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DNA barcoding of brown *Parmeliae* (Parmeliaceae) species: a molecular approach for accurate specimen identification, emphasizing species in Greenland

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Abstract Warming of Arctic and alpine regions has a substantial impact on high-altitude/-latitude ecosystems. Shifting biomes due to climate change may lead to adjustments in species distributions and potential extinctions. Therefore, detailed monitoring is requisite to assess biologically meaningful shifts in community composition and species distributions. Some Arctic-alpine lichens have been shown to be particularly sensitive to climatic shifts associated with global change. However, accurate identification of lichenized fungal species remains challenging and may limit the effective use of lichens in climate change research. Given the inherent difficulties in accurate identification of lichenized fungi and the potential value of efficient identifications for bio-monitoring research, we investigated the utility of DNA barcode identification of the 13 brown *Parmeliae* (Ascomycota) species occurring in Greenland. For these species, we assessed monophyly and genetic distances using the nuclear ribosomal internal transcribed spacer region (ITS), the standard DNA barcode for fungi. We also compared intraspecific distance values to a proposed intra-interspecific threshold value for Parmeliaceae to identify nominal taxa potentially masking previously unrecognized diversity. Our results indicated that the 13 brown *Parmeliae* species occurring in Greenland can be successfully discriminated using the ITS region. All phenotypically circumscribed species were recovered as well-supported, monophyletic clades. Furthermore, our data supported a barcode gap among congeners for all brown *Parmeliae* species investigated here. However, high intraspecific genetic distances suggest the potential for previously unrecognized species-lineages in at least five species: *Melanelia agnata*, *M. hepatizon*, *Montanelia disjuncta*, *M. panniformis*, and *M. tominii*. Our research facilitates effective, long-term bio-monitoring of climate change in Greenland using lichens by providing accurate molecular identification of brown *Parmeliae* specimens.

Keywords Arctic · Barcode gap · Climate change · Genetic distances · Lichens · Molecular systematics

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Introduction

The recent warming of Arctic and alpine regions has had a substantial impact on high altitude/latitude ecosystems (Serreze et al. 2000; Dirnböck et al. 2003; Post et al. 2009). Shifting climatic zones may lead to adjustments of species distributions and potential extinctions if populations are unable to tolerate or adapt *in situ* to the altered environmental conditions or migrate to suitable habitats (Jackson and Overpeck 2000; Bradshaw and Holzapfel 2006; Aitken et al. 2008; Beck et al. 2011). Therefore, contemporary climate change is among the

major factors jeopardizing biodiversity in Arctic and alpine regions (Sykes et al. 1999; Parmesan and Yohe 2003; Araújo and Rahbek 2006). The observed climatic shifts are predicted to promote borealization of Arctic communities and expansion of some species associated with warmer climates into higher altitudes/latitudes (Strum et al. 2001; Kortsch et al. 2012). However, specific responses of many species to the rapid rates of ongoing climate change remain uncertain (Manel et al. 2010), and detailed monitoring will be required to assess biologically meaningful shifts in community composition and species distributions.

High-altitude/-latitude ecosystems are often dominated by bryophytes and lichens (Longton 1997; Jägerbrand et al. 2006). Some components of these cryptogamic communities are particularly sensitive to climatic shifts (McCune 2000; Cornelissen et al. 2001; Jägerbrand et al. 2006; Bjerke 2011), and these may play an important role in monitoring the impact of climate change. For example, the moss genus *Sphagnum* has been used globally as an indicator of climate change (Whinam and Copson 2006). Additionally, Arctic-alpine lichens have been shown to be sensitive to a warmer and more fluctuating winter climate, and winter icing events may have a substantial effect on lichen-dominated ecosystems (Bjerke 2011). However, accurate identification of lichenized fungal species remains challenging and may limit the effective use of lichens in climate change research (Crespo and Pérez-Ortega 2009; Crespo and Lumbsch 2010; Lumbsch and Leavitt 2011).

Recently, molecular-based techniques have been shown to provide a valuable tool for accurate specimen identification in fungi, including lichenized fungi (Begerow et al. 2010; Kelly et al. 2011; Schoch et al. 2012; Leavitt et al. 2013a; Pino-Bodas et al. 2013). The internal transcribed spacer (ITS) region has been shown to successfully discriminate a broad range of fungal species and was formally proposed for adoption as the primary fungal barcode marker (Kelly et al. 2011; Schoch et al. 2012). Kelly et al. (2011) demonstrated the potential to accurately identify a high percentage of specimens to the correct species when using DNA barcoding in a floristic context. However, DNA-based identification is limited by the availability of accurate baseline taxonomic data (Nilsson et al. 2006; Begerow et al. 2010; Orock et al. 2012). Therefore, the development of expertly curated taxonomic and region-specific sequence databases is crucial to the success of DNA-based identifications.

