A new perspective on the macrolichen genus *Platismatia* (Parmeliaceae, Ascomycota) based on molecular and phenotypic data

Olivia A. Asher[1], John Howieson[2] and James C. Lendemer[3,4]

[1] *Macaulay Honors College at Lehman College, City University of New York, Bronx, NY 10468, U.S.A.;* [2] *Royal Botanic Garden Edinburgh, Edinburgh EH3 5NZ, United Kingdom;* [3] *Institute of Systematic Botany, The New York Botanical Garden, Bronx, NY 10458-5126, U.S.A.*

ABSTRACT. Lichens in the genus *Platismatia* are common, widespread and were some of the first to be studied by Western taxonomists. However, few molecular phylogenetic studies of *Platismatia* have been published to date. We present an expanded phylogeny of *Platismatia* inferred from 60 newly generated ITS sequences and 28 existing publicly available sequences. The new phylogeny confirms the delimitation of *P. wheeleri* as monophyletic and distinct from the widespread *P. glauca*, the latter of which was recovered as two separate, highly supported clades, that do not appear to differ in phenotype or biogeography. The western North American endemics *P. herrei* and *P. stenophylla* were not recovered as reciprocally monophyletic and may be an example of recent speciation similar to that also hypothesized for *Alectoria* in the same region. Ancestral state reconstructions of reproductive modes (dominant asexual vs. sexual reproduction; asexual propagule type) suggest that sexual species like *P. tuckermanii* can evolve from primarily asexual ancestors. Evaluation of species distributions suggests that reproductive mode may be related to range size. These data suggest that *Platismatia* could serve as a model for future studies on reproductive mode, biogeography and speciation in lichens.

KEYWORDS. Asexual reproduction, biodiversity, biogeography, Parmeliaceae, species pairs.

The lichens currently placed in the genus *Platismatia* W.L.Culb. & C.F.Culb. have fascinated lichenologists for centuries. These large and highly conspicuous, foliose macrolichens are common and abundant in northern temperate and boreal ecosystems worldwide (Brodo et al. 2001; Kuznetsova et al. 2021; McCune & Geiser 2009; Nilsson 1928; Stenroos et al. 2011; Thell & Moberg 2011; Tripp & Lendemer 2020). Scientific study of *Platismatia* dates to the late 1700s and early 1800s when *P. glauca* (L.) W.L.Culb. & C.F.Culb was first described by Carl Linnaeus and when his student Erik Acharius subsequently described *P. lacunosa* (Ach.) W.L.Culb. & C.F.Culb (Culberson & Culberson 1968). Linnaeus (1753) classified all lichens in a single broadly defined genus *Lichen* L. However, Acharius (1803) transcended that classification and recognized additional genera, one of which was *Cetraria* Ach. As delimited by Acharius, *Cetraria* included the species now placed in *Platismatia* together with many other superficially

[4] Corresponding author's e-mail: jlendemer@nybg.org
DOI: 10.1639/0007-2745-126.1.xxx

similar taxa that came to be treated in other genera such as *Asahinea* W.L.Culb. & C.F.Culb. and *Cetrelia* W.L.Culb. & C.F.Culb. based on phenotypic characters (Culberson & Culberson 1968).

For more than a decade, molecular phylogenetic evolutionary and systematic studies of Parmelioid lichens have challenged many established phenotypic generic delimitations (e.g., Crespo et al. 2010; Divakar et al. 2013, 2017; Pizzaro et al. 2018; Thell et al. 2012, 2018). This has particularly been the case for species that were historically treated in *Cetraria* and which are often collectively referred to as Cetrarioid lichens (Nelsen et al. 2011). In contrast, however, *Platismatia* is one of several Cetrarioid genera whose historical delimitations have been maintained and reaffirmed by more recent molecular studies that recovered it both as monophyletic and strongly supported (e.g., Crespo et al. 2010). At present there are 11 accepted species of *Platismatia* (Culberson & Culberson 1968; Lumbsch et al. 2011) of which seven occur in North America north of Mexico (Esslinger 2021).

Species of *Platismatia* have been incorporated into broader evolutionary studies of lichen fungi, both of the highly speciose Parmeliaceae (Amo da Paz 2011) and the evolutionarily diverse Lecanoromycetes (Miadlikowska et al. 2014). However, despite being widespread and common, there are surprisingly few publicly available sequences of *Platismatia*, nearly all of which belong to the fungal barcoding locus ITS and are derived from studies published over a decade ago (e.g., Thell et al. 1998; Thell & Miao 1999). Relationships among *Platismatia* species have also been little examined, with only one dedicated molecular study that included limited sampling within a small number of species (Thell et al 1998). More recently an additional species, *P. wheeleri* Goward et al., was described from western North America without published molecular data (Lumbsch et al. 2011).

Advancing understanding of species delimitations and inter-species relationships within *Platismatia* is important because the genus has the potential to serve as a model for broader evolutionary and biogeographical studies of the lichen symbioses. *Platismatia* includes multiple examples of hypothesized pairs of sister taxa that differ in reproductive mode, range size and degree of allopatry (Culberson & Culberson 1968; Stone & Root 2021). Species of *Platismatia* also follow multiple classic biogeographic patterns, including Northern Hemisphere intercontinental disjunctions, discontinuous Holarctic distributions and bipolar disjunctions (e.g., Brodo et al. 2001; Culberson & Culberson 1968; Garrido-Benavent & Pérez-Ortega 2017). The aim of this study was to assemble available sequence data for *Platismatia*, which has largely been limited to the fungal barcoding locus ITS, expand sampling for that locus with a focus on North American populations and taxa, and examine relationships within and among species especially with respect to hypothesized pairs of sister species that differ in contrasting reproductive modes. Drawing upon analyses of previously published and newly generated data, we aim to provide a foundation to guide development of *Platismatia* as a tractable system within which to examine the evolution and ecology of foliose macrolichens.

