Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi

Pradeep K. Divakar[1], Ana Crespo[1], Mats Wedin[2], Steven D. Leavitt[3], David L. Hawksworth[1], Leena Myllys[4], Bruce McCune[5], Tiina Randlane[6], Jarle W. Bjerke[7], Yoshihito Ohmura[8], Imke Schmitt[9,10], Carlos G. Boluda[1], David Alors[1], Beatriz Roca-Valiente[1], Ruth Del-Prado[1], Constantino Ruibal[1], Kawinnat Buaruang[1,11], Jano Núñez-Zapata[1], Guillermo Amo de Paz[1], Víctor J. Rico[1], M. Carmen Molina[12], John A. Elix[13], Theodore L. Esslinger[14], Inger Kristin K. Tronstad[15], Hanna Lindgren[4], Damien Ertz[16], Cécile Gueidan[17], Lauri Saag[6], Kristiina Mark[6], Garima Singh[9], Francesco Dal Grande[9], Sittiporn Parnmen[3,18], Andreas Beck[19], Michel Navarro Benatti[20], Dan Blanchon[21], Mehmet Candan[22], Philippe Clerc[23], Trevor Goward[24], Martin Grube[25], Brendan P. Hodkinson[26], Jae-Seoun Hur[27], Gintaras Kantvilas[28], Paul M. Kirika[29], James Lendemer[30], Jan-Eric Mattsson[31], María Inés Messuti[32], Jolanta Miadlikowska[33], Matthew Nelsen[3], Jan I. Ohlson[34], Sergio Pérez-Ortega[35], Andres Saag[6], Harrie J. M. Sipman[36], Mohammad Sohrabi[37], Arne Thell[38], Göran Thor[39], Camille Truong[23], Rebecca Yahr[40], Dalip K. Upreti[41], Paloma Cubas[1] and H. Thorsten Lumbsch[3]

[1]Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza de Ramón y Cajal s/n, 28040 Madrid, Spain; [2]Department of Botany, Swedish Museum of Natural History, PO Box 50007, SE-104 05 Stockholm, Sweden; [3]Science & Education, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA; [4]Botanical Museum, Finnish Museum of Natural History, University of Helsinki, PO Box 7, Helsinki FI-00014, Finland; [5]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, USA; [6]Institute of Ecology & Earth Sciences, University of Tartu, Lai Street 38, Tartu 51005, Estonia; [7]Norwegian Institute for Nature Research (NINA), FRAM – High North Research Centre for Climate and the Environment, NO-9296 Tromsø, Norway; [8]National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan; [9]Biodiversity and Climate Research Centre BiK-F, Senckenberganlage 25, 60325 Frankfurt am Main, Germany; [10]Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Goethe Universität, Max-von-Laue-Str. 13, 85 D-60438 Frankfurt, Germany; [11]Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand; [12]Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, c/ Tulipón s/n., 28933 Móstoles, Madrid, Spain; [13]Research School of Chemistry, Australian National University, Building 137, Canberra, ACT 2601, Australia; [14]Department of Biological Sciences Dept. 2715, North Dakota State University, PO Box 6050, Fargo, ND 58108-6050, USA; [15]Tromsø University Museum, University of Tromsø — The Arctic University of Norway, PO Box 6050, Langnes NO-9037, Tromsø, Norway; [16]Department of Bryophytes-Thallophytes, Domaine de Bouchout, National Botanic Garden of Belgium, 1860 Meise, Belgium; [17]Department of Botany, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK; [18]Department of Medical Sciences, Ministry of Public Health, Tivanon Road, Nonthaburi 11000, Thailand; [19]Department of Lichenology and Bryology, Botanische Staatssammlung, Menzinger Str. 67 D-80638, München, Germany; [20]Instituto de Botânica, Núcleo de Pesquisa em Micologia, Caixa Postal 68041, 04045-972 São Paulo, SP, Brazil; [21]Biodiversity and Animal Welfare Research Group, Department of Natural Sciences, Unitec Institute of Technology, Private Bag 92025, Auckland 1142, New Zealand; [22]Department of Biology, Faculty of Science, Anadolu University, EskiŞehir, Turkey; [23]Conservatoire et Jardin botaniques de la Ville de Genève, CP 60, 1292 Chambésy, Switzerland; [24]UBC Herbarium, Beaty Museum, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; [25]Institute of Plant Sciences, Karl-Franzens-University Graz, Holteigasse 6, 8010 Graz, Austria; [26]Grice Lab, Department of Dermatology, University of Pennsylvania, BRB 1046A, 421 Curie Blvd, Philadelphia, PA 19104, USA; [27]Korean Lichen Research Institute, Sunchon National University, Sunchon 540–742, Korea; [28]Tasmanian Herbarium, Private Bag 4, Hobart, Tas. 7001, Australia; [29]Botany Department, National Museums of Kenya, PO Box 45166-00100, Nairobi, Kenya; [30]Institute of Systematic Botany, The New York Botanical Garden, Bronx, NY 10458-5126, USA; [31]School of Life Sciences, Södertörn University, SE-141 89, Huddinge, Sweden; [32]Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA) – CONICET, Universidad del Comahue, Quintral 1250, 8400, Sán Carlos de Bariloche, Río Negro, Argentina; [33]Department of Biology, Duke University, 27708-0338, Durham, NC 27708, USA; [34]Department of Bioinformatics and Genetics, Swedish Museum of Natural History, PO Box 50007, SE-104 05, Stockholm, Sweden; [35]Departamento de Biogeoquímica y Ecología Microbiana, Museo Nacional de Ciencias Naturales, CSIC, c/ Serrano 115, E-28006, Madrid, Spain; [36]Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Königin-Luise-Straße 6–8, 14195, Berlin, Germany; [37]Iranian Research Organization for Science and Technology (IROST), 15815-115 Tehran, Iran; [38]Biologal Museum, Lund University, Box 117, SE-22100, Lund, Sweden; [39]Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, SE-750 07, Uppsala, Sweden; [40]Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR, UK; [41]National Botanical Research Institute (CSIR), Rana Pratap Marg, Lucknow, 226001 Uttar Pradesh, India

Author for correspondence:
Pradeep K. Divakar
Tel: +34 913942282
Email: pdivakar@farm.ucm.es

Summary

• We studied the evolutionary history of the Parmeliaceae (Lecanoromycetes, Ascomycota), one of the largest families of lichen-forming fungi with complex and variable morphologies, also including several lichenicolous fungi.

New Phytologist

Received: 2 April 2015
Accepted: 9 June 2015

New Phytologist (2015)
doi: 10.1111/nph.13553

Key words: ancestral character reconstruction, Ascomycota, lichenicolous fungi, mutualism, Parmeliaceae, phylogeny, *Raesaenenia*.

• We assembled a six-locus data set including nuclear, mitochondrial and low-copy protein-coding genes from 293 operational taxonomic units (OTUs).
• The lichenicolous lifestyle originated independently three times in lichenized ancestors within Parmeliaceae, and a new generic name is introduced for one of these fungi. In all cases, the independent origins occurred *c*. 24 million yr ago. Further, we show that the Paleocene, Eocene and Oligocene were key periods when diversification of major lineages within Parmeliaceae occurred, with subsequent radiations occurring primarily during the Oligocene and Miocene.
• Our phylogenetic hypothesis supports the independent origin of lichenicolous fungi associated with climatic shifts at the Oligocene–Miocene boundary. Moreover, diversification bursts at different times may be crucial factors driving the diversification of Parmeliaceae. Additionally, our study provides novel insight into evolutionary relationships in this large and diverse family of lichen-forming ascomycetes.

