Reproductive and dispersal strategies shape the diversity of mycobiont-photobiont association in *Cladonia* lichens

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ARTICLE INFO

ABSTRACT

Ecological preferences, partner compatibility, or partner availability are known to be important factors shaping obligate and intimate lichen symbioses. We considered a complex of *Cladonia* species, traditionally differentiated by the extent of sexual reproduction and the type of vegetative propagules, to assess if the reproductive and dispersal strategies affect mycobiont-photobiont association patterns. In total 85 lichen thalli from 72 European localities were studied, two genetic markers for both *Cladonia* mycobionts and *Asterochloris* photobionts were analyzed. Variance partitioning analysis by multiple regression on distance matrices was performed to describe and partition variance in photobiont genetic diversity. Asexually reproducing *Cladonia* in our study were found to be strongly specific to their photobionts, associating with only two closely related *Asterochloris* species. In contrast, sexually reproducing lichens associated with seven unrelated *Asterochloris* lineages, thus being photobiont generalists. The reproductive mode had the largest explanatory power, explaining 44% of the total photobiont variability. Reproductive and dispersal strategies are the key factors shaping photobiont diversity in this group of *Cladonia* lichens. A strict photobiont specialisation observed in two studied species may steer both evolutionary flexibility and responses to ecological changes of these organisms, and considerably limit their distribution ranges.

1. Introduction

Lichens, as one of the most spectacular examples of mutualistic symbiotic associations, result from interdependent relationships between heterotrophic fungi, the mycobionts, and one or more population(s) of photosynthetic partners, the photobionts, these being either green or blue-green algae or both (Hawksworth and Honegger, 1994). The obligate and intimate associations between mycobionts and photobionts can lead to the co-evolution of both partners and to concerted diversification (del Campo et al., 2013; Rambold et al., 1998). These processes are in many cases conditioned by the ecological preferences for one or both partners and by the degree of partner specificity (defined as the potential range of compatible partners for a given symbiont; Rambold et al., 1998), with possibilities ranging from generalist associations for both partners, to strong reciprocal specificity, or any of a range of intermediate outcomes including local ecological specialization (Belinchón et al., 2015; Otálora et al., 2010; Yahr et al., 2004). In

lichens, species distribution and ecological adaptations to a certain niche depend on abiotic conditions, such as substrate, availability and different requirements of light, habitat quality and climate (Bannister et al., 2004; Giordani and Incerti, 2007); however, lichen distributions have also been hypothesized to strongly correlate with the ecological specialization and the physiological responses of the photobionts (e.g., Casano et al., 2011; Peksa and Škaloud, 2011; Yahr et al., 2006).