MATERIALS AND METHODS

Chemical and morphological studies. All specimens of *Platismatia* in the New York Botanical Garden (NY) were examined as part of this study. In addition, specimens from CANL were borrowed on loan and duplicates of recent collections from OSC were sent by Bruce McCune. Jessica Allen (Eastern Washington University) also provided specimens of *P. glauca* and *P. wheeleri* collected in the vicinity of Spokane, Washington. Specimens for which sequence data were generated were examined in detail morphologically. Thalli were examined dry using an Olympus SZ-STB dissecting microscope. Four sequenced specimens (DNA isolates NY4749,

NY4750, NY4751, NY4755) which were identified in the field as *P. wheeleri* but were recovered within *P. glauca* in our analyses tree and appeared phenotypically ambiguous under light microscopy, were examined with a Scanning Electron Microscope (SEM) following the methods of Lendemer et al. (2019). Selected sequenced specimens of *P. glauca, P. tuckermanii* and *P. wheeleri* were also studied with thin layer chromatography (TLC) using Solvent C following the methods of Lendemer (2011) and with a modified solvent ratio of 200 toluene: 30 glacial acetic acid to confirm the presence of atranorin and caperatic acid, as well as to confirm that there was not previously overlooked chemical variation within these taxa that could correspond to monophyletic groups recovered in the molecular analyses.

***Molecular data generation*.** Three methods of DNA extraction and amplification were used. DNA was extracted from ten samples (Table 1) using the DNeasy Plant Mini Kit (Qiagen, Valencia, Spain) as modified by Muscavitch et al. (2017). The primer pair ITS4 and ITS5 (White et al. 1990) were used for amplification. PCR reactions contained 4μL of extracted DNA, 12 μL of EconoTaq® PLUS GREEN 2× Master Mix (Lucigen, Middleton, Wisconsin), 0.25 μL of each primer, and 8.5 μL of purified water. The PCR protocol was denaturation at 95°C for 5 min, 35 cycles at 95°C for 1 min, 62°C for 1 min, 56°C for 1 min, 62°C for 1 min, final extension at 72°C for 7 min, with final storage at 4°C. PCR products were examined on a 1% agarose gel via electrophoresis and stained with ethidium bromide. Unpurified PCR products were sent to the Macrogen U.S.A. for double stranded sequencing.

DNA was extracted from 48 samples (Table 1) using Rapid DNA Isolation Protocol IIa from the DNA Barcoding 101 Protocol (Cold Spring Harbor Laboratory DNA Learning Center 2018). The primers used to amplify the extracted DNA were ITS1F (Gad) M13F (Gardes & Bruns 1993) and ITS4 (Whi) M13R (White et al. 1990). PCR reactions followed the puReTaq Ready-To-Go™ PCR Beads method as outlined in Protocol III from the DNA Barcoding 101 Protocol. The PCR protocol was 94°C for 1 min, followed by 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 45 seconds, with final storage at 4°C. PCR products were examined on a 2% agarose gel using electrophoresis and an ethidium bromide stain. Unpurified PCR products were sent to the Genewiz U.S.A. sequencing facility for bidirectional sequencing.

DNA was extracted from two samples (EDNA18_0051389 and EDNA18_0051620) using the Qiagen Plant DNeasy kit (Qiagen, Germantown, MD, USA) and the protocol recommended by Qiagen with minor adjustments for fungal tissue. The primers ITSF (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used. The PCR protocol was 25 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 20 seconds and extension at 60°C for 4 minutes before being incubated at 4°C. Following the PCR, 5 μL of PCR product was purified using 2 μL of ExoSAP IT (GE Healthcare) before being incubated at 37°C for 15 minutes then heated at 80°C for 15 minutes to inactivate the ExoSAP IT enzymes. After purification, the sample was sequenced using 6.68 μL of ddH_2O, 2 μL of 5× buffer, 0.32 μL of primer (10 μM), 0.5 μL of BigDye and 0.5 μL of purified PCR product per sample for both forward and reverse primers. Sequenced PCR product was then sent to Edinburgh Genomics

Molecular dataset taxon sampling. The molecular dataset used for this study was restricted to sequences of the ITS region because 1) multiple published studies have already demonstrated *Platismatia* to be monophyletic (e.g., Crespo et al. 2010), 2) the goal was primarily to evaluate the monophyly of species as presently delimited and ITS has been proposed as the universal barcode for fungi (Schoch et al. 2012) with widespread use in Parmeliaceae (Nelsen et al. 2011) and 3) available reference sequences of *Platismatia* sequences in GenBank almost entirely belonged to this locus.

All existing ITS sequences (n=29) of *Platismatia* species were downloaded from GenBank on 13 July 2018: *P. erosa* (n=1), *P. glauca* (n=17), *P. herrei* (n=2), *P. norvegica* (n=4), *P. stenophylla* (n=2), and *P. tuckermanii* (n=3). Fifty-eight new ITS sequences were generated: *P. glauca* (n=21), *P. herrei* (n=6), *P. lacunosa* (n=2), *P. norvegica* (n=2), *P. stenophylla* (n=4), *P. tuckermanii* (n=13), and *P. wheeleri* (n=10). A BLASTn search of the newly generated sequences confirmed that they were all assignable to *Platismatia*. *Omphalodium pisacomense* and *Imshaugia aleurites* were selected as outgroups based on their close relationship to *Platismatia* inferred from the phylogeny of Parmelioid lichens published by Crespo et al. (2010). Reference ITS sequences of *O. pisacomense* and *I. aleurites* were also downloaded from GenBank. Voucher data and associated GenBank accession numbers are presented in **Table 1**.

