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Parmelina yalungana resurrected and reported from Alaska, China and Russia

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Abstract. *Parmelina quercina* is a well-studied foliose macro-lichen found on rocks and trees in the Northern Hemisphere. Recent studies support multiple species within *P. quercina* based on material from Europe, North America and western Asia. The identities of *Parmelina quercina* s.lat. reported from eastern Asia and Alaska remain unknown. We compared DNA sequences, secondary chemistry and morphological traits of *Parmelina* from Alaska, Russia and China. These data support the resurrection of *Parmelina yalungana* to accommodate eastern Asian and Alaskan material. *Parmelina yalungana* differs from congeners in ascospore dimensions, geographic range and molecular data from three gene loci. We place *P. yalungana* in the phylogenetic context of the *P. quercina* group using DNA from Alaskan and Russian material.

Keywords. Ascomycota, ascospore size, epiphyte, Parmeliaceae.

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Parmelina Hale (Parmeliaceae) once contained over 40 species (Hale 1976), many of which were later transferred to *Canomaculina* Elix & Hale, *Myelochroa* (Asahina) Elix & Hale, *Parmelinella* Elix & Hale, *Parmelinopsis* Elix & Hale and *Parmotremopsis* Elix & Hale (Elix & Hale 1987). Morphological differences between *Parmelina* and those genera are described elsewhere (e.g., Elix & Hale 1987; Hale 1974) but two recently described genera, *Austroparmelina* A. Crespo, Divakar & Elix and *Remototrachyna* Divakar & A. Crespo are similar and warrant discussion. *Austroparmelina* is restricted to the Southern Hemisphere and differs from *Parmelina* by its thicker excipular cell walls, no dark pigment where the exciple meets the surface of the apothecium, more complex chemistry, sparse cilia and larger ascospores

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(Crespo et al. 2010). *Remototrachyna* and *Parmelina* are both found in the Northern Hemisphere in eastern Asia. *Remototrachyna* differs from *Parmelina* by having dichotomously branching rhizines and distinctive, thick excipular cell walls and larger ascospores (Divakar et al. 2010). As currently defined, *Parmelina* comprises grayish-white foliose macro-lichens distinguished by 1) the presence of lecanoric or gyrophoric acids in the medulla, atranorin in the cortex and the lack of usnic acid; 2) irregularly branching, linear to sublinear lobes that have cilia frequently in the lobe axils or margins (Hale 1974); 3) simple, black rhizines (Divakar et al. 2010); and 4) thin-walled excipular cells that become darkly pigmented at the edge of the apothecium (Crespo et al. 2010).

Since Hale's monograph, several new species of *Parmelina* have been described (Argüello et al. 2007; Clerc & Truong 2008; Núñez-Zapata et al. 2011; Wang et al. 2000), with eight species currently accepted. All grow on trees and rocks in areas of temperate climates in the Northern Hemisphere. *Parmelina* is a well-supported, monophyletic clade sister to *Myelochroa* (Argüello et al. 2007; Crespo et al. 2011; Divakar et al. 2006), with their common ancestor sister to the clade containing *Bulbothrix*, *Parmelinella* and *Remototrachyna* (Crespo et al. 2011).