The degree of partner specificity is usually considered as that of the mycobiont towards the photobiont, and it has been correlated with the distributional range of the mycobionts (e.g., Blaha et al., 2006; Fernández-Mendoza et al., 2011; Muggia et al., 2014). In cosmopolitan lichen species-complexes with wide ecological amplitude, low photobiont specificity apparently allows the mycobiont to establish successful symbioses with locally adapted photobionts in a wide range of habitats (Muggia et al., 2014). Alternatively, a widely distributed, but ecologically more restricted mycobiont species was reported to have a narrower photobiont range, likely explained by habitat-scale factors
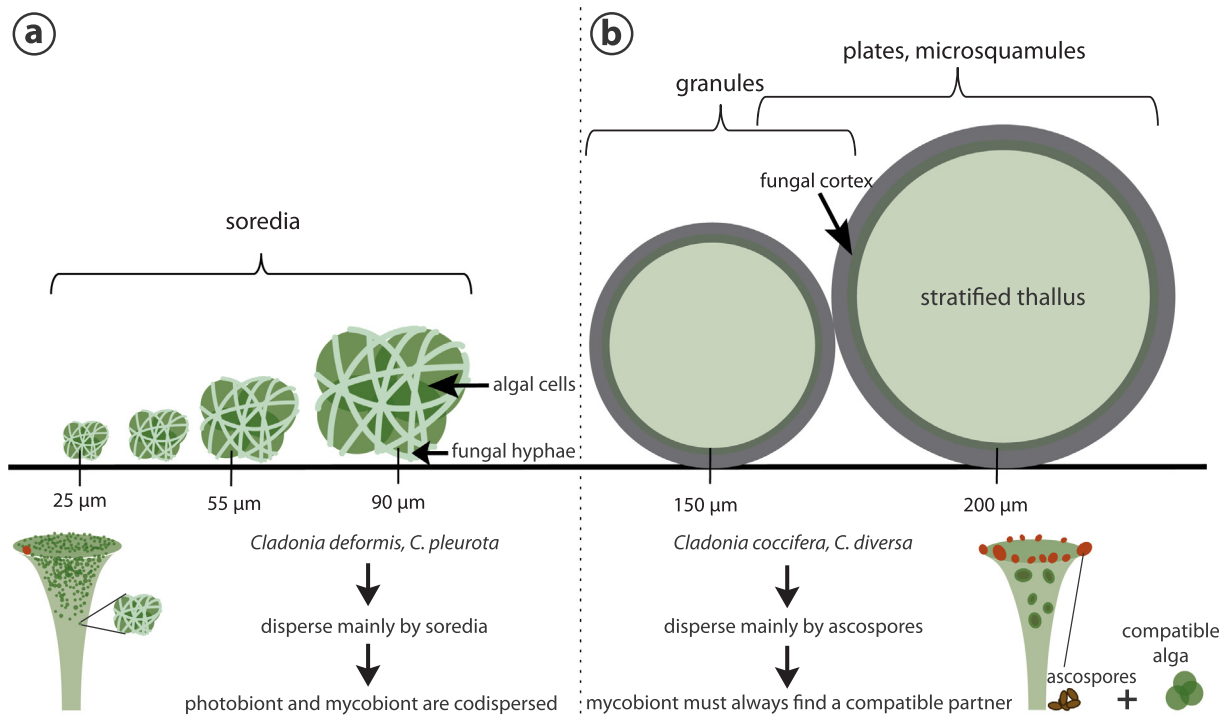
Fig. 1. Schematic representation of *Cladonia* reproductive and dispersal modes. (a) The mycobionts *C. deformis* and *C. pleurota* reproduces mainly asexually and co-disperse together with the photobiont vegetative by soredia, which size is up to 90 μm. (b) The mycobionts *C. coccifera* and *C. diversa* reproduce and disperse predominantly sexually by ascospores, therefore they need to find *de novo* a compatible photobiont; granules, plates and microsquamules are corticated thallus structures of 150–200 μm size.

(Domaschke et al., 2012; Fernández-Mendoza et al., 2011). Similarly, diverse reproductive strategies can result in varying patterns in fungal-algal specificity (Cao et al., 2015; Fedrowitz et al., 2011). On the other hand, interactions between symbionts at the local scale are responsible for observed patterns of selectivity (defined as the locally-observed patterns of association), and these can be influenced by interaction regimes between lichen species and interactions of mycobionts with the available photobionts in particular ecological settings (Belinchón et al., 2015; Yahr, et al., 2006).

While algal cells reproduce clonally by cell division inside the thallus, the different reproductive and dispersal modes which the mycobionts employ account for varying evolutionary advantages and drawbacks of the symbioses. Asexual propagules (in some species even thallus fragments), represent clonal diaspores in which the mycobiont and its compatible algal partner are co-dispersed. Soredia are tiny, abundant, powdery propagules of fungal hyphae wrapped around photobiont cells that detach easily from the thallus. Isidia, granules, plates and microsquamules are outgrowths of the thallus in which photobiont cells are enclosed by a cortex of fungal hyphae. Soredia are lighter and smaller in size than the corticate propagules (see Fig. 1), and potentially allow greater dispersal distances from the parent thallus (Büdel and Scheidegger, 2008). Asexual reproduction circumvents problems of low symbiont availability (Wornik and Grube, 2009), but it reduces the opportunities for adaptive evolution (Eckert, 2002). It is hypothesized that clonal dispersion can lead to high co-evolutionary rate of the two symbionts and their specialization to certain niches but it might decrease the genetic diversity of both partners (Otálora et al., 2012; Wornik and Grube, 2009). Alternatively, a sexually reproducing lichen mycobiont disperses independently by spores and must re-synthesize the thallus with a suitable photobiont. The thallus re-synthesis requires the presence of compatible algae in the environment where the spore germinates and is triggered by the degree of preference by the fungi towards the available photobionts (e.g., Beck et al., 1998; Honegger, 2008, 1993; Ott, 1987). Though sexually reproducing lichen