***Alignment assembly*.** The newly generated sequences were examined, trimmed and error-checked in Sequencher v5.4.6 (Gene Codes Corporation). The cleaned newly generated sequences and those sequences from GenBank were assembled into a single matrix and saved as a NEXUS file in Mesquite 3.31 (Maddison & Maddison 2017). The matrix was then saved in FASTA format, uploaded to MAFFT version 7 (Katoh & Standley 2013) and aligned using default settings with unusual characters allowed. The resulting alignment (**Supplemental Table S1**) was initially examined in Mesquite and AY036994 was found to deviate in many nucleotide positions within highly conserved regions across both other *Platismatia* and the outgroups. AY036994 is currently identified as *P. glauca* in GenBank but was originally published in a phylogenetic study of Parmeliaceae (Molina et al. 2004) where it was identified as *Parmelia pseudolaevior* Asahina, a species since re-assigned to the genus *Nipponoparmelia* (Kurok.) K.H.Moon, Y.Ohmura & Kashiw. (Crespo et al. 2010). Although the identification of AY036994 was subsequently changed to *P. glauca* by the sequence authors, and the sequence shows some similarity to *Platismatia* with BLASTn, we excluded it from our analyses due to the differences from all other sequences in the study as well as the lack of explanation surrounding the change in identification from *Nipponoparmelia* to *Platismatia*. After removal of AY036994, the alignment was manually adjusted in Mesquite. Uncertainties/polymorphisms were converted to missing data and ambiguously aligned regions were excluded. A supplemental alignment of only *Platismatia herrei, P. stenophylla,* and *P. norvegica* sequences (see Table 1 for accession numbers; **Supplemental Table S2** for alignment) was assembled following the same methods as the full alignment described above. Ultimately the molecular dataset analyzed for this study included all species currently reported from North America (Esslinger 2021) with a minimum sample size of two.

***Molecular phylogenetic analyses*.** The alignment was then exported as a PHYLIP file and analyzed using RAxML v8.2x (Stamatakis 2006). A maximum likelihood (ML) topology search and a bootstrapping analysis with 500 replicates were performed using the model GTRGAMMA (implemented as the most complex available in the version of RAxML). The results were visualized in FigTree 1.4.3 (Rambaut 2016). Bayesian Inference (BI) analyses of the NEXUS formatted alignment were also performed using MrBayes 3.2.6 (Huelsenbeck & Ronquest 2001). MrModelTest v2.3 selected SYM+G as the appropriate nucleotide substitution model using Akaike Information Criterion (AIC; Akaike 1973). The model settings from MrModelTest were pasted directly into the Bayes block. The Markov chain Monte Carlo parameters consisted of 10,000,000 generations with four chains and a tree sampled every 100 generations. The first 10,000 trees were discarded as burn-in and TRACER was used to confirm convergence (Rambaut et al. 2014). The results, summarized as a majority rule consensus tree, were visualized in FigTree.

The supplemental alignment of only *Platismatia herrei, P. stenophylla,* and *P. norvegica* sequences, was exported as a PHYLIP file and analyzed using RAxML v8.2x (Stamatakis 2006). A maximum likelihood (ML) topology search and a bootstrapping analysis with 500 replicates were performed on this alignment using the model GTRGAMMA and the results were visualized in FigTree as above.

Distance matrix. Genetic distances were calculated using the GTR+G (General Time Reversable model plus gamma) in PAUP 4.0a169 (Swofford 2003). Microsoft Excel was used to visualize the distance matrix and calculate the mean and standard deviation of the genetic distance between and within species groups.

Trait scoring and ancestral state reconstruction. The R package phytools (Revell 2012) was used to perform ancestral state reconstruction (ASR) for dominant reproductive mode (presumed strictly sexual or dominant lichenized asexual) and for presumed dominant dispersal propagule type (isidia, soredia, or ascospores) following Revell (2015). The most likely RAxML tree was used as the input phylogenetic tree. Reproductive mode and dispersal propagule type were treated as discrete traits. Species that were reported to occasionally reproduce using multiple reproductive modes (i.e., species that reproduce asexually by the production of isidia, but which rarely produce apothecia with hypothetically viable sexual ascospores) were scored according to the dominant mode of reproduction in that species. For the reproductive mode ASR, the equal-rates (ER) model of trait evolution selected based on a higher log-likelihood score than the all-rates-different model (ARD). The symmetrical model (SYM) of trait evolution had an equal log-likelihood to the ER model; however, these models are equivalent when two states are being modeled. For the reproductive propagule ASR, the ER model was also selected as it had a higher log-likelihood score than either the ARD or SYM models. The default continuous-time Markov chain model (Mk) which uses marginal probabilities of the ancestral states was used to determine the likelihood of ancestral traits. In addition, stochastic character mapping, which uses the joint probabilities for the ancestral states, was used as an alternative method to the Mk procedure. The same model (ER) was used for the character mapping in both analyses. The marginal ancestral states from the Mk method and posterior probabilities from the stochastic mapping were then compared by plotting.

Range comparison and distribution mapping. Maps of the ranges of each North American *Platismatia* species represented in this study were created using occurrence data downloaded from the Consortium of North American Lichen Herbaria (CNALH) website on 26 June 2022 (CNALH 2021) and from the NYBG Virtual Herbarium on 3 July 2022. All georeferenced occurrences were plotted using GeoJSON and manually parsed to remove all non-North American occurrences. The remaining georeferenced occurrences for each species were then compared to the range maps for each species published by Brodo et al. (2001) as well as Culberson & Culberson (1968), with the exception of *P. wheeleri*, which was described after those publications. Occurrences mapped outside of the published ranges were manually checked and any points that were erroneously mapped to due to incorrect georeferencing or misinterpretation of the original locality data were removed. Extreme occurrences mapped well outside of the published ranges were also removed if the voucher was not identified by an expert lichenologist. The final parsed occurrences for each taxon were saved and mapped with SimpleMappr (Shorthouse 2010). Published ranges from Brodo et al. (2001) and Stone & Root (2021) were superimposed on the plotted points using Adobe Photoshop CS6.