Introduction

Mutualistic systems include two or more partners that provide services to each other in order to maximize the net fitness of all partners (Bronstein, 1994). Lichens represent an iconic example of mutualistic interactions. However, relatively little is known of the factors driving partner selection in these systems. In many cases, lichenized fungi can form symbiotic associations with a range of photobiont species. For example, under extreme conditions, lichen-forming fungi have been shown to establish symbioses with a broad range of locally available photobionts (Wirtz *et al.*, 2003; Jones *et al.*, 2013). In some cases, multiple distinct algal species may even be found within a single thallus (Del Campo *et al.*, 2013; Muggia *et al.*, 2013; Dal Grande *et al.*, 2014; Sadowska-Des *et al.*, 2014). Furthermore, some fungal genera include both lichen-forming species and species with different biologies (Hawksworth, 2005), and there are single species that can live either in a symbiotic association with algae or alternatively as saprobes on bark (Wedin *et al.*, 2004; Muggia *et al.*, 2011).

A number of studies suggest that the evolution of lichen symbioses occurred independently several times in Ascomycota (Gargas *et al.*, 1995; Gueidan *et al.*, 2008; Schoch *et al.*, 2009). Within some lichen-forming fungal lineages, especially ascomycetes, a few authors have suggested that nonlichenized fungi have evolved from lichenized ancestors (Eriksson, 1981, 2005; Hawksworth, 1982a; Lutzoni *et al.*, 2001), implicitly suggesting that the lichen symbiosis is labile at an evolutionary scale. Kranner & Lutzoni (1999) argued that transitions from a lichenized to a nonlichenized lifestyle would be more likely, with more losses of lichenization than gains, than vice versa, because lichenization would involve complicated physiological adaptations of both partners. However, experimental evidence indicates that shifts to mutualism can happen within a short time frame given suitable ecological conditions (Hom & Murray, 2014). Such shifts have been considered to occur in other fungal nutritional systems, such as endophytes and plant pathogens (Arnold *et al.*, 2009). The presence of both lichenized and nonlichenized forms in several ascomycete clades clearly indicates that transitions from mutualistic to nonmutualistic lifestyles and/or vice versa must have happened during fungal evolution. However, the pathways of such transitions remain largely unsettled.

Lutzoni and coworkers (Lutzoni *et al.*, 2001; Arnold *et al.*, 2009) have proposed that lichenicolous fungi play an important role in the transition from lichenized to other nonlichenized nutritional modes. It has also long been recognized that a single fungal genus can include species with different nutritional strategies (Santesson, 1967; Wedin *et al.*, 2004; Hawksworth, 2005). Lichenicolous fungi represent an ecological group of over 1800 species that form obligate associations with lichens, as parasites, saprotrophs, or commensals (Hawksworth, 1982b, 2003; Richardson, 1999; Lawrey & Diederich, 2003). Some species are clearly pathogenic, such as *Clypeococcum hypocenomycis*, which causes necrosis and degeneration of the host thallus (Hawksworth, 1980), whereas others can form galls or hardly perturb the thallus with no obvious harmful effects, such as some *Nesolechia* or *Phacopsis* species (Triebel *et al.*, 1995; Peršoh & Rambold, 2002). In the latter case, it has been hypothesized that these lichenicolous fungi have a mutualistic relationship with the photobiont of the lichen, whereas there is a competitive relationship with the primary fungal partner (Poelt & Vězda, 1984; Friedl, 1987; Rambold & Triebel, 1992; Peršoh & Rambold, 2002).

While the lichenicolous lifestyle has been suggested to facilitate the transition to different nutritional modes in fungi (see above), there have been multiple origins of the lichenicolous lifestyle, with lineages including mainly or exclusively lichenicolous fungi, and being unrelated to lichen-forming lineages, in both Basidiomycota (Lawrey *et al.*, 2007; Millanes *et al.*, 2011) and Ascomycota (Diederich *et al.*, 2012; Suija *et al.*, 2015). Some transitions from lichenized to lichenicolous lifestyles have, however, been suggested (Diederich *et al.*, 2012; Frisch *et al.*, 2014).

The aim of this paper was to elucidate phylogenetic relationships within Parmeliaceae and to test whether a transition from lichenized to lichenicolous lifestyles happened within a morphologically and chemically diverse clade of lichenized fungi. Parmeliaceae is one of the largest families of lichen-forming ascomycetes with *c*. 2800 species, representing *c*. 15% of the

total species diversity in lichenized fungi. The family has a world-wide distribution, with the highest diversity in the tropics, but members occur across a broad range of habitats, from hyperarid deserts to polar or alpine regions. The family is characterized morphologically by a specific type of ascoma ontogeny and the presence of an ascomatal feature termed the cupulate exciple (Henssen *et al.*, 1981). Most genera in this family form lichens with large and often complex thalli, having either foliose or fruticose growth forms. Thus it was surprising when internal transcribed spacer (ITS) and nuclear ribosomal small subunit (SSU) rDNA data revealed a phylogenetic affiliation of the lichenicolous genera *Phacopsis* and *Nesolechia* with this morphologically complex family (Peršoh & Rambold, 2002). While this placement was initially questioned (Grube & Hawksworth, 2007), it was subsequently confirmed using additional molecular loci (Crespo *et al.*, 2007, 2010). Here, we assess the phylogenetic placement and the age of origin of the lichenicolous habit within Parmeliaceae, in addition to elucidating the broader evolutionary history of other genera in the family. To this end, we have assembled an extended multilocus data set from 293 operational taxonomic units (OTUs) representing 72 of the 80 genera in Parmeliaceae and included five samples from two lichenicolous species. One additional lichenicolous species was included in a more comprehensive single locus (ITS) data set. Based on the results of this study, we discuss the hypothesis that the lichen symbiosis is labile and that lichenicolous fungi can evolve from lichenized ancestors. We also provide an updated hypothesis of phylogenetic relationships and divergence time estimates for Parmeliaceae.

Materials and Methods

Data assembly

Molecular analyses were based on a six-locus data set (two nuclear ribosomal markers: ITS and the nuclear ribosomal large subunit (nuLSU); the mitochondrial SSU (mtSSU) marker; and three protein-coding loci: the largest subunit of RNA polymerase II (*RPB1*), the DNA replication licensing factor mini-chromosome maintenance complex component 7 (*Mcm7*) and the pre-rRNA processing *Trypanosoma* serine–arginine 1 protein (*Tsr1*)) generated from 293 OTUs with representatives from the families Parmeliaceae, Gypsoplacaceae, Lecanoraceae and Cladoniaceae (Supporting Information Table S1). Species from the family Cladoniaceae were used to root the tree following Crespo *et al.* (2007). The sampling focused on the family Parmeliaceae and included 274 species representing 72 of the 80 accepted genera in this family (Thell *et al.*, 2012). DNA sequences of six loci (Table S1) represented a compilation of sequences from previous studies and others generated specifically for this study. The ITS data set included 297 OTUs. Primer sequences and annealing conditions are reported in Table S2. Detailed materials and methods sections, including gene amplification and DNA sequencing, sequence alignments, phylogenetic analyses, hypothesis testing, ancestral state reconstruction, divergence time estimates, and phylogenetic informativeness (PI) are provided in Methods S1.

Results

Phylogenetic analysis

The number of unambiguous nucleotide positions in each data set, variable and parsimony informative sites, and the best-fitting models of evolution selected in jModelTest (Darriba *et al.*, 2012) are summarized in Table S3. Newly generated sequences (582) of ribosomal DNA (ITS, nuLSU, and mtSSU) and low-copy protein-coding genes (*RPB1*, *Mcm7*, and *Tsr1*) for this study are deposited in GenBank under accession numbers KP888160–KP888313 and KR995270–KR995697 (Table S1). Testing for topological incongruence showed no strongly supported conflicts (results not shown) and hence the concatenated six-locus data matrix was used for all subsequent analyses. Effective sample sizes (ESSs) of all estimated parameters were well above 200 in the Bayesian analyses, and the 'compare plot' produced by 'Are We There Yet?' (AWTY) indicated that parallel Markov chain Monte Carlo (MCMC) runs achieved topological convergence (results not shown). A simplified tree depicting phylogenetic relationships at the generic level is shown in Fig. 1, and the full tree containing all terminal taxa is provided in Fig. S1. While our best topology is largely in agreement with the existing phylogenetic reconstructions for the family Parmeliaceae that were based on fewer loci (Crespo *et al.*, 2007, 2010), our results provide improved resolution and increased nodal support for a number of key groups.