Morphologically, *Parmelina* species can be divided into two groups: isidiate species and those without isidia. Isidiate species include *P. cryptotilacea*, *P. pastillifera* and *P. tilacea*, which are not discussed in detail here. All species lacking isidia were, until recently, called *Parmelina quercina* (Willd.) Hale (Argüello et al. 2007; Hale 1976). *Parmelina quercina* s.lat. formerly encompassed species from geographically disjunct populations worldwide that were morphologically variable yet superficially similar until Hale (1976) brought them under the umbrella of the single species concept of *P. quercina*. More recently, *P. quercina* has been treated as a cryptic species complex (Crespo & Pérez-Ortega 2009), whose members differ in ascospore dimensions, upper surface maculation and geographic distribution (Argüello et al. 2007; Clerc & Truong 2008). Based on these morphological characteristics and molecular data, Argüello et al. (2007) argued *P. quercina* s.lat. is actually composed of three taxa: *P. quercina* s.str., *P. coleae* Argüello & A. Crespo and *P. carporrhizans* (Taylor) Poelt & Vezda. They moved North American specimens of *P. quercina* to *P. coleae,* which is distinguished morphologically by an emaculate upper surface and a larger ascospore length/width ratio (Argüello et al. 2007). Two different studies showed *P. carporrhizans* includes European and Macaronesian material with a maculate upper surface (Argüello et al. 2007; Clerc & Truong 2008). *Parmelina quercina* s. str. now only applies to European material with an emaculate upper surface growing epiphytically (Argüello et al. 2007). Clerc & Truong (2008) restored *P. atricha*, another old synonym of *P. quercina*, to accommodate saxicolous European material with small, imbricate central lobes. Wang et al. (2000) described the only sterile member of the *P. quercina* complex, *P. gyrophorica,* from western China, known only from the type locality. It is distinguished from other species in the *P. quercina* complex by having gyrophoric acid, rather than lecanoric acid, as the major medullary compound. Today, the genus *Parmelina* contains eight species worldwide, including *P. atricha*, *P. carporrhizans*, *P. coleae*, *P. cryptotiliacea, P. gyrophoria*, *P. pastillifera*, *P. quercina* and *P. tilacea*.

In 2007, two *Parmelina* specimens morphologically similar to the *P. quercina* group were collected during fieldwork by National Park Service staff in Denali National Park and Preserve, Alaska. We obtained additional *P. quercina* s.lat. material from elsewhere in Alaska, central Russia and western China, including the type specimen of *Parmelia yalungana* (Zahlbr.) (syn. *Parmelina quercina* in Hale (1976)). *Parmelia yalungana* was a species from high elevation forests in Sichuan Province that was synonymized with *P. quercina* by Hale (1976). In this study, we compare our observations of the *P. yalungana* type to Alaskan, Russian and Chinese specimens of *P. quercina* s.lat. in the context of the molecular phylogeny of Argüello et al. (2007) and the morphological characters they identified as informative. Molecular data confirms Alaskan and Russian specimens are not assignable to currently recognized species but consistent with characters of the type of *P. yalungana*. Accordingly, we resurrect the name *P. yalungana* comb. nov. for these specimens.

Materials and Methods

Morphological and chemical methods. Hand cut thin sections of apothecia were examined in distilled water or 10% KOH using standard light microscopy methods. Twenty ascospores from each specimen were measured in distilled water from a minimum of two apothecia. Measurements are reported as (minimum) average (maximum). Thin-layer chromatography (TLC) was performed in solvent systems A and C following Culberson et al. (1981) on aluminum-backed silica gel plates (Merck 5554/7 Silica gel 60 F_{254}). For high-performance liquid chromatography (HPLC), lichen compounds were extracted from liquid nitrogen ground specimens overnight in acetone at 4°C. The dried supernatant was dissolved in methanol, and analyzed using HPLC. We used an Agilent Technologies 1200 series integrated system with a Zorbax Eclipse XDB8-CB column (4.6 150 mm, 5 lm) regulated at 30°C, spectrometric detectors operating at 210, 254, 280, 310 nm, and a flow rate of 0.7 ml/min. Following established protocols (Feige et al. 1993; Lumbsch 2002), two mobile phases, A and B, were used: 1% aqueous orthophosphoric acid (A) and methanol (B). The run started with 30% B for 1 min and was raised to 70% B within 15 min of the start time, then to 100% B during an additional 15 min, followed by isocratic elution in 100% B for the final 20 min. Mobile phase B was decreased to 30% within 1 min and the column was flushed with 30% B for 15 min following each run. Using Agilent Chemstation software, UV spectra of each peak were recorded and computer-matched against a library of ultraviolet spectra from authentic metabolites derived under identical conditions. The correlation of UV spectra with the standards in the library was greater than 99.9% and the retention time matched within 30 seconds for each substance identified. Scanning electron micrographs of the upper surface of 5–10 mm pieces of thallus were acquired using a FEI Quanta 600 FEG scanning electron microscope.