RESULTS

Results of the Maximum Likelihood (MI) and Bayesian Inference (BI) analyses of the *Platismatia* ITS sequences were topologically congruent and recovered most of the currently known species of *Platismatia* (*P. lacunosa, P. norvegica*, *P. tuckermanii, P. wheeleri*) as monophyletic and highly supported (all with ML/BI: 100/1.0; **Fig. 1**). Although only one sequence of *P. erosa* was available for this study, it was recovered in a strongly supported sister relationship (ML/BI: 100/1.0) with the clade of sequences that represent *P. norvegica*. *Platismatia glauca* was also recovered as monophyletic with moderate support (ML/BI: 71/0.98), but the sequences were recovered in two sister clades that were each strongly supported (ML/BI: 98/1.0 and 96/1.0). Sequences of *P. herrei* and *P. stenophylla*, two species endemic to western North America, were recovered intermixed in a clade with very weak support (ML/BL: 10/0).

To examine whether that the relationships between *Platsmatia herrei* and *P. stenophylla* recovered in our broader *Platismatia* dataset were an artifact of excluded variable sites from ambiguous aligned regions, we reanalyzed an alignment restricted to only those taxa, using *P. norvegica* as an outgroup and obtained the same results (**Supplementary Fig. S1**). Analysis of the restricted *P. herrei* and *P. stenophylla* dataset recovered sequences of *P. herrei* as monophyletic with very weak support (ML: 35) and embedded in a clade containing *P. stenophylla* sequences that had weakly supported phylogenetic structure.

Genetic distances within and between clades were congruent with the results from our phylogenetic analyses (**Table 2**). Applying the intraspecific/interspecific genetic distance cutoff proposed by Del Prado et al. (2010) for other groups of Parmeliaceae (i.e., 0.015–0.017 substitutions/site) this criterion supports the current delimitations of *Platismatia erosa, P. lacunosa, P. norvegica, P. tuckermanii* and *P. wheeleri* as distinct species. Although distances between the *P. glauca* clade and other clades were uniformly above the cutoff for intraspecific variation, the distance between the two clades within *P. glauca* was also slightly above the cutoff (0.0196$\pm$0.0021 s/s) while the variation within each clade was well below the cutoff (0.0024$\pm$0.0021 s/s and 0.0015$\pm$0.0021 s/s, respectively). In contrast, the distances between sequences of *P. herrei* and *P. stenophylla* were well below the cutoff (0.0053$\pm$0.0017 s/s).

Morphological analysis of the sequenced specimens of *Platismatia herrei, P. lacunosa, P. norvegica, P. stenophylla,* and *P. tuckermanii* revealed the expected characteristics of each species as delimited by Culberson & Culberson (1968; **Table 3** herein). Apothecia were found on the thalli of *P. lacunosa, P. stenophylla,* and *P. tuckermanii* as expected. Asexual lichenized propagules in the form of isidia were also found on *P. glauca, P. herrei,* and *P. norvegica* also as expected. Asexual lichenized propagules in the form of soredia were found on the majority of *P. wheeleri* samples as expected based on the original description (Lumbsch et al. 2011). However, four specimens (vouchers for isolates NY4749, NY4750, NY4751, NY4755; see **Table 1**) identified in the field as *P. wheeleri* had asexual propagules that appeared morphologically ambiguous when first studied with the dissecting microscope. Scanning electronic microscopy (SEM) of these revealed that the asexual lichenized diaspores in these specimens were at least partially corticate, columnar and derived from an extension of the thallus surface rather than a breakdown of the medulla (**Fig. 2**; see Allen et al. 2012, for discussion and illustrations). Hence, they were treated as degraded or poorly developed isidia rather than soredia. The sequences of these samples were recovered with strong support in clades of sequences derived from typical isidiate *P. glauca* and these results corroborate the SEM studies, indicating potential difficulty in field identification of *P. wheeleri*. All samples of *P. glauca*, *P. tuckermanii* and *P. wheeleri* that

were subjected to thin layer chromatography (TLC) were found to contain atranorin and caperatic acid.

The ancestral state reconstruction (ASR; **Fig. 3**, **Supplementary Figs. S2 and S3**) of reproductive mode in *Platismatia* inferred that there were cases wherein asexual species evolved from sexual ancestors and vice versa. The clade composed of *P. herrei* (asexual) and *P. stenophylla* (sexual) was recovered as likely having had a sexual ancestor. A similar situation was inferred for the clade comprising *P. erosa* (asexual), *P. lacunosa* (sexual), and *P. norvegica* (asexual), where the ancestor was inferred to have more likely been sexual. In contrast the clade containing *P. glauca* (asexual), *P. wheeleri* (asexual), and *P. tuckermanii* (sexual) was inferred to have had an asexual ancestor. The ASR of reproductive propagules (**Fig. 4**, **Supplementary Figs. S4 and S5**) revealed that the only sorediate species, *P. wheeleri*, likely evolved from an isidiate ancestor.