DNA barcode identification may be particularly effective in a regional floristic context (Kelly et al. 2011). A reference database restricted to only the species that are known to occur in a specified region may result in higher levels of specimen discrimination because it is unlikely that all close relatives of a given species will occur in the study area (Chase and Fay 2009; Kelly et al. 2011). However, floristic inventories for lichens are often unreliable and incomplete, and critical comparisons with adjacent regions and closely related taxonomic groups are crucial to avoid the detection of "new" or "cryptic" lineages that are in fact already known from outside the region. Among Arctic and alpine regions, ecosystems in Greenland provide a model scenario for assessing the impact of climate change on biological systems. A potentially important component of climate change research includes accurate floristic surveys of lichen diversity, complementing other biomonitoring methods (Dillman 1996; Bässler et al. 2010; Leavitt and St. Clair 2011; Baird and Hajibabaei 2012).

In order to facilitate climate change research using lichens, we tested the effectiveness of DNA barcoding for specimen identification of a taxonomically difficult group of lichenized fungi in the family Parmeliacae (Ascomycota) occurring in Greenland. The lichen flora of Greenland is particularly well developed (Hansen 1971; Hansen 1995), even in extremely harsh climatic conditions, such as Johannes V. Jensen Land, the northernmost land on earth (Hansen 2009). A number of studies have evaluated the potential impact of climate change on Greenland lichens (Hansen 2010, 2011a, 2012a, b; Jensen 2012). These indicate that a warmer climate may result in more favorable conditions for some lichens in specific habitats and unfavorable conditions for others. For some lichens, increased growth rates also correspond to warming temperatures (Hansen 2012a). Significant changes in lichen community composition have been observed at a number of permanent plots in northeast Greenland (Jensen 2012), and careful monitoring will be important for continued climate change research.

Within Parmeliaceae, the brown *Parmeliae* (Esslinger 1977) have received increased attention recently and constituted one of the best best-studied groups in the family (Blanco et al. 2004a; Divakar et al. 2010; Arup and Berlin 2011; Divakar et al. 2012; Leavitt et al. 2012a, b, 2013b). The brown *Parmeliae* are a polyphyletic assemblage of genera having a dark brown to medium brown thallus color and usually lacking atranorin or usnic acid in the cortex (Esslinger 1977; Blanco et al. 2004a). The majority of species are found in four genera: *Melanelia* Essl., *Melanelixia* O. Blanco et al., *Melanohalea* O. Blanco et al., and *Montanelia* Divakar et al. (Esslinger 1977; Blanco et al. 2004a; Crespo et al. 2010; Divakar et al. 2012; Thell et al. 2012). *Melanelia* sensu stricto is restricted to a small clade of saxicolous lichens in the cetrarioid clade, and *Cetrariella commixta*, formerly treated as *Melanelia*, is also recovered within the cetrarioid clade (Blanco et al. 2004a; Thell et al. 2004; Thell et al. 2009; Nelsen et al. 2011). The genus *Melanelixia* belongs to the *Melanohalea* clade and includes ca. 15 corticolous, foliose species distributed predominantly in temperate regions in the Northern Hemisphere (Blanco et al. 2004a; Thell et al. 2012). The closely related genus *Melanohalea* includes ca. 22 species that are primarily distributed in the Northern Hemisphere where they generally occur on bark and wood, with some exceptions (Blanco et al. 2004a; Thell et al.

2012; Leavitt et al. 2013b). Lastly, *Montanelia* was recently segregated from *Melanelia* based on morphological and chemical characters and multi-locus phylogenetic reconstructions and includes ca. five species (Divakar et al. 2012).

A number of other genera have traditionally been treated within the brown *Parmeliae*, including *Allantoparmelia* (3), *Almbornia* and *Neofuscelia*, *Pleurosticta* (2 species), and a single species in the cetraroid genus *Cetrariella*, *C. commixta* (Blanco et al. 2004a; Thell et al. 2004; Crespo et al. 2010; Thell et al. 2012). The parmelioid genera *Almbornia* and *Neofuscelia* have been reduced to synonymy under *Xanthoparmelia* (Blanco et al. 2004b) and are not treated here. Similarly, the arctic-alpine genus *Allantoparmelia* is more closely related to *Brodoa* (Elix 1993) and is not included in this study.

A total of 13 brown *Parmeliae* species have been reported for Greenland, including *Cetrariella commixta* (Nyl.) A. Thell & Kärnefelt, *Melanelia agnata* (Nyl.) Thell, *M. hepatizon* (Ach.) Thell, *M. stygia* (L.) Essl., *Melanohalea elegantula* (Zahlbr.) O. Blanco et al., *M. exasperatula* (Nyl.) O. Blanco et al., *M. infumata* (Nyl.) O. Blanco et al., *M. olivacea* (L.) O.