Parmeliaceae s. lat. (node 2) was strongly supported as monophyletic, and the sister-group relationship of *Protoparmelia s. str.* with all other Parmeliaceae (node 3) was also strongly supported. The largest clade within Parmeliaceae, the Parmelioid clade (node 4), was strongly supported. Within the Parmelioid group, a previously unsupported relationship of the *Cetrelia*, *Parmotrema*, and *Xanthoparmelia* clades (node 8) received strong support. Also, a clade consisting of the *Cetrelia*, *Melanohalea*, *Parmotrema*, and *Xanthoparmelia* clades (node 5) received strong support. The genus *Usnea* formed a strongly supported sister group to the monotypic genus *Cornicularia* (node 7). *Menegazzia* spp. formed a monophyletic group with *Coelopogon*, which was strongly supported (node 13). Also, the genera *Oropogon* and *Sulcaria* formed a strongly supported monophyletic group (node 12). The Alectorioid (node 11), Cetrarioid (node 15), Hypogymnioid (node 16), and Psiloparmelioid (node 14) groups were all recovered with strong support. A new clade, the Anzioid group, encompassing species from the genera *Anzia*, *Pannoparmelia*, *Phacopsis* and *Protousnea*, was also recovered with strong support (node 10).

Phylogenetic placement of lichenicolous species

In the six-locus data set, specimens from the lichenicolous genera *Nesolechia* and *Phacopsis* were represented by a single species of each genus. Additionally, another species of *Phacopsis* was included in the ITS data set. Our results confirmed that both genera belong to Parmeliaceae. However, the two lichenicolous genera were recovered in distantly related lineages: *Nesolechia*

New Phytologist

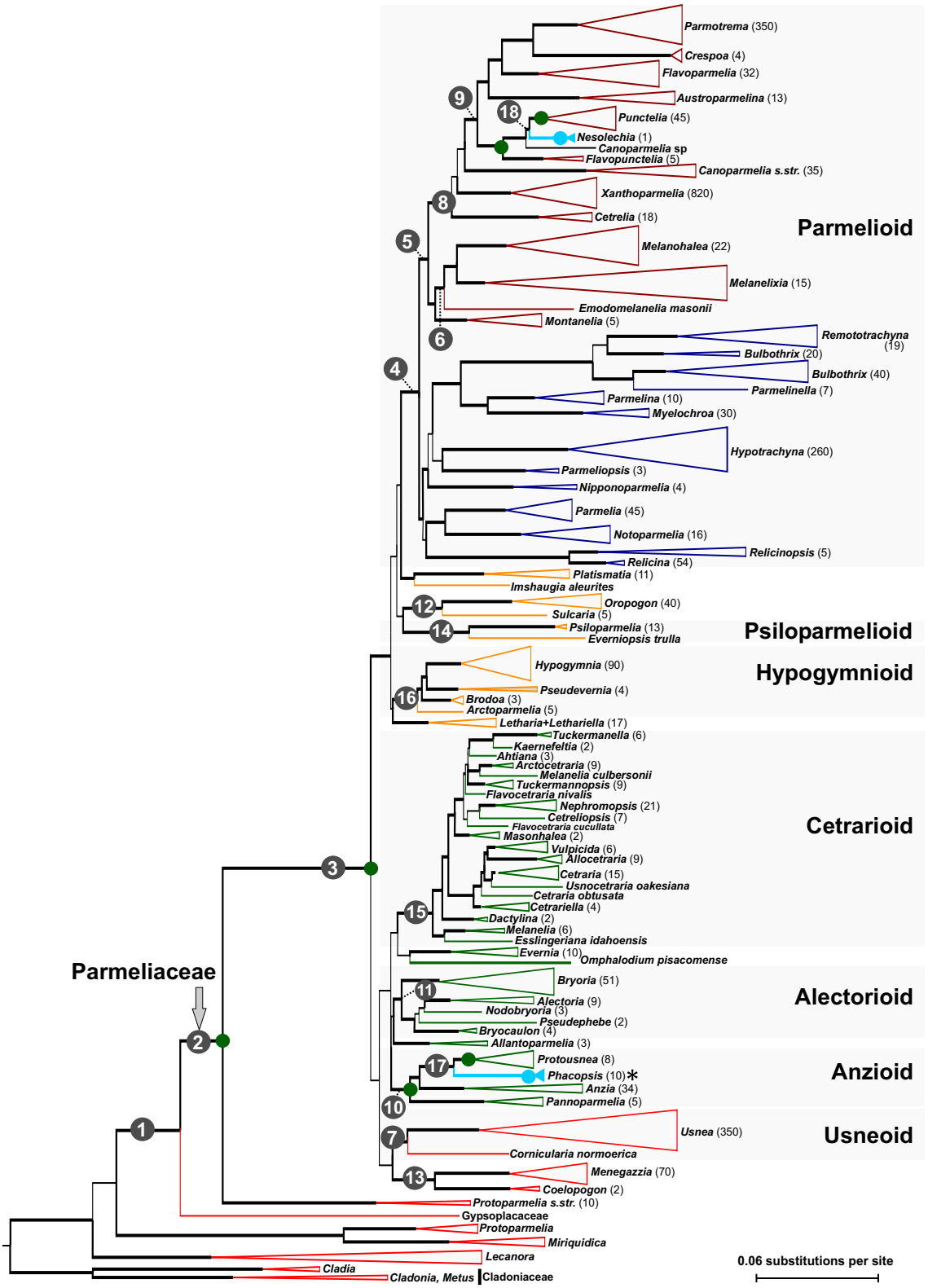

Fig. 1 Cartoon tree showing phylogenetic relationships among major lineages of Parmeliaceae. The tree is derived from a six-locus phylogeny (see Supporting Information Fig. S1). Supported nodes are collapsed to generic level where applicable. The number of species currently accepted in each genus is shown in parentheses. Branches that received strong support in RAxML (bootstrap values ≥ 70%) and/or Bayesian inference (posterior probabilities ≥ 0.95) are in bold. Strongly supported principal nodes are indicated as 1–18. All triangle colors correspond to single figures in Fig. S1. Lichenized (green circles) and lichenicolous (blue circles) ancestral character states are plotted on the node of interest over the tree. **Phacopsis huuskonenii* (placed in the new genus *Raesaenenia* in this paper).

oxyspora (two samples) formed a well-supported sister group with the foliose genus *Punctelia* in the Parmelioid group (node 18), whereas '*Phacopsis' huuskonenii* (three samples) was a sister to the genus *Protousnea* (node 17) in the Anzioid group (Fig. 1) and clearly represents a genus distinct from *Phacopsis vulpina.* That was not surprising as the ascospores in the two species are quite different (Hawksworth, 1978). The new generic name *Raesaenenia* is therefore introduced for *P. huuskonenii* here (Box 1). *Phacopsis vulpina*, represented by a single ITS sequence, formed a sister relationship with the *Relicina* + *Pseudoparmelia* clade in the Parmelioid group in the single-locus ITS analysis (data not shown). Alternative hypothesis testing strongly rejected monophyly of these lichenicolous species ($P < 0.001$ in Shimodaira–Hasegawa (SH) and expected likelihood weight (ELW) tests). Ancestral character reconstruction analyses under maximum parsimony and maximum likelihood optimization criteria estimated the common ancestors of nodes 17 and 18 as being lichenized, therefore suggesting that a transition from lichenized to lichenicolous lifestyle occurred independently in each of the three clades.

