, on fallen conifer twigs and branches, December 2000, *McCune 25752* (holotype OSC; isotypes BM, CANL, DUKE, US). PARATYPES. Coos Co., near Eel Creek Campground, 2–4 km from Pacific Ocean, 43°35.3′ N 124°11.4′ W, elevation 15 m, dunes forested with *Pinus contorta*, *Picea sitchensis*, *Arctostaphylos* sp., and *Myrica californica*, *Miadlikowska 13.08.00–5* (DUKE).

Thallus foliose, loosely adnate, to 10 cm in diameter; upper surface gray to brown, glabrous, ± glossy, often with pale angular to reticulate maculae; lobes sublinear, smooth to weakly reticulate-ridged, mostly 2–10 mm broad, margins often slightly upturned; soralia marginal and laminal, yellowish; medulla pale to deep yellow; lower surface corticate, cream to tan or brownish, tomentose, the tomentum cream to dark brown; pseudocyphellae yellow, conspicuous, on the lower surface. Apothecia not seen. Pycnidia not seen. Photobiont–cyanobacteria.

Taxonomy and relationships.—*Pseudocyphellaria crocata* and *P. perpetua* are similar in their yellowish soredia and rich brown to gray thallus. However, the soralia in *P. perpetua* are mostly marginal, while those of *P. crocata* are mainly laminal, but also marginal in part. Furthermore, the medulla (apart from the soralia) of *P. crocata* is white while it is yellow in *P. perpetua*. Although the color of the upper cortex varies with exposure to light in both species, it tends toward gray in *P. perpetua* and brown in *P. crocata*. In mixed populations on the Oregon coast, the two species are readily distinguished at arm's length. Based on our limited taxon sampling *P. crocata* shares a most recent common ancestor with *P. perpetua* (Fig. 3A,B,D and Fig. 4).

The yellow medulla and marginal soralia have led people to identify specimens of *P. perpetua* as *P. aurata*, but that species has a green photobiont. Furthermore, it is on a distinct and distantly related lineage based on LSU and ITS nrDNA (Fig. 4).

Two clades are recognizable within *P. perpetua*, based on ITS nrDNA and the combined ITS and LSU nrDNA data sets (Fig. 3C,D and Fig. 4). One clade was composed only of the Oregon samples. At present we choose to consider this intraspecific variation. Morphological and chemical characters apparently do not support considering these clades as separate species.

Chemistry.—The upper cortex is with spot tests negative except cortex UV+ pale bluish; medulla and soralia K+ somewhat deeper yellow, C+ or-

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island hit 1,000 times out of 1,000 random-addition-sequence) based on the LSU nrDNA alone, CI (excluding uninformative characters) = 0.8542, RI = 0.8678. — C. Single most likely tree (hit 100 times out of 100 random-addition-sequence) based on the ITS nrDNA alone. — D. Single most parsimonious tree (hit 1,000 times out of, 1,000 random-addition-sequence) based on the ITS nrDNA alone, CI (excluding uninformative characters) = 0.8866, RI = 0.9236. When monophyletic, a thin line was used to circumscribe *P. perpetua*, and its morphologically most similar species *P. crocata*.