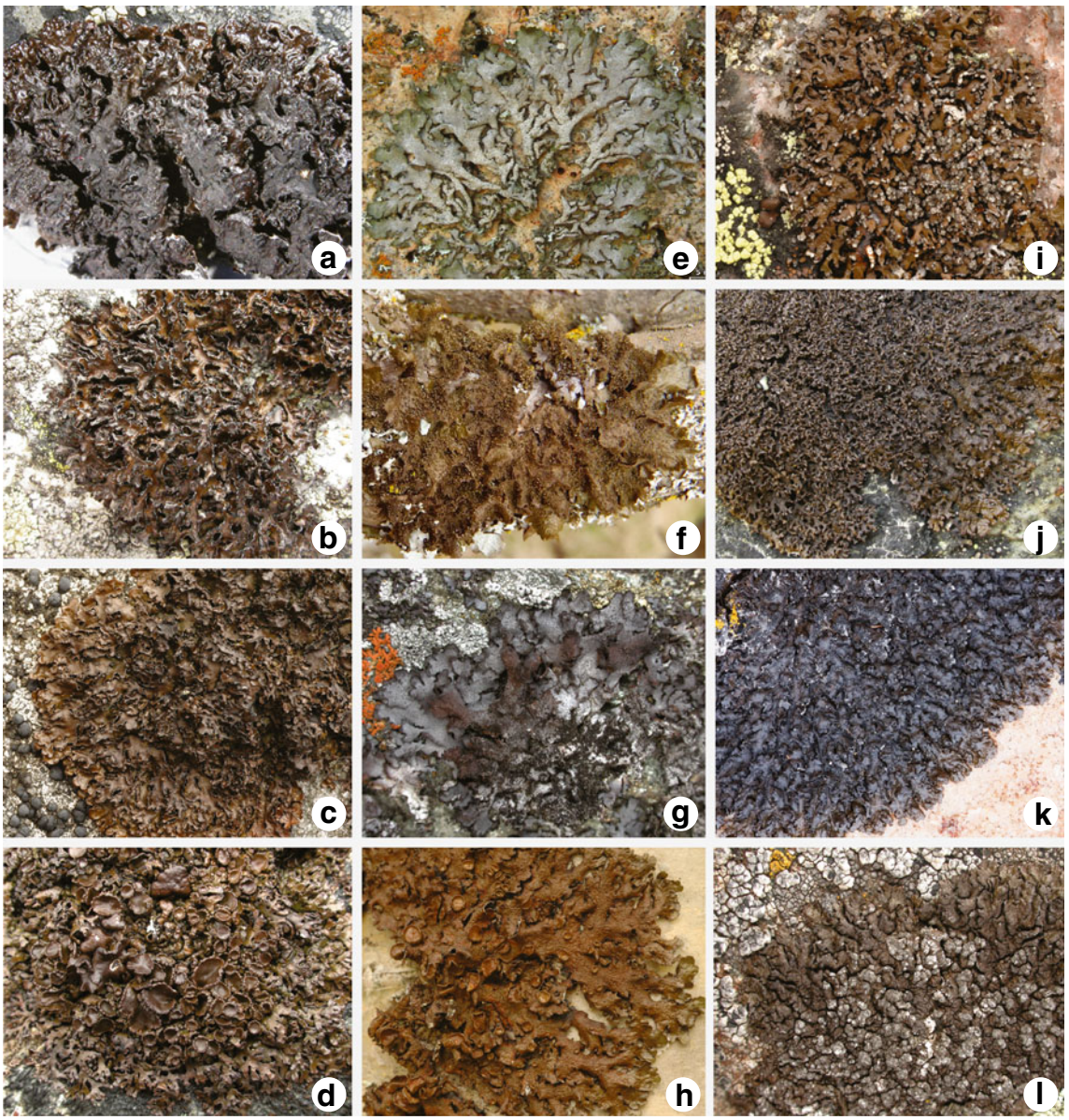

Fig. 1 Brown *Parmeliae* species occurring in Greenland. **a** – **d** Cetrarioid clade: **a** *Cetrariella commixta*; **b** *Melanelia agnata*; **c** *Melanelia hepatizon*; **d** *Melanelia stygia*. **e** – **h** The genus *Melanohalea*: **e** *Melanohalea elegantula*; **f** *Melanohalea exasperatula*; **g** *Melanohalea infumata*; **h** *Melanohalea olivacea*. **i** – **l** The genus *Montanelia*: **i** *Montanelia disjuncta*; **j** *Montanelia panniformis*, **k** *Montanelia sorediata*, and **l** *Montanelia tominii*. *Melanohalea septentrionalis* is not shown, but closely resembles *M. olivacea* (shown in panel **h**). Photo credits: Curtis Björk (**f**), Jason Hollinger (**e**), Chris Parrish (**k**), and Einar Timdal (**a**, **b**, **c**, **d**, **g**, **h**, **i**, **j**, **l**)

Blanco et al., *M. septentrionalis* (Lynge) O. Blanco et al., *Montanelia disjuncta* (Erichsen) Divakar et al., *M. panniformis* (Nyl.) Divakar et al., *M. sorediata* (Ach.) Divakar et al., and *M. tominii* (Oxner) Divakar et al. (Kristinsson et al. 2010) (Fig. 1). Discrimination among closely related brown *Parmeliae* species can be extremely challenging, and specimens with deviating morphological character states are not uncommon, particularly in harsh Arctic habitats. Furthermore, specimen identification may require the use of thin layer chromatography (Culberson 1972; Orange et al. 2001) to elucidate diagnostic chemical characters (Esslinger 1977, 1978).