DNA extraction, PCR amplification and sequencing. DNA extractions were performed by grinding thallus portions with a drill equipped with a plastic Eppendorf pestel in 50 µl CTAB buffer, transferred to a FastDNA lysing matrix A tube (Bio101) and combined with an additional 400 µl CTAB buffer. The lysing matrix tube was then placed in a fast prep machine and macerated twice for 20 sec. After maceration, the tubes were placed in a water bath at 60°C for 20 min and then centrifuged for 10 min at 14,000 rpm. The 400 µl of supernatant was then transferred to 1.5 ml tubes, mixed with 500 µl chloroform:isoamyl alcohol (24:1), and centrifuged at 14,000 rpm for 20 min., after which 300 µl of supernatant was removed. DNA in the supernatant was then further cleaned using the GeneCleanIII Kit following the recommended protocol.

PCR amplifications were performed for the internal transcribed spacer region of nuclear ribosomal DNA (ITS, including ITS1, 5.8s and ITS2), large subunit nuclear ribosomal DNA (nuLSU), and mitochondrial ribosomal small subunit DNA (mtSSU) with the primers ITS1F (Gardes & Bruns 1993)/ITS4 (White et al. 1990), LR5/LR0R (Vilgalys 2003) and mtSSU1/mtSSU3R (Zoller et al. 1999), respectively. PCR reactions were performed in either an iCycler or MyCycler (BioRad, Hercules, CA) using MasterAmp 2X PCR premix E (Epicenter, Madison WI) and Novagen Taq polymerase. Reaction conditions were the same as those used in Johnson et al. (2009). PCR products were cleaned using the GeneClean III kit following the manufacturer's instructions and sequenced using the Macrogen (Seoul, South Korea) sequencing service with the primers used for the initial amplifications.

Phylogenetic analyses. Raw sequence reads returned from Macrogen were edited using the program CodonCode Aligner, version 2.0.6 (Dedham, MA). Individual genes sequenced as part of this study (**Table 1**) were combined with those generated in Argüello et al. (2007) except those for *Austroparmelina elixia* (Arguello & A. Crespo) A.Crespo, Divakar & Elix. An initial alignment was obtained using the program MAFFT (Katoh and Toh 2008) and inspected manually with the program BioEdit (Hall 1999). Ambiguously aligned regions were manually excluded from analysis.

Analyses were conducted with the program RAxML 7.2.6 (Stamatakis 2006) with 100 rapid bootstrap replicates using the nucleotide substitution model GTR-GAMMA. To assess phylogenetic

Table 1. Specimens of *Parmelina yalungana* and *P. coleae* sampled as a part of this study, including information on location, collection numbers and GenBank accession numbers.

			GenBank numbers		
Species	**Locality**	**Collection number**	**nuITS**	**mtSSU**	**nuLSU**
P. yalungana 1	Alaska, USA	P.R. Nelson–07–0804A	GU979823	GU979826	GU942440
P. yalungana 2	Alaska, USA	L.L. Lasselle–07–101	GU979824	GU979827	GU942441
P. yalungana 3	Buryatia, Russia	Urbanavichus & Urbanavichene 81796	GU979821	-	GU979825
P. coleae 5	Oregon, USA	B. McCune 28110	GU979822	GU979828	GU942439

relationships in the *Parmelina quercina* group, the three genes were first inspected for conflict by comparison of maximum likelihood (ML) trees. Two genes were considered to be in conflict where differing topologies received bootstrap support greater than 70 percent (Hillis & Bull 1993; Weins 1998). A final concatenated dataset was then generated from all non-conflicting sequences for use in final analyses. The maximum likelihood trees of the concatenated dataset were constructed under the same conditions described above except 1000 bootstrap replicates were performed, and the nucleotide substitution model applied separately to each gene partition.