Divergence time estimates

Overall, the estimated ages for major clades in Parmeliaceae are similar to the estimations from a previous study based on a more limited sampling (Amo de Paz *et al.*, 2011), and thus the results are not repeated here. Rather, we focus on clades that were not supported in the previous phylogenetic analysis, as well as on dating the origin of lichenicolous lifestyle within the family.

The estimated ages for selected nodes are listed in Table S4 and shown in Fig. 2. Within the Cretaceous, the split of Parmeliaceae from its sister group Gypsoplacaceae was estimated at 126 million yr ago (Ma; 95% highest posterior density (HPD) = 101.21–151.77 Ma; node 1), the split of core Parmeliaceae from *Protoparmelia* at 112 Ma (95% HPD = 92.97–135.47 Ma; node 2), and the split of the *Parmotrema* + *Xanthoparmelia* + *Cetrelia* clades from the *Melanohalea* clade at 68 Ma (95% HPD = 56.87–81.74; node 5). During the Paleocene, the split of *Emodomelanelia* from *Melanelixia* + *Melanohalea* was estimated at 62 Ma (95% HPD = 50.77–74.63 Ma; node 6) and the split of the *Austroparmelina* + *Flavoparmelia* + *Parmotrema* clade from the *Nesolechia* + *Flavopunctelia* + *Punctelia* clade at 55 Ma (95% HPD = 44.85–65.69 Ma; node 9). The crown ages of both the Anzioid and Alectorioid clades were estimated at *c.* 54 Ma (95% HPD = 46.98–61.98 and 46.07–64.26 Ma; nodes 10 and 11). The crown ages of three major groups in Parmeliaceae were estimated at 38 Ma for the Cetrarioid (95% HPD = 30.02–46.16 Ma), 38 Ma for the Hypogymnioid (95% HPD = 27.44–48.90 Ma), and 46 Ma for the Psiloparmelioid (95% HPD = 39.68–62.45 Ma), which dates them to the Eocene. The splits of the lichenicolous species of *Nesolechia* and *Phacopsis* studied from their sister taxa (*Punctelia* and *Protousnea*, respectively) were estimated to have occurred *c.* 25 Ma (95% HPD = 18.34–31.75 and 13.51–41.25 Ma; nodes 18 and 17).

Box 1 *Raesaenenia*: a new generic name for *Phacopsis huuskonenii*.

Raesaenenia D. Hawksw., Boluda & H. Lindgr., **gen. nov**
MycoBank MB 812847

Etymology: In honor of the astute Finnish lichenologist Veli Johannes Paavo Bartholomeus Räsänen (1888–1955) who first described the type species.

Diagnosis: Ascomata resembling those of *Phacopsis* in structure, but differing in the subcylindrical ascospores with thickened caps of wall tissue at each end.

Type species: *Raesaenenia huuskonenii* (Räsänen) D. Hawksw. *et al.* (syn. *Phacopsis huuskonenii* Räsänen).

Raesaenenia huuskonenii (Räsänen) D. Hawksw., Boluda & H. Lindgr., **comb. nov.**
MycoBank MB 812848

Basionym: *Phacopsis huuskonenii* Räsänen, *Lichenoth. Exs.*, fasc. 21 no. 525 (1949).

Type: Finland: Savonia borealis: Pielavesi, Sävia, Lähdemäki, on *Bryoria capillaris* on *Picea excelsa*, 6 March 1949, *K. Huuskonen* (Räsänen, *Lichenoth. Exs.*, fasc. 21 no. 525) (K-IMI 209424 – isotype).

Descriptions and illustrations: Hawksworth (1978), Hafellner (1987), Triebel & Rambold (1988), and Triebel *et al.* (1995).

Phylogenetic informativeness

Based on a per-site comparison, the *Tsr1* gene fragment produced higher PI across relative time units compared with *Mcm7*, *RPB1*, ITS, mtSSU and nuLSU (Fig. S2). *Mcm7* had higher PI values than *RPB1*, ITS, mtSSU, and nuLSU. *RPB1* had a higher PI for older time units (beyond 40 Ma) and ITS had a higher PI for younger time units (before 30 Ma). The two ribosomal markers (nuLSU and mtSSU) showed lower PI values.

Discussion

This study is the first to give conclusive support to the hypothesis that lichenicolous fungi evolved several times within the predominantly lichen-forming fungal family Parmeliaceae, as first suggested by Peršoh & Rambold (2002). The evolution of lichenicolous fungi in a large family that otherwise includes large and morphologically derived lichen-forming fungi is consistent with the hypothesis that there are cases where the lichen symbiosis is labile and fungi with different lifestyles can evolve from lichenized ancestors. These fungi appear to have a mutualistic relationship with the photobionts of the lichens but an antagonistic relationship to the primary fungal partner through competition for resources provided by the photobiont (Poelt & Vězda, 1984; Friedl, 1987; Peršoh & Rambold, 2002). However, the ultrastructural relationship between the partners has not been investigated in these cases, as it has in some other lichenicolous species (de los Ríos & Grube, 2000). The possibility that these fungi are actually lichenized and share the algal partner with the