Given the difficulties in accurate specimen identification using traditional phenotypic characters and the potential value of precise identifications for bio-monitoring research, here we investigate the utility of DNA barcode identification of the 13 brown *Parmeliae* species occurring in Greenland. Specifically, we used genetic information from the ITS region to reconstruct a phylogeny to assess monophyly and genetic divergence among species. We also assessed intra- and interspecific genetic distances and compared intraspecific distance values to a proposed intra-interspecific threshold value for Parmeliaceae (Del-Prado et al. 2010) to identify nominal taxa potential masking previously unrecognized diversity.

Materials and Methods

Taxon Sampling

A total of 13 brown *Parmeliae* species have been reported for Greenland (Kristinsson et al. 2010), belonging to three major groups: (1) the cetrarioid clade, (2) the genus *Melanohalea*, and (3) the genus *Montanelia* (Blanco et al. 2004a; Crespo et al. 2007; Crespo et al. 2010; Divakar et al. 2012). In this study, we sampled a total of 372 specimens representing each of the 13 brown *Parmeliae* species known to occur in Greenland (Supplementary Table S1). Our sampling of the cetrarioid clade included: *Cetrariella commixta* (9 specimens), *Melanelia agnata* (3), *M. hepatizon* (13), and *M. stygia* (6). Sampling in the genus *Melanohalea* included: *M. elegantula* (100 specimens), *M. exasperatula* (104), *M. infumata* (11), *M. olivacea* (35), and *M. septentrionalis* (31). Lastly, the genus *Montanelia* included: *M. disjuncta* (25 specimens), *M. panniformis* (8), *M. sorediata* (6), and *M. tominii* (21). In this study we only evaluated brown *Parmeliae* species know to occur in Greenland. Species from the genera *Melanelixia* and *Pleurosticta* do not occur in Greenland and were not included here. Although *Cetrariella delisei* occurs in Greenland, this species deviates morphologically from other brown *Parmeliae* species with an erect foliose thallus, similar to some *Cetraria* species, and was not included.

For a number of species we were unable to obtain fresh material from collections made in Greenland (i.e., *Melanohalea exasperatula*, *M. olivacea*, *M. septentrionalis*, *Montanelia panniformis*, *M. sorediata*, and *M. tominii*), and these species were represented exclusively by proxy material collected outside of Greenland. *Melanohalea exasperatula*, *M. olivacea*, and *M. septentrionalis* have recently been shown to have broad, intercontinental distributions (Leavitt et al. 2013b), and we assume that populations from Greenland likely fall within the range of sampled variation. In this study, *Montanelia* species not represented by collections made in Greenland were represented by individuals collected from multiple disjunct populations, and we assume these collections adequately characterize genetic diversity within these species. Although identifications were generally based on traditional morphological and chemical characters, final determinations for a number of morphologically ambiguous specimens were confirmed using phylogenetic reconstructions of ITS sequence data. Our sampling (66 sequences) was augmented by 306 ITS sequences obtained from GenBank (Supplementary Table S1).

DNA extraction, PCR amplification, and sequencing

For all new material collected for this study, we extracted total genomic DNA from a small thallus section using the Prepease DNA Isolation Kit (USB, Cleveland, OH, USA) and following the leaf extraction protocol. The complete internal transcribed spacer region (ITS1, 5.8S, ITS2; ~500 bp) was amplified using Ready-To-Go PCR Beads (GE Healthcare) and primers ITS1f (White et al. 1990) with ITS4a (Larena et al. 1999). Cycling parameters for amplifying the ITS marker followed Crespo et al. (2007). PCR products were cleaned using ExoSAP-IT (USB, Cleveland, OH, USA), following the manufacturers' instructions. We sequenced complementary strands from cleaned PCR products using the same primers used for amplification. Sequencing reactions were performed using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, USA). Products were then run on an ABI 3730 automated sequencer according to established protocols (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics at the Field Museum, Chicago, IL, USA.

Sequence assembly and multiple sequence alignments

Contigs were assembled and edited using the program Sequencher v4.10 (Gene Codes Corporation, Ann Arbor, MI). We confirmed sequence identity using a 'megaBLAST' search in the GenBank nucleotide sequence database (Wheeler et al. 2006). Sequences from the complete brown *Parmeliae* data set, including individuals from the cetrarioid clade (33 specimens), *Melanohalea* (281), and *Montanelia* (60), were aligned using the program MAFFT v6, implementing the G-INS-I alignment algorithm, '200PAM / K=2' scoring matrix, and with an offset value of 0.0, with the remaining parameters set to default values. Intraspecific sequences were re-aligned independently for

subsequent comparisons of intraspecific genetic distances for each of the 13 species sampled for this study. Intraspecific sequences were aligned for each of the 13 sampled species using the G-INS-I alignment algorithm, '1PAM / K=2' scoring matrix, with an offset value of 0.2, with the remaining parameters set to default values for all species-specific alignments.