Maps of the range of each species in North America (**Fig. 5**) revealed different distribution patterns between pairs of sister species. *Platismatia herrei* (**Fig. 5A**) and *P. stenophylla* (**Fig. 5B**), have largely sympatric ranges on the West Coast of North America, although *P. herrei* which is isidiate is more widely distributed than *P. stenophylla* which is strictly sexually reproducing. *Platismatia norvegica*, which is isidiate, is found along the northern coastal regions of North America (**Fig. 5D**), while *P. lacunosa* which is strictly sexually reproducing occurs only in Western North America (**Fig. 5C**). *Platismatia tuckermanii*, which is strictly sexually reproducing is endemic to Eastern North America (**Fig. 5G**), while *P. wheeleri* which is sorediate occurs only in arid inland Western North America (**Fig. 5F**). *Platismatia glauca* is the only species in the genus that appears be truly widespread, including large areas of both Eastern and Western North America (**Fig. 5E**). Though some asexual species have similar ranges to their sexual relatives, notably *P. herrei* and *P. stenophylla*, in general the asexual species such as *P. glauca* and *P. norvegica* have larger ranges than closely related sexual species *P. tuckermanii* and *P. lacunosa*. The exception to this is *P. wheeleri*, which reproduces primarily asexually and is entirely allopatric with both its sexual relative (*P. tuckermanii*), while also mostly sympatric with the asexually reproducing *P. glauca*.

DISCUSSION

The majority of *Platismatia* species were recovered as highly supported and monophyletic, largely affirming existing species delimitations based on chemical and morphological characters. The most notable of these is *P. wheeleri* which was described just over a decade ago (Lumbsch et al. 2011), has not appeared in any phylogenetic analysis, and is not represented by any publicly available sequences on GenBank. Our results represent independent confirmation that *P. wheeleri* is indeed a distinct species. *Platismatia wheeleri* was described based on the presence of soredia, differing from *P. glauca* which produces isidia. Our morphological analysis using SEM of the specimens initially identified as *P. wheeleri* but recovered as *P. glauca* in our phylogenetic analyses revealed the presence of superficially ambiguous asexual propagules that in the field or under a dissecting scope could easily be mistaken for soredia. It is possible that the soredia present on some *P. glauca* specimens as described by Culberson & Culberson (1968) are actually degraded isidia. Another possibility is that these sorediate *P. glauca* specimens are representatives of *P. wheeleri.* The morphological differentiation between *P. glauca* and *P. wheeleri* has been extensively discussed by Allen et al. (2012). Recently the ecology of the two species also has been studied in detail in the Pacific Northwest of North America (Stone & Root 2021).

Our molecular phylogenetic analyses recovered *Platismatia glauca* in two distinct highly supported groups. Additionally, the genetic distance between these two groups was higher than expected for variation within a species, and higher than the variation within each *P. glauca* group (**Table 2**). The presence of two separate groups suggests that *P. glauca* as presently delimited may consist of two cryptic species. However, we detected no chemical, morphological or geographic differences between the two groups. We suggest that extensive additional geographic sampling, together with evaluation using additional loci, should be carried out if the cryptic entities were to be taxonomically recognized.

The delimitation of *Platismatia herrei* and *P. stenophylla* also requires further investigation. The species were not recovered as reciprocally monophyletic, the genetic distance between them was below that between other pairs of species, and the clade comprising the sequences of the species was itself poorly supported. It is possible that *P. herrei* and *P. stenophylla* are recently diverged, as has been suggested for the lack of resolution for other morphologically distinct macrolichen endemics from the Pacific Northwest of North America (McMullin et al. 2016). It is also possible that the taxa as presently defined based on reproductive mode are morphological variants of the same species and instead should be treated as conspecific. Again, further investigation with increased sampling and additional loci is needed.

The results of the ancestral state reconstruction (ASR) for reproductive mode are particularly interesting. In the *Platismatia glauca, P. wheeleri,* and *P. tuckermanii* clade, a sexual species, *P. tuckermanii*, likely evolved from a primarily asexual ancestor. This suggests a shift to dominant sexual reproduction and loss of asexual reproduction through lichenized propagules occurred in *P. tuckermanii*. The ASR for propagule type also inferred that *P. wheeleri*, a sorediate species, likely evolved from an isidiate ancestor. This supports other studies that suggest species with dominant asexual reproductive modes are not necessarily evolutionary dead ends (e.g., Tripp 2016), a logical conclusion considering that most asexually reproducing species also rarely reproduce sexually (e.g., Lendemer et al. 2015).

Although we did not explicitly quantify the range size of the species included our phylogenetic analyses, the overall distributions suggest that species with dominant lichenized asexual reproductive modes have larger ranges than those do not produce lichenized propagules (e.g., *Platismatia glauca* vs. *P. tuckermanii*). Nonetheless, this is not absolute as *P. herrei* and *P. stenophylla* have similar ranges, although that of *P. stenophylla* does appear to be smaller than that of *P. herrei*. Further, *P. wheeleri* appears to be a narrowly distributed species that produces small lichenized propagules (soredia), in contrast to its relative *P. glauca* which also produces large lichenized propagules (isidia). While preliminary, these patterns suggest that *Platismatia* could provide useful insights into the processes that underly range sizes in foliose lichens.

CONCLUSIONS

In this study we expanded the available selection of sequences of *Platismatia*, examined the evolutionary relationships of *Platismatia* species using multiple types of data (molecular data, morphology, and chemistry) and conducted an ancestral state reconstruction of reproductive mode which reinforces that sexual species can evolve from ancestors with dominant asexual reproduction. Future research into *Platismatia* should focus on sequencing other loci, especially for *P. herrei* and *P. stenophylla.* Additionally, sequencing efforts for three Asian species *P. formosana*, *P. interrupta*, and *P. regenerans* are needed as no sequences of these species are currently available. Although filling the remaining taxonomic gaps in molecular data

should be a priority, more comprehensive sampling of each species across its geographic range and climate gradients is needed. We also recommend additional exploration into the biogeography of *Platismatia*, particularly the relationships between reproductive mode, distribution and range size.