New Phytologist

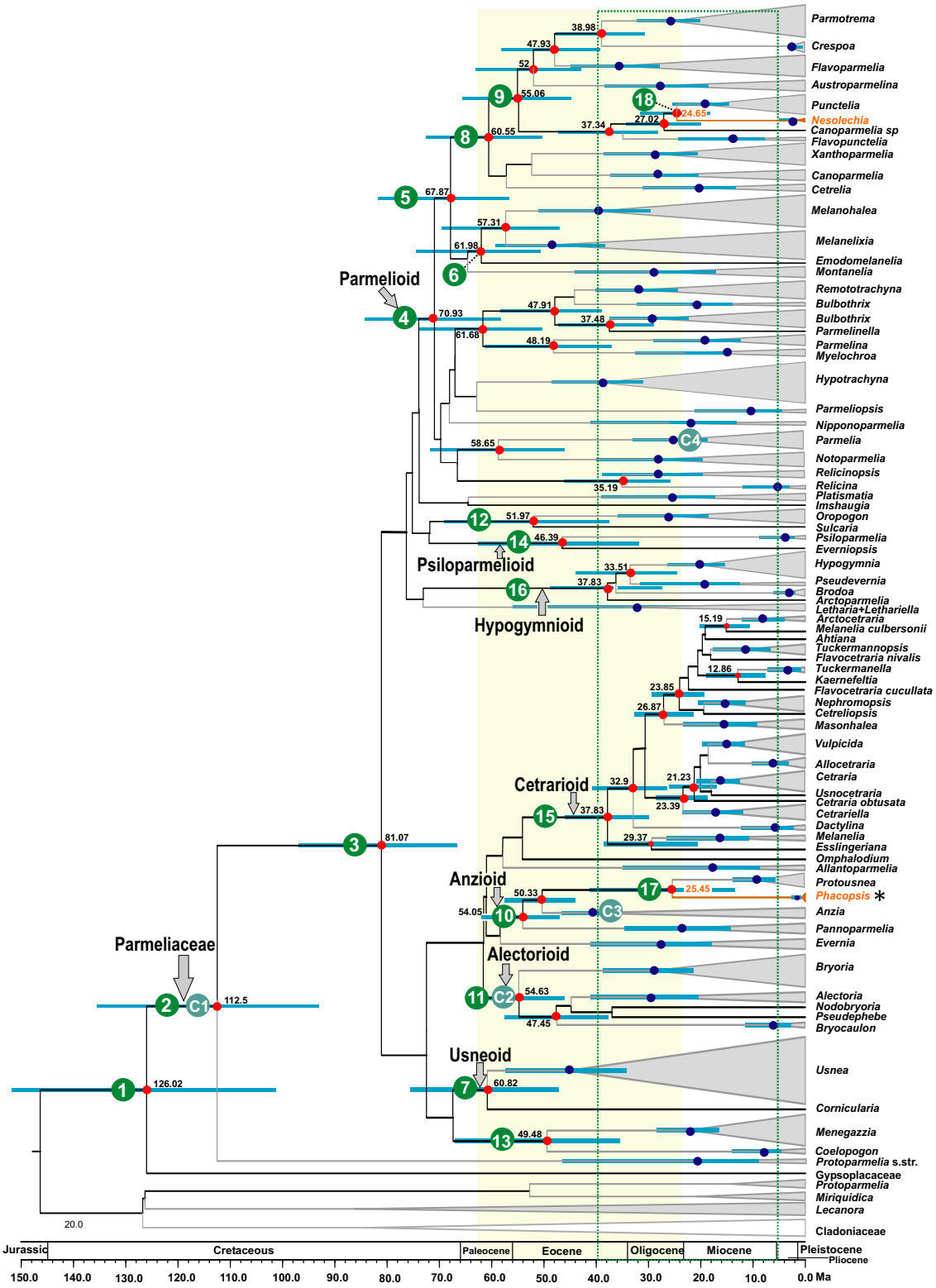

Fig. 2 Timing of Parmeliaceae diversification. Chronogram derived from the maximum clade credibility tree estimated with the uncorrelated Bayesian relaxed molecular clock model method in BEAST (Drummond *et al.*, 2012). Mean ages and their 95% highest posterior density bars are shown above nodes. The nodes indicated by (C) represent calibration nodes: C1, crown node of Parmeliaceae; C2, crown node of Alectorioid clade; C3, crown node of *Anzia*; C4, crown node of *Parmelia*. Supported nodes are collapsed to generic level where applicable. The node splits are indicated in red circles and radiations in blue circles. Major splits period are highlighted with pale yellow rectangle and radiation periods with a green dotted line. Strongly supported principal and other interesting nodes are indicated as 1–18. **Phacopsis huuskonenii* (placed in the new genus *Raesaenenia* in this paper).

lichen's fungal partner cannot be ignored, as several lichenized lichenicolous fungi are known (Hawksworth, 1988, 2003; Rambold & Triebel, 1992).

According to our estimates, the two distantly related lichenicolous genera in Parmeliaceae originated around the same time (*c.* 25 Ma in the late Oligocene). In the late part of the Oligocene, the Earth experienced a warming period, after a long cooling period in the early Oligocene that resulted in growth of the Antarctic ice sheets (Zachos *et al.*, 2008). This warming period, however, was interrupted by cooling periods, such as the Mi-1 glaciation (Zachos *et al.*, 2001; Wilson *et al.*, 2008) at the Oligocene–Miocene boundary *c.* 24 Ma. It has been shown previously that major splits within Parmeliaceae are associated with these climatic shifts (Amo de Paz *et al.*, 2011), and the origin of lichenicolous taxa in Parmeliaceae appears to be related to a major shift in the Earth's climate as well. A phylogenetic analysis of ITS data, including sequences from the lichenicolous *Phacopsis vulpina* (the type species of the genus), suggests the possibility of a third transition within the family. This species formed an independent lineage, sister to the *Relicina* + *Pseudoparmelia* clade (data not shown). However, despite several attempts, we were unable to obtain additional loci from the type species *P. vulpina* and other species of *Phacopsis* because of difficulties in obtaining fresh material, culturing the material, and obtaining PCR products and uncontaminated sequences. Therefore we could not verify the possibility of additional transitions from lichen-forming to lichenicolous lifestyles within the family with additional loci.

The monophyly of Parmeliaceae and its sister relationship with the monotypic family Gypsoplacaceae were strongly supported. Similar relationships have been found in previous studies (Arup *et al.*, 2007; Crespo *et al.*, 2007, 2010; Singh *et al.*, 2013). By contrast, in a recent class-wide study of Lecanoromycetes, the sister relationship between Parmeliaceae and Gypsoplacaceae was not recovered, and the alternative affiliation of the latter family with Malmideaceae and other families within Lecanorineae received high bootstrap (BS) support in selected analyses (Miadlikowska *et al.*, 2014). Here, we provide evidence of a strongly supported sister-group relationship of Gypsoplacaceae and Parmeliaceae within Lecanorineae using a larger sampling of loci and taxa (BS = 100% and posterior probability (pp) = 1.00; Fig. 1). However, the sister relationship of Parmeliaceae is dependent on the selection of the outgroup and the rooting of the tree. While phylogenetic relationships within the family were largely similar to those reported previously using a four-locus data set (Crespo *et al.*, 2010), our new analyses showed an increased number of resolved nodes and previously unrecognized relationships, which are discussed here. The Parmelioid, Hypogymnioid, and Psiloparmelioid clades, and the *Oropogon* + *Sulcaria* and *Platismatia* + *Imshaugia* clades formed a well-supported monophyletic group (pp = 0.97) while the remaining taxa in Parmeliaceae clustered within an unsupported group (Figs 1, S1). While our study supports the placement of the genera *Alectoria*, *Bryoria*, *Bryocaulon*, *Nodobryoria*, and *Pseudephebe* in the Alectorioid clade, the genus *Sulcaria*, considered a member of the Alectorioid clade in previous studies, is shown to be outside the Alectorioid clade and closely related to *Oropogon*. The close relationship between *Oropogon* and *Sulcaria* is not surprising as both genera are characterized morphologically by having septate to muriform brown ascospores and cyphellae-like perforations. The beard lichens, classified in the genus *Usnea*, formed a sister-group relationship with *Cornicularia,* whereas *Menegazzia* spp., which previously formed a sister-group relationship with *Usnea* (Crespo *et al.*, 2010), formed a well-supported sister-group relationship with *Coelopogon* (Fig. 1). The Cetrarioid core group was reconstructed here as monophyletic with strong support, with *Melanelia* and *Esslingeriana* as sister to the other cetrarioid genera. This core group including *Melanelia* was either unsupported or weakly supported in previous studies (Thell *et al.*, 2009; Miadlikowska *et al.*, 2014). Within the Cetrarioid core group, two well-supported clades were recovered in our analysis: the *Cetraria* clade and the *Nephromopsis* clade, the latter unsupported in earlier studies (Thell *et al.*, 2009; Crespo *et al.*, 2010; Nelsen *et al.*, 2011). Within the Parmelioid clade, two major groups were recovered here for the first time: a strongly supported group (BS = 86%; pp = 1.00) including the *Parmotrema*, *Xanthoparmelia*, *Cetrelia*, and *Melanohalea* clades, including almost 80% of the total species diversity of Parmelioid lichens; and a clade that received support in the MrBayes (Huelsenbeck & Ronquist, 2001) analysis only (pp = 0.95), comprising the rest of the Parmelioid species, including the *Nipponoparmelia*, *Hypotrachyna*, *Parmelia*, and *Parmelina* clades (Figs 1, S1). Furthermore, a novel strongly supported clade (BS = 100; pp = 1.00) which included species of the genera *Anzia*, *Pannoparmelia*, and *Protousnea*, and the lichenicolous species of *Phacopsis*, was recovered in Parmeliaceae for the first time and recognized as the Anzioid clade. The genus *Anzia* was part of the *Parmelina* clade in Miadlikowska *et al.* (2014).