Phylogenetic analysis and genetic distance estimates

In order to assess whether sequences from the brown *Parmeliae* data set form species-specific clades, we used the program RAxML v7.3.2 (Stamatakis 2006; Stamatakis et al. 2008) to reconstruct a maximum likelihood (ML) gene tree from the ITS alignment of all sampled specimens. We used the 'GTRGAMMA' model, which includes a parameter (Γ) for rate heterogeneity among sites, but chose not to include a parameter for estimating the proportion of invariable sites (Stamatakis 2006; Stamatakis et al. 2008). A search combining 200 separate maximum likelihood searches (to find the optimal tree) and 1,000 pseudoreplicates to evaluate bootstrap support for each node was conducted. Species were scored as successfully discriminated if sequences formed a species-specific clade with≥70 bootstrap support (Kelly et al. 2011).

We calculated pairwise distances to characterize both intra- and interspecific variation within and among sampled brown *Parmeliae* species. Pairwise distances can be viewed as a rough measure for the overall sequence divergence (Del-Prado et al. 2010). A previous study suggested a threshold between intra- and interspecific distances close to 0.015–0.017 s/s (Del-Prado et al. 2010), and we used this threshold to identify nominal species potentially masking unrecognized species-level lineages. We used PAUP* (Swofford 2002) to compute average genetic distances based on pairwise comparisons of all sequences within each species individually and pairwise interspecific distances. Pairwise distances between different haplotypes were reported as the number of nucleotide substitutions per site (s/s). The majority of *Melanohalea* sequences obtained from GenBank were missing the first 88 base pairs of the ITS1 region. Due to the missing data, our calculations may provide underestimates of genetic distance values, particularly for *M. olivacea* and *M. septentrionalis*.

Results

The complete brown *Parmeliae* ITS data matrix consisted of 370 sequences and 522 aligned nucleotide position characters (Supplementary Table S1; TreeBase ID: 14288). All new sequences generated for this study have been deposited in GenBank under accession nos. KF257934 – KF257999. Samples sizes for each species, number of haplotypes, and alignment lengths are summarized in Table 1.

Phylogenetic analysis

All traditional brown *Parmeliae* species were recovered as monophyletic clades with strong statistical support [bootstrap support (BS)≥88%] in the ITS gene tree (Fig. 2a; Electronic Supplementary Material, Fig. S1). Additionally, the cetrarioid and *Melanohalea* clades were recovered as monophyletic with strong statistical support (BS≥84%) in the ITS topology, although the genus *Montanelia* was recovered with only weak statistical support (BS<50%). Well-supported phylogenetic sub-structure was identified within *Melanelia agnata*, *M. hepatizon*¸ *M. stygia*, *Montanelia panniformis*, *M. sorediata*, and *M. tominii* (Supplementary Material, Fig. S1).

ITS genetic distances

The distribution of intraspecific pairwise distances for each species is shown in Fig. 2b. Clade-specific (i.e., cetrarioid, *Melanohalea*, and *Montanelia* clades) intra- and interspecific genetic distances are shown in Fig. 2c. Mean distance values, standard deviations, and the ranges of intraspecific distances within the 13 brown *Parmeliae* species occurring in Greenland are reported in Table 1. A slight overlap in intra- and interspecific distances was identified in comparisons among all brown *Parmeliae* species taken collectively (Fig. 2b). However, in clade-specific comparisons (i.e., cetrarioid, *Melanohalea*, and *Montanelia* clades), a barcode gap was detected for each clade, with the exclusion of outlier values in the *Melanohalea* clade (Fig. 2c).

The interquartile range of genetic distances for the majority of species fell below the estimated 0.015–0.017 s/s intra-interspecific threshold (Fig. 2b, c). However, the interquartile range for *Melanelia agnata*, *M. hepatizon*, *Montanelia panniformis*, and *M. tominii* included the proposed intra-interspecific threshold. Similarly, the mean values+the standard error for *Melanelia agnata*, *M. hepatizon*, *Montanelia panniformis*, and *M. tominii* surpassed the proposed 0.015–0.017 s/s intra-interspecific threshold (Table 1). Overall, the complete range of intraspecific distances fell below the estimated intra-interspecific threshold for the following species: *Cetrariella commixta*, *Melanelia stygia*, *Melanohalea elegantula*, *Melanohalea infumata*, and *Montanelia sorediata* (excluding outliers).