Results

Phylogenetic placement. DNA amplification was successful for the three specimens from Denali and Russian specimen but unsuccessful for Delta Junction and Chinese specimens, despite several attempts. We were able to obtain the genes of interest from Denali and Russian specimens except mtSSU for *Parmelina yalungana* 3 (**Table 1**). Individual gene datasets consisted of 585 nucleotides positions for mtSSU, 494 for ITS and 849 for nuLSU. No conflict was observed between loci and all three datasets were concatenated. The final tree generated using ML had a log likelihood of -4817.32. Accessions of *P. yalungana* compose a monophyletic lineage, sister to *P. quercina* (**Fig. 3**). Clades supported with significant bootstrap values do not differ from those in Argüello et al. (2007), although the overall topology of the tree differs slightly.

Taxonomy

Parmelina yalungana (Zahlbr.) P.R. Nelson & Kepler *comb. nov.* **Fig. 2**

Mycobank MB 801760

Parmelia yalungana Zahlbruckner, Hedwigia 74: 206. 1934. Type: China: sichuan province. Mountains between Litang and Yalung rivers, between Muli Gomba and Baurong and Wa-Erh-Dje, on *Picea*, alt. 4250 m., Jul., 1928, *J.F. Rock 16720* (w).

Description. Thallus foliose, loosely adnate distally to overlapping and tightly appressed centrally, irregularly branching; corticolous on trees and shrubs. Lobes 1–4 mm wide, plane to weakly convex; lobe margins parallel to diverging with simple, black cilia ca. 0.5 mm long. Upper surface white to grey, matt to weakly shining, smooth with occasional cracks or wrinkles centrally, emaculate but with confluent fenestrations or pores under SEM (**Figs. 1A & B**). Lower surface black with abundant, simple rhizines 0.5–1.5 mm long. Isidia and soredia absent. Apothecia 1.5–3.5 mm wide, common, lecanorine, substipitate; disc brown, plane to weakly concave; margin smooth to weakly crenulate; amphithecium with thick, clear cortex, occasionally with black retrorse rhizines; epihymenium reddish brown 7–20 μm; hymenium clear 25–62 μm; hypothecium clear 22–62 μm; exciple clear, composed of thin-walled paraplectenchyma, 12–32 μm thick; paraphyses sparingly branched, 5–10 μm wide at tip. Ascospores hyaline, broadly ellipsoid to subglobose, 1-celled, 8 per ascus, (6.3)–7.8–(10) × (3.8)–5.3–(6.5) μm. Pycnidia small, black, immersed in thalli. Conidia clear, bacilliform to slightly curved, 6–9 × 1 μm.