Our results provide evidence for the divergence between the species-rich Parmeliaceae and the monotypic Gypsoplacaceae to have occurred in the early Cretaceous (mean age = 126 Ma; 95% HPD = 101–151 Ma; Fig. 2; node 1; Table S4). Divergence estimates between these families have not been inferred in previous molecular dating studies because of a lack of Gypsoplacaceae in the data set (Amo de Paz *et al.*, 2011). The origin of the family Parmeliaceae, represented by the divergence of the crustose genus *Protoparmelia* s.str. (node 2; Fig. 2) from the remaining part of the family (node 3), was here estimated to have occurred in the early Cretaceous (mean age = 112 Ma, 95% HPD = 92–135 Ma) which is almost the same as previously estimated (108 Ma; Amo de Paz *et al.*, 2011). Divergence time estimates for the Parmelioid clade were largely similar to those estimated before (Amo de Paz *et al.*, 2011) and thus are not discussed further here. The origin of the Cetrarioid clade was represented by an initial split of *Melanelia* + *Esslingeriana* at *c.* 37 Ma. However, the earliest divergent lineage was not recovered in the Parmelioid crown, and this may be attributable to the occurrence of lineage extinction events in this clade. The origin of the Usneoid clade (node 7) was estimated as mid-Paleocene, with *Cornicularia* representing the earliest divergent lineage; the Anzioid clade (node 10) in the early Eocene, with *Pannoparmelia* representing the earliest divergent lineage; the Psiloparmelioid clade (node 14) in the mid-Eocene; and the Hypogymnioid clade (node 16) originated at the

Eocene–Oligocene boundary. The age of the Alectorioid clade was estimated to be slightly older (*c.* 54 Ma; Table S4) than the previous estimates (*c.* 47 Ma, Amo de Paz *et al.*, 2011; 49 Ma, Abbas & Guo, 2015), although both estimates fall within the same stratigraphic intervals. A general trend emerging from these data is that the Paleocene, Eocene and Oligocene were key periods when diversification of major lineages within Parmeliaceae occurred, with subsequent radiation happening primarily during the Oligocene and Miocene (see Fig. 2). This may also be linked to the separation of the Southern Hemisphere landmasses (Abbas & Guo, 2015). Diversification bursts at different times may also be crucial factors driving the diversification of Parmeliaceae (Edwards & Donoghue, 2013; Christin *et al.*, 2014). Parmeliaceae shows relatively recent diversification patterns in comparison with other studied lichenized fungal groups (Prieto & Wedin, 2013; Beimforde *et al.*, 2014). High levels of species diversity are also found in many recently evolved groups of angiosperms (Magallon & Sanderson, 2001), with clades such as Apocynaceae, Arecaceae, Burseraceae, Casuarinaceae and Oleaceae, which were reported to have pronounced diversification in the Oligocene and Miocene (Magallon, 2010; De-Nova *et al.*, 2012; Bacon *et al.*, 2012).

The increased taxon and locus sampling, especially the addition of low-copy protein-coding markers such as *RPB1*, *Mcm7* and *Tsr1*, substantially improved the level of phylogenetic resolution and support within Parmeliaceae. Previous comparative studies have shown that low-copy protein-coding markers provide better nodal support than ribosomal markers (Schoch *et al.*, 2009). The *Mcm7* and *Tsr1* loci have been shown to outperform other genetic markers in resolving phylogenetic relationships in Ascomycota (Aguileta *et al.*, 2008; Schmitt *et al.*, 2009). Use of these protein-coding genes has become increasingly common in systematic studies within Ascomycota, including lichen-forming fungi (James *et al.*, 2006; Hofstetter *et al.*, 2007; Crespo *et al.*, 2010; Schmitt *et al.*, 2010; Leavitt *et al.*, 2013; Otálora *et al.*, 2013; Miadlikowska *et al.*, 2014). The PI of ribosomal markers (nuLSU, nuSSU, and mtSSU) and protein-coding genes (*RPB1*, *RPB2*, and *Mcm7*) was assessed for the higher level relationships in the Ascomycota tree of life (Schoch *et al.*, 2009; Raja *et al.*, 2011); however, it was never profiled for any of the major groups of lichenized fungi. The results of our PI analyses showed that *Tsr1* (625 bp) had the highest PI among the tested markers at this phylogenetic scale (Fig. S2). Moreover, *Tsr1* was the main contributor in resolving clades at both the higher and lower taxonomic levels. In our PI analysis, *Mcm7* (512 bp) performed worse than *Tsr1*, but better than *RPB1* and ITS (Fig. S2). ITS (345 bp) performed better than *RPB1* (663 bp) at the species level, whereas *RPB1* outperformed ITS at the generic and higher taxonomic levels. The commonly used ribosomal markers, nuLSU (791 bp) and mtSSU (724 bp), were outperformed by all other markers assessed here (Fig. S2). Thus, our results suggest that the phylogenetic power of *Tsr1* has a great potential to contribute significantly toward more stable relationships among lichenized fungi in Lecanoromycetes.

While our study provides an improved level of phylogenetic resolution within Parmeliaceae, some deep-level relationships, at the backbone and among some of the major clades, still remained unresolved. Whether this is a result of adaptive radiations in the early evolution of Parmeliaceae is unclear. Phylogenomic approaches have been shown to help to resolve deep-level node relationships in different organisms, including fungi (Soltis *et al.*, 2011; Ebersberger *et al.*, 2012; Timme *et al.*, 2012; Zhou *et al.*, 2012; Shen *et al.*, 2013; Ampio *et al.*, 2014), and thus a phylogenomic approach is a logical next step to elucidate deep-level relationships within the Parmeliaceae in the future.

Acknowledgements

We are indebted to Adriano A. Spielmann, Joel Mercado, Robert Egan, Udeni Jayalal and Daniel Sanchez Mata for providing a few samples, and to Rosario G. Gavilán for assisting in collecting expeditions. We also thank editors and anonymous reviewers for valuable comments and suggestions. This work was supported by the Spanish Ministerio de Ciencia e Innovación (projects CGL2010-21646/BOS, CGL2011-25003 and CGL2013-42498-P), the Universidad Complutense-Banco Santander (GR3/14), Comunidad Autónoma de Madrid (REMEDINAL S-2009/AMB-1783), the National Science Foundation ('Hidden diversity in parmelioid lichens'; DEB-0949147), the Swedish Research Council (VR621-2009-5372 and VR 621-2012-3990), the Academy of Finland (grant 1133858), the Estonian Science Foundation (grant 9109), and the European Regional Development Fund (Center of Excellence FIBIR). G.S. was supported by a fellowship from the German Academic Exchange Service (DAAD). I.K.K.T. was supported by a grant from Tromsø University Museum.

References

Abbas A, Guo S-y. 2015. Estimated divergenece of some lineages in Parmeliaceae inferred by Bayesian analysis on RPB2 sequence data. *Journal of Fungal Research* **13**: 73–78.

Aguileta G, Marthey S, Chiapello H, Lebrun MH, Rodolphe F, Fournier E, Gendrault-Jacquemard A, Giraud T. 2008. Assessing the performance of single-copy genes for recovering robust phylogenies. *Systematic Biology* **57**: 613–627.

Amo de Paz G, Cubas P, Divakar PK, Lumbsch HT, Crespo A. 2011. Origin and diversification of major clades in parmelioid lichens (Parmeliaceae, Ascomycota) during the Paleogene inferred by Bayesian analysis. *PLoS ONE* **6**: e2816.

Ampio E, Meusemann K, Szucsich NU, Peters RS, Meyer B, Borner J, Petersen M, Aberer AJ, Stamatakis A, Walsl MG *et al.* 2014. Decisive data sets in phylogenomics: lessons from studies on the phylogenetic relationships of primarily wingless insects. *Molecular Biology and Evolution* **31**: 239–249.

Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A, Hofstetter V, Kauff F, Lutzoni F. 2009. A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Systematic Biology* **58**: 283–297.

Arup U, Ekman S, Grube M, Mattsson J-E, Wedin M. 2007. The sister group relation of Parmeliaceae (Lecanorales, Ascomycota). *Mycologia* **99**: 42–49.

Bacon CD, Baker WJ, Simmons MP. 2012. Miocene dispersal drives island radiations in the palm tribe Trachycarpeae (Arecaceae). *Systematic Biology* **61**: 426–442.