Discussion

Accurate specimen identification is important to effectively monitor the impact of changing climate on species distribution and abundance. In this study we investigated the utility of DNA barcode identification of 13 lichen-forming fungal species (Parmeliaceae) occurring in Greenland. Our results indicate that all studied brown *Parmeliae* species in Greenland

Table 1 Mean genetic distance values (given as number of nucleotide substitutions per site) and range of intraspecific distances for brown *Parmeliae* species occurring in Greenland. Numbers within parentheses indicate the number of sampled individuals/number of unique haplotypes, and values following mean genetic distance represent standard deviations. Bolded taxon labels are species with average+standard deviations greater than the proposed 0.015–0.017. intraspecific/interspecific threshold

Species	Alignment length (bp)	Mean	Range
Cetrariella conmixta (11/6)	494	0.002±0.002	0.0–0.006
Melanelia agnata (3/3)	494	0.013±0.008	0.004–0.020
Melanelia hepatizon (13/8)	494	0.013±0.009	0.0–0.028
Melanelia stygia (6/3)	498	0.007±0.005	0.0–0.014
Melanohalea elegantula (100/25)	494	0.002±0.002	0.0–0.014
Melanohalea exasperatula (104/23)	490	0.004±0.004	0.0–0.026
Melanohalea infumata (11/9)	494	0.002±0.002	0.0–0.005
Melanohalea olivacea (35/28)	491	0.009±0.005	0.0–0.023
Melanohalea septentrionalis (31/13)	490	0.005±0.006	0.0–0.020
Montanelia disjuncta (25/20)	496	0.009±0.007	0.0–0.032
Montanelia panniformis (8)	498	0.012±0.007	0.0–0.021
Montanelia sorediata (6/5)	494	0.010±0.005	0.0–0.018
Montanelia tominii (21/15)	500	0.018±0.011	0.0–0.040
Interspecific (372/159)	522	0.089±0.026	0.016–0.146

can be successfully discriminated using the nuclear ribosomal ITS region, the standard DNA barcode for fungi. All phenotypically circumscribed species were recovered as well-supported, monophyletic clades. Furthermore, our data support a barcode gap among congeners (i.e., *Melanohalea*, *Montanelia*, and the cetrarioid clade) for all brown *Parmeliae* species investigated here. However, high intraspecific genetic distances suggest the potential for previously unrecognized species lineages in at least five species: *Melanelia agnata*, *M. hepatizon*, *Montanelia disjuncta*, *M. panniformis*, and *M. tominii*.

Our data support the use of the ITS as an efficient barcoding marker for the brown *Parmeliae* in Greenland. This is consistent with results from temperate ecosystems, where for most genera – with the exception of *Cladonia* (Cladoniaceae) and *Physcia* (Physciaceae) – a barcoding gap was found (Kelly et al. 2011). Subsequently, other species within the genus *Cladonia* have also been shown to lack a barcoding gap using ITS data, which was explained by incomplete lineage sorting, radiations, or hybridizations (Pino-Bodas et al. 2013). In contrast to species within *Cladonia* and *Physcia*, the ITS has been shown to be a reliable marker to discriminate among species in Parmeliaceae (Del-Prado et al. 2010). Likewise, species in the *Rhizoplaca melanophthalma* complex (Lecanoraceae) can be reliably identified using ITS sequence data (Leavitt et al. 2013a). The general success of DNA-based identification of lichen-forming fungal specimens will likely prove valuable with the application of next-generation sequencing for assessing community composition from environmental samples (O'Brien et al. 2005; Medinger et al. 2010), assuming the development of high-quality reference databases.

Hidden diversity in phenotype-based species has frequently been demonstrated in lichenized fungi (Lumbsch and Leavitt 2011). In Parmeliaceae, the comparisons of inter- and intraspecific genetic distances have been shown to provide a practical tool for identifying the potential for species complexes masked under a single nominal species (Del-Prado et al. 2010). Furthermore, Del-Prado et al. (2010) proposed a general threshold between intra- and interspecific distances, close to 0.015–0.017 substitutions/site, for Parmeliaceae. Based on this threshold, additional research will be essential to accurately characterize species level diversity in two species in the genus *Melanelia* (*M. agnata* and *M. hepatizon*) and three *Montanelia* species (*M. disjuncta M. panniformis*, and *M. tominii*). This threshold also suggests the potential for additional lineages within *Melanohalea olivacea* and *M. septentrionalis*, although a recent study provided no evidence for biogeographic or well-supported phylogenetic substructure within either of these species (Leavitt et al. 2013b). Due to differences in population size, mutation rate, and time since speciation, coalescent depths among species may vary considerably (Monaghan et al. 2009; Fujita et al. 2012; Collins and Cruickshank 2012) and may explain the relatively high genetic distances in *M. olivacea* and *M. septentrionalis*. Rather than relying on general intra-interspecific thresholds, thresholds can be estimated directly from the data for taxon-specific optimization (Collins and Cruickshank 2012). However, additional in-depth assessments of the brown *Parmeliae* species with high genetic distance values will ultimately be required to accurately circumscribe species within these complexes.