ACKNOWLEDGMENTS

Jessica Allen (EWU) and her students generously provided fresh material of *P. glauca* and *P. wheeleri* for use in this study. Bruce McCune (OSU) provided fresh material of species from the Pacific Northwest, while Troy McMullin (CANL) provided fresh material from northern North America. Rebecca Yahr is thanked for her collaboration and communication regarding the work and sequences of the second author. Erin Tripp (COLO) is thanked for discussion on ancestral state reconstructions and providing the R code from Tripp (2016) which aided the lead author in developing her code for the analyses. The SEM and associated processing equipment were supported by NSF-MRI grant 1828479 to PIs (Ambrose, Little and Michelangeli) at The New York Botanical Garden. Christine Marizzi (Harlem DNA Lab, CSHL) provided assistance and facilities for the sequencing of 48 of our new samples. The first author's work was supported by Research Initiative for Scientific Enhancement (RISE) program at Lehman College, City University of New York. The last author's participation in the project derives from NSF Award 2115190 (to PI Lendemer) at NYBG. Author contributions: OAA and JCL conceived of the project. OAA performed the morphological study, DNA sequencing, phylogenetic analysis, and ancestral state reconstruction, and led writing of the manuscript. JCL obtained specimens from the field and from herbaria, provided input on morphological, and genetic data analysis, created the map figures, and contributed to editing and revision of the manuscript. JH extracted and sequenced the DNA from samples EDNA18_0051389 and EDNA18_0051620.

LITERATURE CITED

Acharius, E. 1803. Methodus qua omnes detectos Lichenes: Secundum organa carpomorpha, ad genera, species et varietates. F.D.D. Ulrich, typis C.F. Marquard, Stockholm.

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267–281. In: B. N. Petrov & F. Csaki F (eds.), Proceedings of the 2nd International Symposium on Information Theory. Akademiai Kiado, Budapest.

Allen, J. L., B. P. Hodkinson & C. R. Björk. 2012. A major range expansion for *Platismatia wheeleri*. North American Fungi 7(10): 1–12.

Amo de Paz, G., P. Cubas, P. K. Divakar, H. T. Lumbsch & A. Crespo. 2011. Origin and diversification of major clades in Parmelioid lichens (Parmeliaceae, Ascomycota) during the Paleogene inferred by bayesian analysis. PLoS ONE 6(12): e28161.

Brodo, I. M., S. D. Sharnoff & S. Sharnoff. 2001. Lichens of North America. Yale University Press, New Haven & London.

CNALH. 2021. http//:lichenportal.org/cnalh/index.php. [Accessed on 31 August 2021.]

Cold Spring Harbor Laboratory DNA Learning Center. 2018. IIa: Isolate DNA from plant, fungal, or animal Samples. Pages 11–12. In: Using DNA Barcodes to Identify and Classify Living Things. https://dnabarcoding101.org/files/using-dna-barcodes.pdf

Crespo, A., F. Kauff, P. K. Divakar, R. del Prado, S. Pérez-Ortega, G. A. de Paz, Z. Ferencova, O. Blanco, B. Roca-Valiente, J. Núñez-Zapata, P. Cubas, A. Argüello, J. A. Elix, T. L. Esslinger, D. L. Hawksworth, A. M. Millanes, M. C. Molina, M. Wedin, T. Ahti, A. Aptroot, E. Barreno, F. Bungartz, S. Calvelo, M. Candan, M. J. Cole, D. Ertz, B.

Goffinet, L. Lindblom, R. Lücking, F. Lutzoni, J.-E. Mattsson, M. I. Messuti, J. Miadlikowska, M. D. Piercey-Normore, V. J. Rico, H. Sipman, I. Schmitt, T. Spribille, A. Thell, G. Thor, D. K. Upreti & H. T. Lumbsch. 2010. Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. Taxon 59: 1735–1753.

Culberson, W. L. & C. F. Culberson. 1968. The lichen genera *Cetrelia* and *Platismatia* (Parmeliaceae). Contributions from the United States National Herbarium 34: 449–558.

Del-Prado P. C., T. H. Lumbsch, P. K. Divakar, O. Blanco, G. Amo de Paz, M. C. Molina & A. Crespo. 2010. Genetic distances within and among species in monophyletic lineages of Parmeliaceae (Ascomycota) as a tool for taxon delimitation. Molecular Phylogenetics and Evolution 56:125–133.

Divakar, P. K., A. Crespo, J. Núñez-Zapata, A. Flakus, H. J. M. Sipman, J. A. Elix & H. T. Lumbsch. 2013. A molecular perspective on generic concepts in the *Hypotrachyna* clade (Parmeliaceae, Ascomycota). Phytotaxa 132(1): 21–38.

Divakar, P. K., A. Crespo, E. Kraichak, S. D. Leavitt, G. Singh, I. Schmitt & H. T. Lumbsch. 2017. Using a temporal phylogenetic method to harmonize family- and genus-level classification in the largest clade of lichen-forming fungi. Fungal Diversity 84: 101–114.

Esslinger, T. L. 2021., A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the Continental United States and Canada, version 24. Opuscula Philolichenum 20: 100–394.

Gardes, M. & T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Garrido-Benavent, I. & S. Pérez-Ortega. 2017. Past, present, and future research in bipolar lichen-forming fungi and their photobionts. American Journal of Botany 104: 1660–1674.

Huelsenbeck, J. P. & F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Katoh, K. & D. M. Standley 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.

Kuznetsova, E. S., I. S. Stepanchikova, I. F. Skirina, S. V. Chesnokov & D. E. Himelbrant. 2021. A revision of the lichen genus *Platismatia* (Parmeliaceae) in Russia, with a key to the species. Novosti Sistematiki Nizshikh Rastenii 55: 179–194.

Lendemer, J. C. 2011. A review of the morphologically similar species *Fuscidea pusilla* and *Ropalospora viridis* in eastern North America. Opuscula Philolichenum 9: 11–20.