Beimforde C, Feldberg K, Nylinder S, Rikkinen J, Tuovila H, Dörfelt H, Gube M, Jackson DJ, Reitner J, Seyfullah LJ *et al.* 2014. Estimating the Phanerozoic

history of the Ascomycota lineages: combining fossil and molecular data. *Molecular Phylogenetics and Evolution* **78**: 386–398.

Bronstein JL. 1994. Our current understanding of mutualism. *Quarterly Review of Biology* **69**: 31–51.

Christin P-A, Spriggs E, Osborne CP, Strömberg CA, Salamin N, Edwards EJ. 2014. Molecular dating, evolutionary rates, and the age of the grasses. *Systematic Biology* **63**: 153–165.

Crespo A, Kauff F, Divakar PK, Amo G, Arguello A, Blanco O, Cubas P, del Prado R, Elix JA, Esslinger TL *et al.* 2010. Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* **59**: 1735–1753.

Crespo A, Lumbsch HT, Mattsson JE, Blanco O, Divakar PK, Articus K, Wiklund E, Bawingan PA, Wedin M. 2007. Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear *RPB1* gene. *Molecular Phylogenetics and Evolution* **44**: 812–824.

Dal Grande F, Beck A, Cornejo C, Singh G, Cheenacharoen S, Nelsen MP, Scheidegger C. 2014. Molecular phylogeny and symbiotic selectivity of the green algal genus *Dictyochloropsis* s.l. (Trebouxiophyceae): a polyphyletic and widespread group forming photobiont-mediated guilds in the lichen family Lobariaceae. *New Phytologist* **202**: 455–470.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

De-Nova J, Medina R, Carlos Montero J, Weeks A, Rosell JA, Olson ME, Eguiarte LE, Magallon S. 2012. Insights into the historical construction of species-rich Mesoamerican seasonally dry tropical forests: the diversification of *Bursera* (Burseraceae, Sapindales). *New Phytologist* **193**: 276–287.

Del Campo EM, Catalá S, Gimeno J, Hoyo AD, Martínez-Alberola F, Casano LM, Grube M, Barreno E. 2013. The genetic structure of the cosmopolitan three-partner lichen *Ramalina farinacea* evidences the concerted diversification of symbionts. *Fems Microbiology Ecology* **83**: 310–323.

Diederich P, Lawrey JD, Sikaroodi M, van den Boom PPG, Ertz D. 2012. *Briancoppinsia*, a new coelomycetous genus of Arthoniaceae (Arthoniales) for the lichenicolous *Phoma cytospora*, with a key to this and similar taxa. *Fungal Diversity* **52**: 1–12.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.

Ebersberger I, de Matos Simoes R, Kupczok A, Gube M, Voigt K, Kothe E, von Haeseler A. 2012. A consistent phylogenetic backbone for the Fungi. *Molecular Biology and Evolution* **29**: 1319–1334.

Edwards EJ, Donoghue MJ. 2013. Is it easy to move and easy to evolve? Evolutionary accessibility and adaptation. *Journal of Experimental Botany* **64**: 4047–4052.

Eriksson O. 1981. The families of bitunicate Ascomycetes. *Opera Botanica* **60**: 1–209.

Eriksson OE. 2005. Ascomyceternas ursprung och evolution – Protolichenes-hypotesen. *Svensk Mykologisk Tidskrift* **26**: 22–29.

Friedl T. 1987. Thallus development and phycobionts of the parasitic lichen *Diploschistes muscorum*. *Lichenologist* **19**: 183–191.

Frisch A, Thor G, Ertz D, Grube M. 2014. The Arthonialean challenge: restructuring Arthoniaceae. *Taxon* **63**: 727–744.

Gargas A, DePriest PT, Grube M, Tehler A. 1995. Multiple origins of lichen symbioses in Fungi suggested by SSU rDNA phylogeny. *Science* **268**: 1492–1495.

Grube M, Hawksworth DL. 2007. Trouble with lichen: the re-evaluation and re-interpretation of thallus form and fruit body types in the molecular era. *Mycological Research* **111**: 1116–1132.

Gueidan C, Villasenor CR, de Hoog GS, Gorbushina AA, Untereiner WA, Lutzoni F. 2008. A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Studies in Mycology* **61**: 111–119.

Hafellner J. 1987. Studien über lichenicole Pilze und Flechten V. Über die Gattung *Phacopsis* Tul. emend. Körber (Lecanorales). *Herzogia* **7**: 343–352.

Hawksworth DL. 1978. Notes on British lichenicolous fungi: II. *Notes from the Royal Botanic Garden Edinburgh* **36**: 181–198.

Hawksworth DL. 1980. Notes on British lichenicolous fungi: III. *Notes from the Royal Botanic Garden Edinburgh* **38**: 165–183.

Hawksworth DL. 1982a. Co-evolution and the detection of ancestry in lichens. *Journal of the Hattori Botanical Laboratory* **52**: 323–329.

Hawksworth DL. 1982b. Secondary fungi in lichen symbioses: parasites, saprophytes and parasymbionts. *Journal of the Hattori Botanical Laboratory* **52**: 357–366.

Hawksworth DL. 1988. The variety of fungal–algal symbioses, their evolutionary significance and the nature of lichens. *Botanical Journal of the Linnean Society* **96**: 3–20.

Hawksworth DL. 2003. The lichenicolous fungi of Great Britain and Ireland: an overview and annotated checklist. *Lichenologist* **35**: 181–232.

Hawksworth DL. 2005. Life-style choices in lichen-forming and lichen-dwelling fungi. *Mycological Research* **109**: 135–136.

Henssen A, Keuck G, Renner B, Vobis G. 1981. The lecanoralean centrum. In: Reynolds DR, ed. *Ascomycete systematics: the Luttrellian concept*. New York, NY, USA: Springer-Verlag, 138–234.

Hofstetter V, Miadlikowska J, Kauff F, Lutzoni F. 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). *Molecular Phylogenetics and Evolution* **44**: 412–426.

Hom EFY, Murray AW. 2014. Niche engineering demonstrates a latent capacity for fungal–algal mutualism. *Science* **345**: 94–98.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J *et al.* 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**: 818–822.

Jones TC, Hogg ID, Wilkins RJ, Green TGA. 2013. Photobiont selectivity for lichens and evidence for a possible glacial refugium in the Ross Sea Region, Antarctica. *Polar Biology* **36**: 767–774.

Kranner I, Lutzoni F. 1999. Evolutionary consequences of transition to a lichen symbiotic state and physiological adaptation to oxidative damage associated with poikilohydry. In: Lerner HR, ed. *Plant responses to environmental stresses: from phytohormones to genome reorganization*. New York, NY, USA: Marcel Dekker, 591–628.

Lawrey JD, Binder M, Diederich P, Molina MC, Sikaroodi M, Ertz D. 2007. Phylogenetic diversity of lichen-associated homobasidiomycetes. *Molecular Phylogenetics and Evolution* **44**: 778–789.

Lawrey JD, Diederich P. 2003. Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist* **106**: 81–120.

Leavitt SD, Esslinger TL, Spribille T, Divakar PK, Lumbsch HT. 2013. Multilocus phylogeny of the lichen-forming fungal genus *Melanohalea* (Parmeliaceae, Ascomycota): insights on diversity, distributions, and a comparison of species tree and concatenated topologies. *Molecular Phylogenetics and Evolution* **66**: 138–152.

Lutzoni F, Pagel M, Reeb V. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* **411**: 937–940.

Magallon S. 2010. Using fossils to break long branches in molecular dating: a comparison of relaxed clocks applied to the origin of angiosperms. *Systematic Biology* **59**: 384–399.

Magallon S, Sanderson MJ. 2001. Absolute diversification rates in angiosperm clades. *Evolution* **55**: 1762–1780.