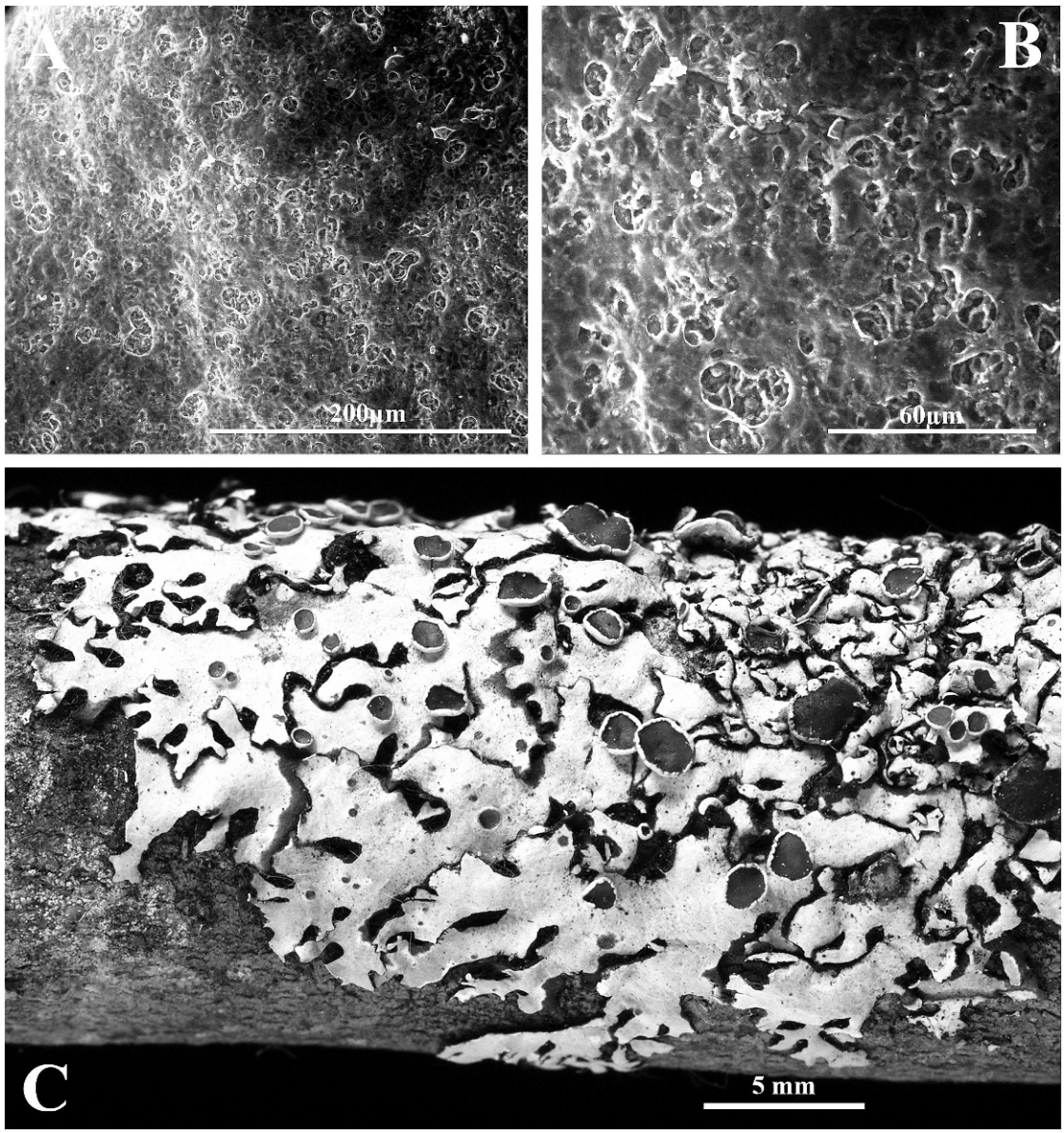

Figure 1. *Parmelina yalungana.* **A & B.** Scanning electron micrographs of the upper surface (*P.R. Nelson PRN -07-804A*) at two different magnifications showing the confluent fenestrations **C.** An image of the same specimen taken with a digital SLR camera with a 100 mm macro-lens.

Chemistry. Cortex: atranorin (major); K+ yellow, PD+ pale yellow. Medulla: lecanoric acid (major), stictic acid (minor, infrequent); C+ red.

Etymology. Presumably named after the adjacent Yalung river.

Distribution and ecology. Recent taxonomic studies of the *Parmelina quercina* complex have included specimens from across the geographic range with the exception of two large areas where it is known to occur: eastern Asia and Alaska. *Parmelina quercina* has been reported from many provinces in China (Wei 1991), Japan (Kurokawa 2003), eastern Russia (Chabanenko 2002) and Alaska (Stair 1947). Despite recent work on *P. quercina*, the identities of specimens from many of these areas remain unresolved. *Parmelina quercina* s. str. is a