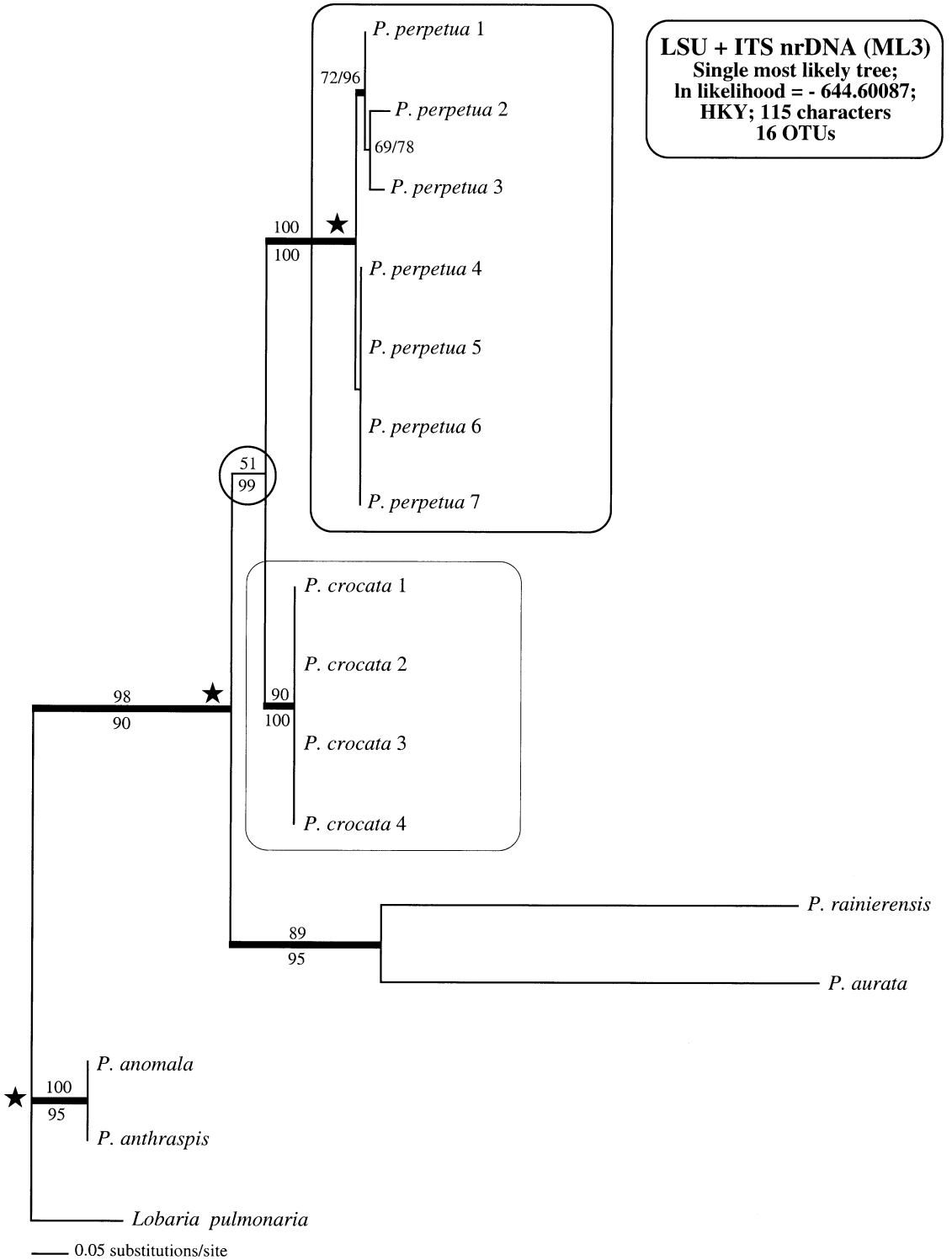

FIGURE 4. Single most likely tree resulting from a combined analysis of LSU and ITS nrDNA data sets. Bootstrap supports greater than 50% are provided for each internode, which are shown with thicker lines if BS is ≥70%. Bootstrap values from MP3 are shown below each internal branch or after a slash. Bootstrap values associated with ML3 are above internal branches or before a slash. Stars indicate nodes that received significant posterior probability support at p level ≥0.95 with B/MCMC. Circle indicates a node with the greatest difference between BS support derived from the MP3 and ML3 analyses.

angish (fleeting), KC+ orangish (fleeting), P+ orange, UV- or dark reddish; containing unidentified pulvinic acid derivatives and terpenoids. The terpenoid composition differed from the pattern observed for *P. crocata* (under study by J. M.)

Etymology.—The epithet refers to Cape Perpetua, a prominent headland on the Oregon coast, named for St. Perpetua by Capt. James Cook in 1778. The only large populations known for this species in Oregon occur in the Cape Perpetua area. The epithet also alludes to the long historical continuity of the old *Picea sitchensis* forests that it frequents, and which had already stood for centuries at the time of Cook's discovery.

Ecology.—So far the species is known only from oceanic and suboceanic areas in North America and Far East Russia. It occurs on both conifers and hardwoods. The closed *Picea sitchensis* forests on the Oregon coast are often so dense that it grows only in the upper to mid-canopy. It is most frequently encountered on fallen branches.

In Oregon, associated lichens included a diverse array of epiphytic cyanolichens (*Lobaria, Nephroma, Parmeliella parvula, Peltigera collina, Polychidium contortum, Pseudocyphellaria,* and *Sticta*), as well as various green algal lichens (e.g., *Hypogymnia, Platismatia,* and *Sphaerophorus*).

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APPENDIX 1.—Alignment of ITS nrDNA sequences from one *Lobaria* and 16 *Pseudocyphellaria* individuals. Boxes delimit homologous ambiguously aligned regions of the alignment. Numbers on the right side of each box represent character states for a new character resulting from coding ambiguous regions using INAASE (Lutzoni et al. 2000). -, gaps; *, variable site; #, INAASE character for ambiguous regions. All sites without the * symbol on the line below the alignment are constant.