fungi have to re-establish the symbiosis *de novo* every time, this type of reproduction increases the genotypic diversity and the successful dissemination by long range dispersal (e.g., Bailey, 1976; Belinchón et al., 2015; Pyatt, 1973; Werth et al., 2006). In addition, many closely related lichen species present either sexual or asexual reproductive structures, or both, and they proved to be, therefore, ideal subjects to investigate the dispersal patterns and the genetic diversity of the symbionts (Bannister et al., 2004; Cao et al., 2015; Otálora et al., 2012; Wornik and Grube, 2009).

The genus *Cladonia* is a group of lichenized fungi for which asexually and sexually reproducing taxa are known, and in some species both vegetative propagules and apothecia producing ascospores are present on the same thallus. *Cladonia* is distributed world-wide and is one of the species-richest and morphologically most distinctive genera of lichenized fungi (over 400 described species; Ahti, 2000; Stenroos et al., 2002). *Cladonia* species are also known for their high specificity towards the green algal photobiont genus *Asterochloris* (Trebouxiophyceae; Bačkor et al., 2010; Beiggi and Piercey-Normore, 2007; Nelsen and Gargas, 2006; Piercey-Normore et al., 2010; Škaloud et al., 2015; Yahr et al., 2004, 2006). In the same way, the genus *Asterochloris* has been found to associate only with a limited number of lichenized fungal genera which share similar ecological conditions, and these are correlated with the environmental factors preferred by *Asterochloris* photobionts (Peksa and Škaloud, 2011).

Recent phylogenetic analyses coupled with microscopic observations disentangled 20 phylogenetic lineages within the genus *Asterochloris*, and seven new species were described and characterized by genetic diversity, morphological and anatomical traits (Moya et al., 2015; Škaloud et al., 2015). The species diversity of *Asterochloris* was recorded across multiple, ecologically diverse lichen species (Škaloud et al., 2015; Škaloud and Peksa, 2010) but has never been investigated within a group of closely related lichens so far.

In the *Cladonia-Asterochloris* symbioses different patterns of specificity of the mycobionts towards the photobiont have already been

documented (Piercey-Normore and DePriest, 2001; Yahr et al., 2004, 2006). The role of different factors possibly shaping the algal-fungal association in *Cladonia* symbiosis was evaluated on *Cladonia subtenuis* by Yahr et al. (2006). The authors demonstrated that geographic position and habitat are the best predictors of algal genotype distribution. However, the relation of the photobiont diversity and the dispersal mode(s) of the mycobionts has not been studied so far in this group of lichens.