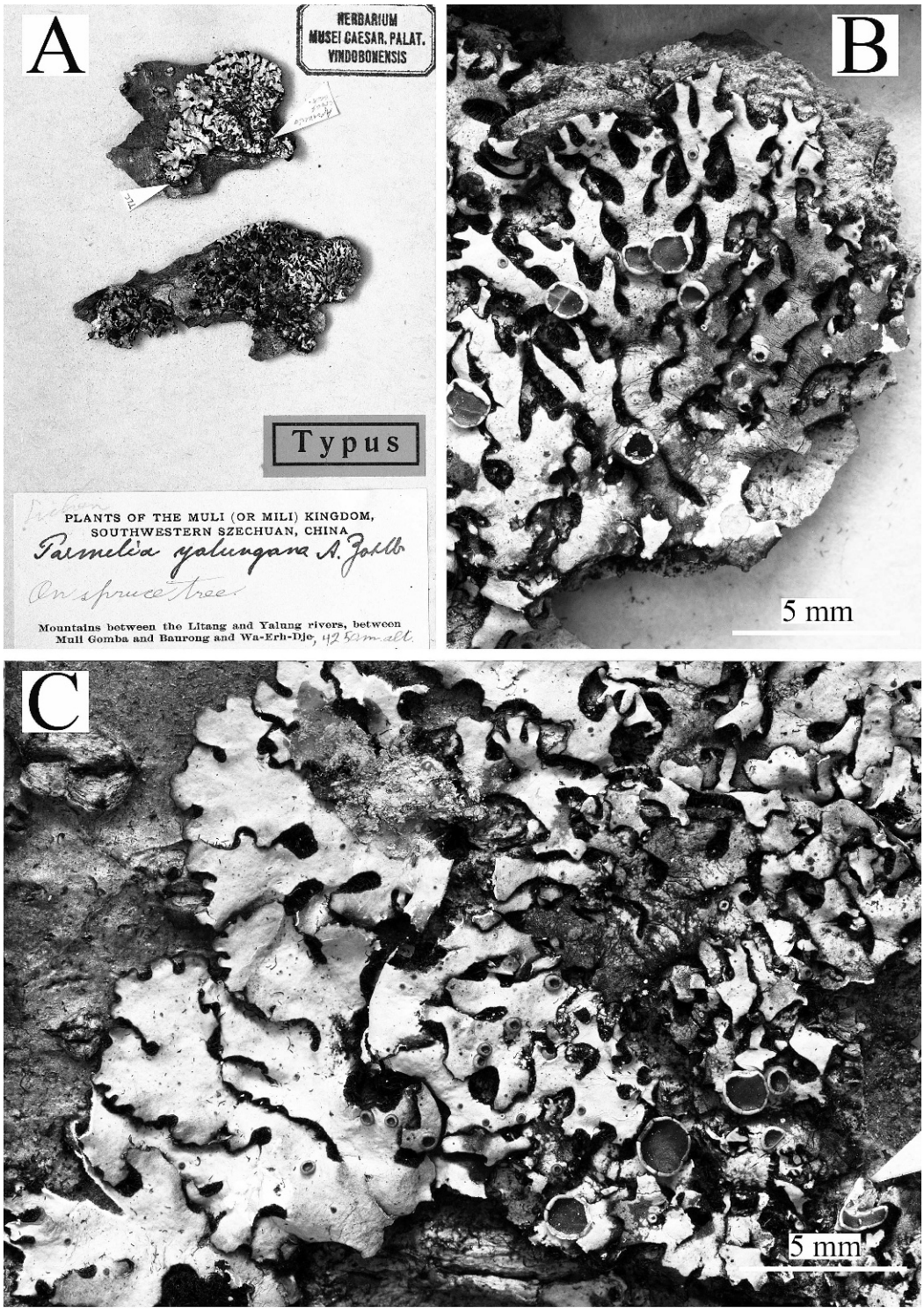

Figure 2. Habit of *Parmelina yalungana*. **A.** Type specimen **B.** Narrow lobed portion of specimen **C.** Wide lobed portion of specimen.