Lendemer, J. C., N. Noell & J. L. Allen. 2015: The *Parmotrema* acid test: A look at species delineation in the *P. perforatum* group 40 y later. Mycologia 107: 1120–1129

Lendemer, J. C., J. H. Hoffman & J. W. Sheard. 2019. *Rinodina brauniana* (Physciaceae, Teloschistales), a new species with pseudoisidia from the southern Appalachian Mountains of eastern North America. The Bryologist 122: 111–121.

Linnaeus, C. 1753. Species plantarum exhibentes plantas rite cognitas ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Impensis Laurentii Salvii, Holmiæ Stockholm.

Lumbsch, H. T., T. Ahti, S. Altermann, G. Amo de Paz, A. Aptroot, U. Arup, A. Bárcenas Peña, P. A. Bawingan, M. N. Benatti, L. Betancourt, C. R. Björk, K. Boonpragob, M. Brand, F. Bungartz, M. E. S. Cáceres, M. Candan, J. L. Luis Chaves, P. Clerc, R. Common, B. J. Coppins, A. Crespo, M. Dal-Forno, P. K. Divakar, M. V. Duya, J. A. Elix, A. Elvebakk,

J. D. Fankhauser, E. Farkas, L. Itatí Ferraro, E. Fischer, D. J. Galloway, E. Gaya, M. Giralt, T. Goward, M. Grube, J. Hafellner, J. E. Hernández M., M. A. Herrera Campos, K. Kalb, I. Kärnefelt, G. Kantvilas, D. Killmann, P. Kirika, K. Knudsen, H. Komposch, S. Kondratyuk, J. D. Lawrey, A. Mangold, M. P. Marcelli, B. McCune, M. I. Messuti, A. Michlig, R. Miranda González, B. Moncada, A. Naikatini, M. P. Nelsen, D. O. Øvstedal, Z. Palice, K. Papong, S. Parnmen, S. Pérez-Ortega, C. Printzen, V. J. Rico, E. Rivas Plata, J. Robayo, D. Rosabal, U. Ruprecht, N. Salazar Allen, L. Sancho, L. Santos De Jesus, T. Santos Vieira, M. Schultz, M. R. D. Seaward, E. Sérusiaux, I. Schmitt, H. J. M. Sipman, M. Sohrabi, U. Søchting, M. Z. Søgaard, L. B. Sparrius, A. Spielmann, T. Spribille, J. Sutjaritturakan, A. Thammathaworn, A. Thell, G. Thor, H. Thüs, E. Timdal, C. Truong, R. Türk, L. Umaña Tenorio, D. K. Upreti, P. van den Boom, M. Vivas Rebuelta, M. Wedin, S. Will-Wolf, V. Wirth, N. Wirtz, R. Yahr, K. Yeshitela, F. Ziemmeck, T. Wheeler, R. Lücking. 2011. One hundred new species of lichenized fungi: a signature of undiscovered global diversity. Phytotaxa 18: 1–127.

Maddison, W. P. & D. R. Maddison. 2017. Mesquite: A modular system for evolutionary analysis. Version 3.31. http://mesquiteproject.org.

Miadlikowska, J., F Kauff, F. Högnabba, J. C. Oliver, K. Molnár, E. Fraker, E. Gaya, J. Hafellner, V. Hofstetter, C. Gueidan, M. A. G. Otálora, B. P. Hodkinson, M. Kukwa, R. Lücking, C. Björk, H. J. M. Sipman, A. R. Burgaz, A. Thell, A. Passo, L. Myllys, T. Goward, S. Fernández-Brime, G. Hestmark, J. C. Lendemer, H. T. Lumbsch, M. Schmull, C. L. Schoch, E. Sérusiaux, D. R. Maddison, A. E. Arnold, F. Lutzoni & S. Stenroos. 2014. A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. Molecular Phylogenetics and Evolution 79: 132–168.

Molina, M., A. Crespo, O. Blanco, H. T. Lumbsch & D. L. Hawksworth. 2004. Phylogenetic relationships and species concepts in *Parmelia* s. str. (Parmeliaceae) inferred from nuclear ITS rDNA and β-tubulin sequences. The Lichenologist 36: 37–54.

McCune, B. & L. Geiser. 2009. Macrolichens of the Pacific Northwest. Second Edition. Oregon State University Press, Corvallis.

McMullin, R. T., J. C. Lendemer, H. E. Braid & S. G. Newmaster. 2016. Molecular insights into the lichen genus *Alectoria* (Parmeliaceae) in North America. Botany 94: 165–175.

Muscavitch, Z. M., J. C. Lendemer & R. C. Harris. 2017. A review of the lichen genus *Phlyctis* in North America (Phlyctidaceae) including the description of a new widespread saxicolous species from eastern North America. The Bryologist 120: 388–417.

Nelsen, M. P., N. Chavez, E. Sackett-Hermann, A. Thell, T. Randlane, P. K. Divakar, V. Rico & H. T. Lumbsch. 2011. The cetrarioid core group revisited (Lecanorales: Parmeliaceae). The Lichenologist 43: 537–551.

Nilsson, G. 1928. *Cetraria norvegica* (Lynge) DR in Fennoskandia. Svensk Botanisk Tidskrift 22: 515–527.

Pizarro, D., P. K. Divakar, F. Grewe, S. D. Leavitt, J.-P. Huang, F. Dal Grande, I. Schmitt, M. Wedin, A. Crespo, H. T. Lumbsch. 2018. Phylogenomic analysis of 2556 single-copy protein-coding genes resolves most evolutionary relationships for the major clades in the most diverse group of lichen-forming fungi. Fungal Diversity 91: 31–41.