Miadlikowska J, Kauff F, Högnabba F, Oliver JC, Molnár K, Fraker E, Gaya E, Hafellner J, Hofstetter V, Gueidan C *et al.* 2014. Multigene phylogenetic synthesis for 1307 fungi representing 1139 infrageneric taxa, 312 genera and 66 families of the class Lecanoromycetes (Ascomycota). *Molecular Phylogenetics and Evolution* **79**: 132–168.

Millanes AM, Diederich P, Ekman S, Wedin M. 2011. Phylogeny and character evolution in the jelly fungi (Tremellomycetes, Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* **61**: 12–28.

Muggia L, Baloch E, Stabentheiner E, Grube M, Wedin M. 2011. Photobiont association and genetic diversity of the optionally lichenized fungus *Schizoxylon albescens*. *FEMS Microbiology Ecology* **75**: 255–272.

Muggia L, Vancurova L, Skaloud P, Peksa O, Wedin M, Grube M. 2013. The symbiotic playground of lichen thalli – a highly flexible photobiont association in rock-inhabiting lichens. *FEMS Microbiology Ecology* **85**: 313–323.

Nelsen MP, Chavez N, Sackett-Hermann E, Thell A, Randlane T, Divakar PK, Rico VJ, Lumbsch HT. 2011. The cetrarioid core group revisited (Lecanorales: Parmeliaceae). *Lichenologist* **43**: 537–551.

Otálora MA, Aragón G, Martínez I, Wedin M. 2013. Cardinal characters on a slippery slope – a re-evaluation of phylogeny, character evolution, and evolutionary rates in the jelly lichens (Collemataceae s. str). *Molecular and Phylogenetic Evolution* **68**: 185–198.

Peršoh D, Rambold G. 2002. *Phacopsis* – a lichenicolous genus of the family Parmeliaceae. *Mycological Progress* **1**: 43–55.

Poelt J, Vězda A. 1984. *Rhizocarpon inimicum* spec. nov. eine weitere parasitische Flechte auf *Lecanora rupicola* spec. coll. *Herzogia* **6**: 469–475.

Prieto M, Wedin M. 2013. Dating the diversification of the major lineages of Ascomycota (Fungi). *PLoS ONE* **8**: e65576.

Raja HA, Schoch CL, Hustad VP, Shearer CA, Miller AN. 2011. Testing the phylogenetic utility of MCM7 in the Ascomycota. *MycoKeys* **1**: 63–94.

Rambold G, Triebel D. 1992. The inter-lecanoralean associations. *Bibliotheca Lichenologica* **48**: 1–201.

Richardson DHS. 1999. War in the world of lichens: parasitism and symbiosis as exemplified by lichens and lichenicolous fungi. *Mycological Research* **103**: 641–650.

de los Ríos A, Grube M. 2000. Host–parasite interfaces of some lichenicolous fungi in the Dacampiaceae (Dothideales, Ascomycota). *Mycological Research* **104**: 1348–1353.

Sadowska-Des AD, Dal Grande F, Lumbsch HT, Beck A, Otte J, Hur JS, Kim JA, Schmitt I. 2014. Integrating coalescent and phylogenetic approaches to delimit species in the lichen photobiont *Trebouxia*. *Molecular Phylogenetics and Evolution* **76**: 202–210.

Santesson R. 1967. On taxonomical and biological relations between lichens and non-lichenized fungi. *Botaniska Notiser* **120**: 497–498.

Schmitt I, Crespo A, Divakar PK, Fankhauser J, Herman-Sackett E, Nelsen MP, Nelson NA, Rivas Plata E, Shimp AD, Widhelm T *et al.* 2009. New primers for single-copy protein-coding genes for fungal systematics. *Persoonia – Molecular Phylogeny and Evolution of Fungi* **23**: 35–40.

Schmitt I, Fankhauser JD, Sweeney K, Spribille T, Kalb K, Lumbsch HT. 2010. Gyalectoid *Pertusaria* species form a sister-clade to *Coccotrema* (Ostropomycetidae, Ascomycota). *Mycology* **1**: 75–83.

Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z *et al.* 2009. The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* **58**: 224–239.

Shen X, Liang D, Feng J, Chen M, Zhang P. 2013. A versatile and highly efficient toolkit including 102 nuclear markers for vertebrate phylogenomics, tested by resolving the higher level relationships of the Caudata. *Molecular Biology and Evolution* **30**: 2235–2248.

Singh G, Divakar PK, Dal Grande F, Otte J, Parnmen S, Wedin M, Crespo A, Lumbsch HT, Schmitt I. 2013. The sister-group relationships of the largest family of lichenized fungi, Parmeliaceae (Lecanorales, Ascomycota). *Fungal Biology* **117**: 715–721.

Soltis D, Smith S, Cellinese N. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* **98**: 704–730.

Suija A, Ertz D, Lawrey JD, Diederich P. 2015. Multiple origin of the lichenicolous life habit in Helotiales, based on nuclear ribosomal sequences. *Fungal Diversity* **70**: 55–72.

Thell A, Crespo A, Divakar PK, Kärnefelt I, Leavitt SD, Lumbsch HT, Seaward MRD. 2012. A review of the lichen family Parmeliaceae – history, phylogeny and current taxonomy. *Nordic Journal of Botany* **30**: 641–664.

Thell A, Högnabba F, Elix JA, Feuerer T, Kärnefelt I, Myllys L, Randlane T, Saag A, Stenroos S, Ahti T *et al.* 2009. Phylogeny of the cetrarioid core (Parmeliaceae) based on five genetic markers. *Lichenologist* **41**: 489–511.

Timme R, Bachvaroff TR, Delwiche CF. 2012. Broad phylogenomic sampling and the sister lineage of land plants. *PLoS ONE* **7**: e29696.

Triebel D, Rambold G. 1988. Cecidonia and Phacopsis (Lecanorales): zwei lichenicole Pilzgattungen mit cecidogenen Arten. *Nova Hedwigia* **47**: 279–309.

Triebel D, Rambold G, Elix JA. 1995. A conspectus of the genus *Phacopsis* (Lecanorales). *Bryologist* **98**: 71–83.

Wedin M, Döring H, Gilenstam G. 2004. Saprotrophy and lichenization as options for the same fungal species on different substrata: enzironmental plasticity and fungal lifestyles in the *Stictis-Conotrema* complex. *New Phytologist* **164**: 459–465.

Wilson GS, Pekar SF, Naish TR, Passchier S, DeConto R. 2008. Chapter 9 The Oligocene-Miocene boundary – Antarctic climate response to orbital forcing. In: Fabio F, Martin S, eds. *Developments in earth and environmental sciences*. Oxford, UK: Elsevier, 369–400.

Wirtz N, Lumbsch HT, Green TGA, Turk R, Pintado A, Sancho L, Schroeter B. 2003. Lichen fungi have low cyanobiont selectivity in maritime Antarctica. *New Phytologist* **160**: 177–183.

Zachos JC, Dickens GR, Zeebe RE. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* **451**: 279–283.

Zachos JC, Pagani M, Sloan LC, Thomas EW, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**: 686–693.

Zhou X, Xu S, Xu J, Chen C, Zhou K, Yang G. 2012. Phylogenomic analysis resolves the interordinal relationships and rapid diversification of the laurasiatherian mammals. *Systematic Biology* **61**: 150–164.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Phylogenetic relationships among almost all accepted genera of Parmeliaceae.

Fig. S2 Phylogenetic informativeness profiles for six loci.

Table S1 Voucher information and GenBank accession numbers of the samples studied

Table S2 Primers and annealing conditions used for amplification and sequencing

Table S3 Genetic variability of the genes used in this study

Table S4 Mean and range of divergence time estimations for Parmeliaceae

Methods S1 Materials and methods.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.