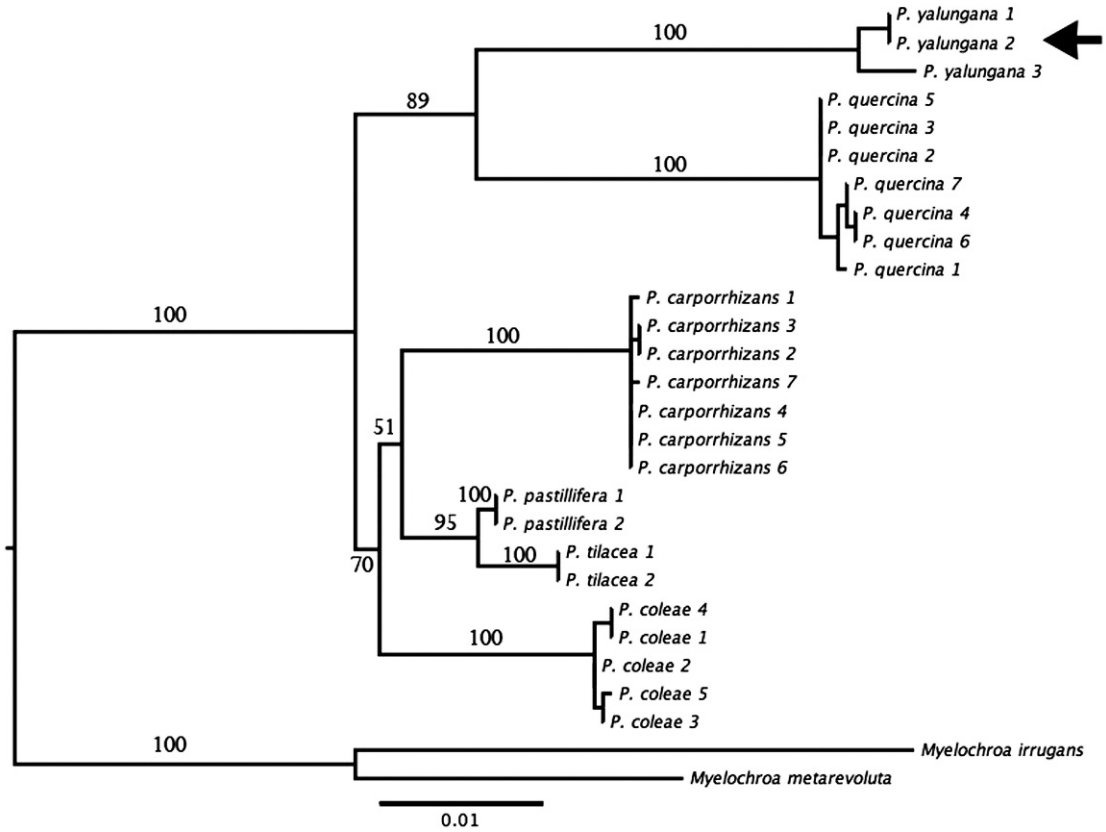

Figure 3. Maximum likelihood tree from a combined multigene dataset (mtSSU, nuLSU, ITS) showing the relationship of *Parmelina yalungana* to other taxa in the *P. quercina* group. Numbers on branches denote ML bootstrap percentages. The scale bar indicates the number of changes per site. Treebase accession number: S13520.

Mediterranean and continental European species, separated from eastern Asian records (China, Russia, Japan) by thousands of kilometers. The one Alaskan record (Stair 1947) of *P. quercina* is from the coastal rainforest in Yakutat, nearly 2000 km away from the next nearest record of the genus, a specimen of *P. coleae* from southern Oregon, U.S.A. *Parmelina coleae* occurs mostly in California but is occasionally found south to northern Mexico, eastern Arizona and north to central Oregon (CNALH; GBIF; McCune & Geiser 2009). All these *P. coleae* localities experience a Mediterranean climate whereas the climate in Yakutat, Alaska is hypermaritime and much colder. Geography is important in delimiting other species of the *P. quercina* group (Argüello et al. 2007) and for other closely related genera (Crespo et al. 2010; Divakar et al. 2010). Given this, it seems likely that eastern Asian (China, Russia and Japan) and Alaskan material represent a distinct taxon or taxa.

Discussion

Parmelina yalungana can be can be distinguished from its congeners by the 1) emaculate upper surface, 2) epiphytic substrate and 3) much shorter ascospores (5.3 × 7.3 μm). There was some morphological variation within the *P. yalungana* specimens we examined. One specimen from Alaska showed small, imbricate lobes (**Fig. 1C**) reminiscent of *P. atricha* (see Figure 6 in Clerc & Truong (2008)). The type specimen also had very narrow (<1mm) lobes (**Fig. 2B**) to wide (ca. 4 mm) lobes (**Fig. 2C**). Ascospore dimensions also varied slightly but consistently along geographic lines. Chinese specimens, including the type, had ascospores closer to 8 μm long, whereas North American and Russian specimens had ascospores slightly more than 9 μm long, on average. We found the upper cortex maculation not to be a good distinguishing character for North American *Parmelina*. *P. coleae* (see

Figure 2 in Argüello et al. (2007)) and *P. yalungana* (**Figs. 1A & B**) both have a weakly pored upper cortex. No significant chemical differences were detected between *P. yalungana* and other species. All species in the *P. quercina* group have atranorin and lecanoric acid.