Rambaut, A. 2016. FigTree: tree figure drawing tool. Version 1.4.3.2006–2016.

Rambaut, A., M. A. Suchard, D. Xie & A. J. Drummond. 2014. Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer.

Revell, L. J. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3: 217–223.
Revell, L. J. (2015, August 15). Ancestral state reconstruction & visualizing ancestral states on a phylogeny. http://www.phytools.org/eqg2015/asr.html
Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen & Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences, U.S.A. 109: 6241–6246.
Shorthouse, D. P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. http://www.simplemappr.net
Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.
Stenroos, S., T. Ahti, K. Lohtander & L. Myllys. 2011. Suomen Jäkäläopas. Kasvimuseo, Luonnontieteellinen keskus- museo, Helsinki.
Stone, D. & H. T. Root. 2021. A niche for *Platismatia wheeleri*. Evansia 38: 137–145.
Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
Thell, A. & V. Miao. 1999. Phylogenetic analysis of ITS and group I intron sequences from European and North American samples of cetrarioid lichens. Annales Botanici Fennici 35: 275–286.
Thell, A. & R. Moberg. 2011. Parmeliaceae. Nordic Lichen Flora 4: 1–184.
Thell, A., M. Berbee & V. Miao. 1998. Phylogeny within the genus *Platismatia* based on rDNA ITS sequences (lichenized Ascomycota). Cryptogamie. Bryologie, lichenologie 19: 307–319.
Thell, A., A. Crespo, P. K. Divakar, I. Kärnefelt, S. D. Leavitt, H. T. Lumbsch & M. R. D. Seaward. 2012. A review of the lichen family Parmeliaceae. Nordic Journal of Botany 30: 641–664.
Thell, A., I. Kärnefelt, M. R. D. Seaward. 2018. Splitting or synonymizing – genus concept and taxonomy exemplified by the Parmeliaceae in the Nordic region. Graphis Scripta 30: 130–137.
Tripp, E. A. 2016. Is asexual reproduction an evolutionary dead end in lichens? The Lichenologist 48: 559–580.
Tripp, E. A. & J. C. Lendemer. 2020. Field Guide to the Lichens of Great Smoky Mountains National Park. University of Tennessee Press, Knoxville.
White, T. J., T. Bruns, S. Lee & J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322. In: M. A. Innis, M. A., D. H. Gelfand, J. J. Sninsky & T. J. White (eds.) PCR Protocols: A Guide to Methods and Applications, Academic Press, New York.

---

manuscript received July 9, 2022; accepted November 9, 2022.

Supplementary documents online:

Supplementary Table S1. Nexus formatted alignment of *Platismatia* ITS sequences used in molecular phylogenetic analyses in this study.

Supplementary Table S2. Nexus formatted alignment of *Platismatia herrei* and *P. stenophylla* ITS sequences used in molecular phylogenetic analyses in this study.

Supplementary Fig. S1. Phylogeny of *Platismatia herrei* and *P. stenophylla* inferred from ITS sequence data and presented as the most likely tree with *P. norvegica* selected as outgroup. Support is displayed as maximum likelihood bootstrap (ML-BP) values and branches with ≥70 ML-BP are thickened.

Supplementary Fig. S2. Ancestral state reconstruction of dominant reproductive mode created using stochastic character mapping across the phylogeny of *Platismatia* mapped on the most likely RAxML tree with *Imshaugia aleurites* and *Omphalodium pisacomense* selected as outgroups.

Supplementary Fig. S3. Comparison of probabilities for the ancestral state reconstruction of dominant reproductive mode obtained using the continuous-time Markov chain model and stochastic character mapping, respectively.

Supplementary Fig. S4. Ancestral state reconstruction of dominant asexual dispersal propagule per species created using stochastic character mapping across the phylogeny of *Platismatia* mapped on the most likely RAxML tree with *Imshaugia aleurites* and *Omphalodium pisacomense* selected as outgroups.

Supplementary Fig. S5. Comparison of probabilities for the ancestral state reconstruction of propagule type per species obtained using the continuous-time Markov chain model and stochastic character mapping, respectively.

Figure 1. Phylogeny of *Platismatia* inferred from ITS sequence data and presented as the most likely tree with *Imshaugia aleurites* and *Omphalodium pisacomense* selected as outgroups. Support is displayed as maximum likelihood bootstrap (ML-BP) values and Bayesian posterior probabilities (BI). Branches with ≥70 ML-BP and ≥0.95 BI are thickened.

Figure 2. Scanning electron micrographs of isidia in sequenced vouchers of *Platismatia glauca* that were initially mistaken for *P. wheeleri* due to the macroscopic resemblance to soredia. A-C from isolate NY-4749, D from isolate NY-4750.

Figure 3. Ancestral state reconstruction of dominant reproductive mode created using a continuous-time Markov chain model across the phylogeny of *Platismatia* mapped on the most likely RAxML tree with *Imshaugia aleurites* and *Omphalodium pisacomense* selected as outgroups.

Figure 4. Ancestral state reconstruction of dominant asexual dispersal propagule per species created using a continuous-time Markov chain model across the phylogeny of *Platismatia* mapped on the most likely RAxML tree with *Imshaugia aleurites* and *Omphalodium pisacomense* selected as outgroups.

Figure 5. Ranges of *Platismatia* species in North America based on specimens examined for this study at NY (black dots), georeferenced occurrence data downloaded from CNALH (gray dots) and overlaid with the approximate ranges as mapped by Brodo et al. (2001), or Stone and Root (2021) for *P. wheeleri*, shaded in red. Maps are organized to present comparative ranges of related species: *P. herrei* (A) and *P. stenophylla* (B), *P. lacunosa* (C) and *P. norvegica* (D), *P. glauca* (E) and *P. wheeleri* (F) and *P. tuckermanii* (G).