Here we considered a complex of four red-fruited *Cladonia* species (*C. deformis* (L.) Hoffm., *C. pleurota* (Flörke) Schaer, *C. coccifera* (L.) Willd. and *C. diversa* Asperges ex S. Stenroos) which are characterized by sharing chemical patterns (presence of usnic acid derivates and zeorin occasionally accompanied by porphyrilic acid) but are traditionally differentiated by the type of vegetative propagules (soredia, granules, plates or microsquamules) and by the incidence of producing sexually reproductive structures (apothecia with ascospores; Fig. 1). Because esorediate species (*C. coccifera* and *C. diversa*) present heavy, corticated, vegetative propagules (Fig. 1; plates, granules or microsquamules) which are produced in small amounts and are firmly attached to the potential surface, and regularly build sexual reproductive structures (apothecia, producing spores in large amounts), they have been considered here to reproduce mainly sexually. In contrast, sorediate species (*C. pleurota* and *C. deformis*) are seldom recovered with apothecia (Ahti et al., 2013) and therefore their primary dispersal mode has been hypothesized to be asexual and to depend on the small, light and ecorticate soredia produced in large amounts. Indeed, Molina et al., (2013) demonstrated that the viability of spores of the mixed lichen species *Physconia grisea* is much lower compared to its related sexual species (*P. distorta*). The four species treated here occur in habitats with low rate of competition by vascular plants, e.g., on sandy and rocky acidic soils, on soil in rock crevices; they are seldom found on bark or rotten wood on siliceous bedrock. In Europe the sorediate species *C. deformis* and *C. pleurota* are common in the Northern Scandinavian countries and in Central Europe. In British Isles, Western and Southern Europe they are usually restricted to mountains. On the other hand the esorediate taxa *C. coccifera* and *C. diversa* have broader distributions, and they dominate in areas where sorediate species are very rare (e.g., British Isles, Western Europe). *C. coccifera* is widespread in Europe growing from arctic to warm temperate areas (Ahti et al., 2013). C. *diversa* shows oceanic tendencies, is rather rare in the area of Fennoscandia and avoids high altitudes (Ahti and Steinová, personal observation). These four species as traditionally circumscribed have been previously shown not to be supported by molecular data (Steinová, et al., 2013), and therefore in this study, we focus on their reproductive traits as specimens, rather than at the species level for all statistical tests.

In this context, we aimed to test whether the type of reproductive strategy is the key factor shaping photobiont diversity in a complex of *Cladonia* lichen species across a broad geographical scale. In particular, we tested two main hypotheses: (i) the shared dispersal strategy of mycobiont and photobiont via soredia correlates with a higher specificity of mycobionts towards their photobionts (compared to esorediate lichens which reproduce mainly sexually); (ii) the photobiont diversity of esorediate lichens is determined by the mycobiont sexual reproduction and not by the vegetative dispersal of their propagules (plates, granules or microsquamules). To strengthen these hypotheses, the recovered photobiont diversity was tested against the genetic distance of the mycobionts, the geographic, the climatic and the reproductive variances.

2. Material and methods

2.1. Taxon sampling

A total of 85 lichen thalli from 72 localities in Europe were included in this study (see Table 1, Fig. 2). The lichen material was freshly collected or retrieved from herbarium collections (BG, C, CBFS, GZU, H, MACB, NMW, PL, PRA). The starting dataset for the mycobionts was the one published by Steinová et al. (2013) and it was here complemented with additional 44 new samples. A total of 43 specimens traditionally ascribed to the two sorediate species, *C. deformis* and *C. pleurota*, and 42 specimens ascribed to the esorediate species, *C. coccifera* and *C. diversa*, were used for molecular analyses. At five localities in the Czech Republic and in Germany we collected both sorediate and esorediate specimens growing up to 10 m from each other (Table 2). The specimens were determined using morphological and chemical characters. The presence of zeorin, as key trait of this lichen species complex, was confirmed by thin-layer chromatography (TLC) according to Orange et al. (2001).

2.2. DNA extraction, PCR and sequencing

Dry lichen material was ground to powder and was used for DNA extraction following either the CTAB protocol (Cubero et al., 1999) or the DNA extraction kit InstaGene Matrix (Bio-Rad). Genetic loci were analyzed for both the mycobionts and the photobionts. The fungal ITS region and an intron-containing portion of the β-tubulin gene were amplified as described in Steinová et al. (2013). The algal ITS rRNA gene was amplified using the algal-specific amplification primers ITS1T and ITS4T (Kroken and Taylor, 2000). The actin type I locus was amplified with primers actin_F and actin_R (Cocquyt et al., 2010). PCR conditions were applied as in Steinová et al. (2013) and Muggia et al. (2014). The PCR products were visualized on a 1% agarose gel stained with ethidium bromide and subsequently cleaned using the QIAquick PCR Purification Kit (Genomed) according to manufacturer's instructions. PCR products were sequenced with the same forward and reverse primers used for the PCR amplifications at Macrogen Corp. (Amsterdam, The Netherlands).