The impact of contemporary climate change in terrestrial Arctic ecosystems has been well documented. Major vegetation shifts, particularly increasing the biomass of vascular plants, have been shown to adversely affect lichens (Cornelissen et al. 2001; Van Wijk et al. 2004). In *Cassiope tetragona* heaths in

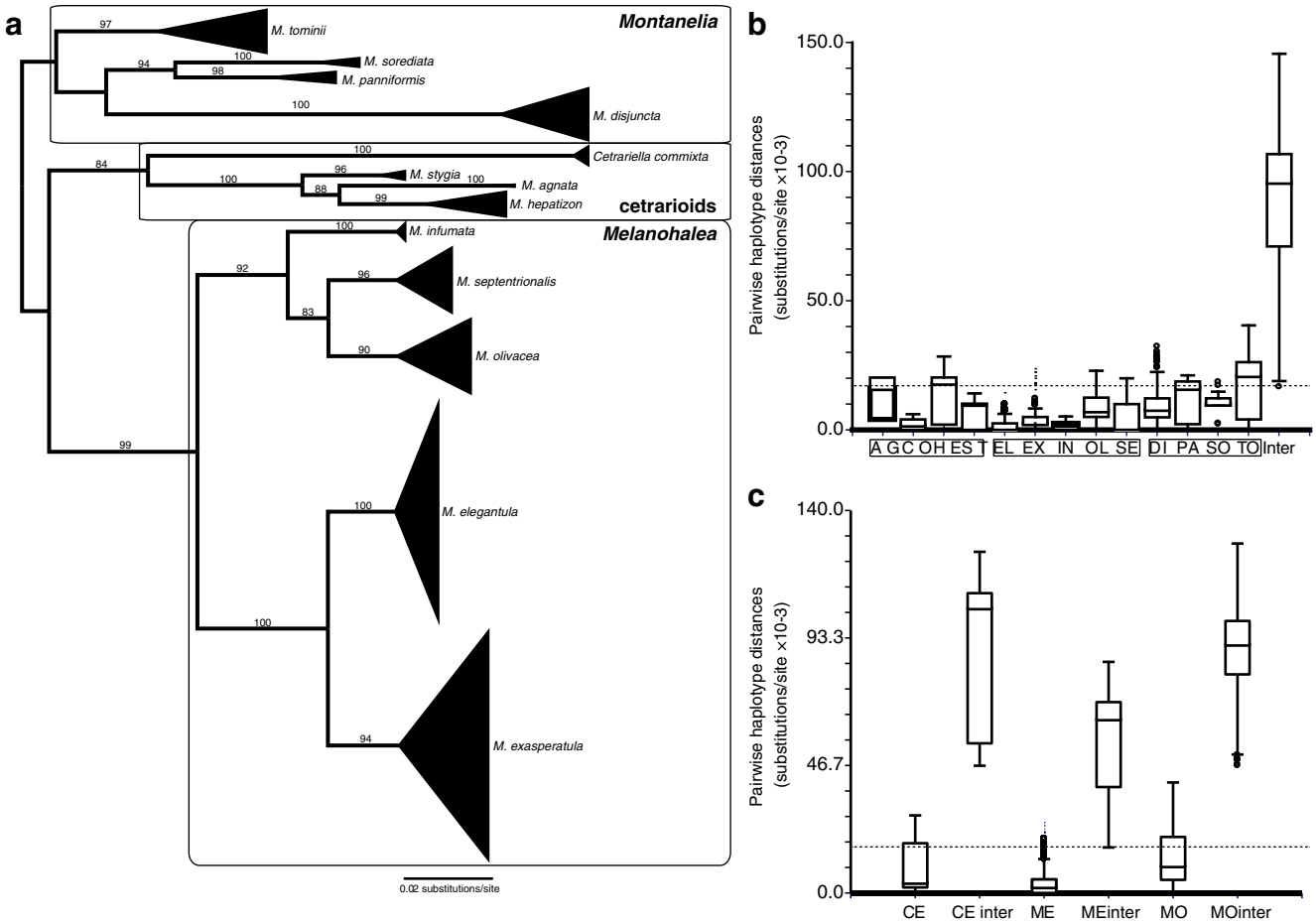

Fig. 2 ITS phylogeny and genetic distances from the 13 brown *Parmeliae* species occurring in Greenland. **a** Cartoon representation of the maximum likelihood ITS topology obtained from 372 brown *Parmeliae* specimens. Values at each node indicate non-parametric bootstrap support; only support values >50% are shown (complete ITS topology is shown in Supplementary Figure, S1). **b** Box plots of ITS genetic distances estimated for each species and all interspecific distances. 'CO'=*Cetrariella commixta*; 'AG'=*Melanelia agnata*; 'HE'=*M. hepatizon*; 'ST'=*M. stygia*; 'EL'=*Melanohalea elegantula*; 'EX'=*M. exasperatula*; 'IN'=*M. infumata*; 'OL'=*M. olivacea*; 'SE'=*M. septentrionalis*; 'DI'=*Montanelia disjuncta*; 'PA'=*M. panniformis*; 'SO'=*M. sorediata*; 'TO'=*M. tominii*. **c** Box plots of pooled intraspecific ITS genetic distances relative to interspecific distances for each of the three major clades sampled here: cetrarioid clade (CE); *Melanohalea* (ME); *Montanelia* (ME). In each box plot, the box shows the interquartile range (IRQ) of the data. The IRQ is defined as the distance between the 75th and the 25th percentile. The solid line within each box represents the median length. The dashed line running across the entire x-axis indicates a threshold between intra- and interspecific distances close to 0.015–0.017 s/s proposed by Del-Prado et al. (2010)

Greenland, certain lichens, including *Cetrariella delisei*, *Flavocetraria nivalis*, *Peltigera leucophlebia*, *P. rufescens*, and *Stereocaulon alpinum*, appear to be favored by an earlier spring and snow melt associated with warming temperatures (Jensen 2012). Similarly, increased growth rates have been documented for *Rhizocarpon geographicum*, *Umbilicaria hyperborea*, *U. virginis*, and *Xanthoria elegans* in the proglacier valley at the Mittivakkat Glacier in southeast Greenland (Hansen 2012a). In contrast, a drier microclimate in *Dryas-Carex rupestris* heaths appears to have a negative effect on some lichens, including *Peltigera venosa* and *Solorina bispora* (Jensen 2012). These heaths are exposed to strong winds and heavy frosts during the winter, and *Peltigera venosa* and *Solorina bispora* appear to simply dry out and die on the gravelly soil in these areas (Hansen, personal observation). In northwest Greenland (Qaanaaq), it appears that drier summers are unfavorable for lichens associated with snow patches and oceanic conditions, such as *Solorina crocea* (Hansen 2011b). Other lichens at Qaanaaq, including *Cladonia mitis* and *Dactylina arctica*, appear to survive because they are adapted to grow in mossy tussocks, which retain water better than the uppermost soil layer (Hansen 2011b).