The Alaskan localities of *Parmelina yalungana* are from two different climatic zones, one maritime and the other continental. The locality in Denali is a mature mixed *Picea glauca-Betula* forest in a toe slope, side drainage alluvial fan at the base of the Yentna River valley on the south side of the Alaska Range. This area has a strong maritime influence from the Cook Inlet to the south. Three specimens were collected there from branches of a single, fallen *Betula neoalaskana* by two different collectors (**Table 1**) during the same lichen inventory. Before falling, this tree was apparently growing on a rocky outcrop approximately 5 meters immediately above a small stream. There were no other trees on this little outcrop, making it exceptionally exposed in terms of light (no neighboring trees) at the same time being humidified by living next to a creek. This tree had a very high cover of *Parmelia sulcata* Taylor, among which the 3 specimens of *Parmelina yalungana* were collected. Relative to the surrounding forest, this tree exhibited an aberrant lichen community. The surrounding site had numerous cyanolichens indicative of its humid climate, including *Lobaria pulmonaria* (L.) Hoffm., *Collema furfuraceum* (Arnold) Du Rietz, *Nephroma isidiosum* (Nyl.) Gyelnik, and *Sticta* cf. *wrightii*. The second Alaskan locality was north of the Alaska Range in interior Alaska near Delta Junction, where the climate is more continental. The forest was mainly *Picea glauca* with an *Alnus* sp. understory. There were many *Parmelina* thalli observed growing on *Alnus* sp., but no information available about the surrounding lichen community. The ecology and distribution of *P. quercina* in eastern Asia is not discussed here but it remains unclear whether these reports actually are *P. yalungana*. Future studies should focus on elucidating the identity of other reports of *P. quercina* in China, Japan and elsewhere in Asia.

Additional specimens examined. China: yunnan prov. Zhongdian County, Daxueshan Mt., Kuangchang, on *Quercus* sp., alt. 3850m, 28°30′N, 99°49′E, Sept. 13, 2003, *Wang Li Song 03-22724* (kun); Zhongdian County, on tree, alt. 3450 m, 27°48′N, 99°48′E, Aug. 7 19, 1993, *Wang Li Song 93-13771* (kun); Western slope of Baimaxueshan Mt, head of Wu Rd in oak forest, alt. 3400–3600 m, 28°20′N, 99°03′E, July 22, 1981, *Wang Li Song 2421* (kun); Zhongdian County, vicinity of Nixi village in oak forest, alt. 2600 m, 28°02′N, 99°31′E, July 5, 1981, *Wang Li Song 1920* (kun). sichuan prov. Xiaojin County, on *Quercus* sp., alt. 3500 m, 31°02′N, 102°26′E, Aug. 26, 2005, *Xiao Yue Qin 05-53* (kun) tibet. Milin County, on *Rhododendron* sp., alt. 3020 m, Aug. 26, 2007, *Wang Li Song, Niu Dong Ling, Zheng Chuanwei 07-28732* (kun). Russia: buryatia republic. Baikalskii Zapovednik Nature Reserve, along Pereemnaya River, alt. 600 m., 8.VII.1996, *Urbanavichus & Urbanavichene 81796* (m), U.S.A.: alaska. Denali National Park, upper Yentna River valley, growing on *Betula* sp., alt. 146 m., 62.3836°N, 151.8551°W, Aug. 28, 2007, *P.R. Nelson PRN -07-804A* (ala), *L. Lasselle LLL-07-101* (denali national park and preserve herbarium); Quartz Lake State Recreation Area, white spruce (*Picea glauca*) swamp, on *Alnus* sp., alt. 345 m, 64°11′27.44″N, 145°51′5.76″W, Jul. 30, 2011, *T. Spribille & F. Fernández s.n.*

Acknowledgments

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