While specific changes in brown *Parmeliae* communities have not yet been observed in Greenland, we predict that a number of brown *Parmeliae* species in Greenland may increase in relative abundance with the forecast borealization of the Arctic. For example, both *Melanohalea elegantula* and *M. exasperatula* are common corticolous species in boreal zones and into lower latitudes and appear to be spreading in some areas (Otte et al. 2005). These species may respond favorably

to increased vascular plant biomass and warmer temperatures in the Arctic. Similarly, the distribution and abundance of *Montanelia disjuncta* and *M. tominii*, both commonly found on rocks in montane regions in North America and Europe (Otte et al. 2005), would likely expand in Arctic regions with the predicted warming and drying trend. Other species generally restricted to open habitats in boreal, alpine, and Arctic zones, including *Cetrariella commixta*, *Melanelia agnata*, *M. hepatizon*, and *Melanohalea infumata*, will likely respond negatively to the forecast climate change. However, the specific responses of brown *Parmeliae* to climate change and shift in vegetation are largely unknown, and careful monitoring will be required to ascertain their response.

Although each of the 13 brown *Parmeliae* species known to occur in Greenland was included in this study, 6 of these were not represented by collections made in Greenland (*Melanohalea exasperatula*, *M. olivacea*, *M. septentrionalis*, *Montanelia panniformis*, *M. sorediata*, and *M. tominii*). Ultimately, additional studies will be required to empirically confirm that the Greenland populations of these species do, in fact, fall within the range of variation identified in this current study. However, the three *Melanohalea* species occurring in Greenland have recently been shown to have broad, intercontinental distributions with no evidence for biogeographic or well-supported phylogenetic substructure within any of the species (Leavitt et al. 2013b). This suggests that it would be unlikely that populations of *Melanohalea* spp. in Greenland represent distinct lineages or have a substantial impact on estimates of intraspecific genetic distances. Results from this study indicate that each of the three *Montanelia* species found in Greenland form distinct, well-supported monophyletic clades (Fig. 2). However, the average intraspecific genetic distances for both *M. panniformis* and *M. tominii* surpassed the intra-interspecific threshold (Table 1) proposed for Parmeliaceae (Del-Prado et al. 2010). Additional research will be required to assess species boundaries within these *Montanelia* species and the potential for distinct lineages occurring in Greenland. In the meantime, our study indicates that DNA barcoding can be an effective tool for discriminating among morphologically similar *Montanelia* species complexes.

In conclusion, we show that DNA barcoding can be used to successfully discriminate among all brown *Parmeliae* species occurring in Greenland. This study may facilitate effective, long-term climate change bio-monitoring research in Greenland using lichens by providing accurate specimen identifications regardless of taxonomic expertise through molecular identification. Furthermore, we have identified a number of potential species complexes that will require additional research to fully characterize species diversity within these groups.

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