2.3. Sequence alignment and phylogenetic analyses

The new obtained sequences were assembled using the software SeqAssem (Hepperle, 2004) and checked for their identity in the GenBank database by blast similarity search (Altschul et al., 1990). The sequences were aligned using MAFFT v.6 software (Katoh et al., 2002) under the QINS-I strategy. Ambiguous SNPs and aligned regions were estimated using the program Gblocks v.0.91b (Castresana, 2000) and were excluded from the alignment. Beginning and ending parts of the sequences containing missing data were also removed from the alignment. For a number of specimens we were unable to generate sequences for all of the selected loci. Additional mycobiont and photobiont sequences were retrieved from the previous study by Steinová et al. (2013) and from GenBank and included in the dataset (Table 1). Identical sequences were removed to speed-up the analyses.

Two different multilocus alignments were prepared for the phylogenetic analyses: (i) the fungal ITS rRNA concatenated with β-tubulin genes alignment, (ii) the algal ITS rRNA gene concatenated with actin alignment. Photobiont sequences were selected to encompass all known lineages of *Asterochloris* (Bačkor et al., 2010; Škaloud et al., 2015) for which data of both loci were available.

The phylogenetic network analyses of *Cladonia* mycobionts was conducted with the program SplitsTree 4 (Huson and Bryant, 2006) as in our previous study (Steinová et al., 2013), because the β-tubulin gene tree was incongruent with the ITS-based phylogeny. The consensus network based on the combined dataset of ITS rRNA and β-tubulin genes sequences was reconstructed using NeighborNet analysis option.

We used single locus trees analyses to detect possible phylogenetic conflicts between the *Asterochloris* photobiont ITS rRNA and the actin genes. As both phylogenies resulted in congruent topologies we used the concatenated dataset for the final analysis.

The phylogenetic analyses were performed with Bayesian inference (BI), Maximum Likelihood (ML) and weighted Maximum Parsimony

Table 1

List of the environmental samples used in the molecular analyses. DNA extraction numbers, voucher numbers, geographic origin and NCBI accessions for the new sequences (bold) obtained for both the mycobionts and the photobionts are reported.

Taxon name	DNA extraction No.	Collection No. (herbarium)	Locality	Photobiont ITS	Photobiont actin	Mycobiont ITS	Mycobiont β-tubulin
Cladonia coccifera	CL31	Haffelner 66608 (GZU)	Austria, Stubalpe, Größenberg	**KT989915**	–	HE611155	HE611207
	CL32	Haffelner 66785 (GZU)	Austria, Stubalpe, Ofnerkogel	**KT989916**	**MK049185**	HE611156	HE611208
	CL39	Haffelner 66214 (GZU)	Austria, Stubalpe, Lichtengraben	**KT989919**	**MK049186**	HE611157	HE611209
	CL52	Bouda 778 (PRC)	Czech Rep., Novohradské hory, Kraví hora	**KT989908**	**MK049180**	HE611158	HE611210
	CL60	Peksa 359 (PL)	Czech Rep., Lužické hory, Studenec	**KT989888**	**MK049177**	HE611159	HE611211
	CL68	Vondrák 4800 (CBFS)	Czech Rep., Kašperské hory, Obří hrad	**KT989909**	–	**KU053034**	**MK049205**
	CL86[1]	Steinová 97 (PRC)	Czech Rep., Brdy, Žďár	**KT989907**	–	**KU053046**	**MK049206**
	CL90	Steinová 43 (PRC)	Czech Rep., Krkonoše, Velká Kotelní jáma	**KT989920**	**MK049187**	HE611160	HE611212
	CL93	Steinová 81 (PRC)	Czech Rep., Českosaské Švýcarsko, Křepelčí důl	**KT989910**	–	HE611161	HE611213
	CL105	Steinová 401 (PRC)	Spain, Somosierra, arroyo de la Peña del Chorro	KP318669	**MK049183**	HE611162	HE611214
	CL124[2]	Steinová 160 (PRC)	Czech Rep., Sedlčansko, Drbákov-Albertovy skály	**KT989890**	–	**KU053015**	**MK049208**
	CL141	Steinová 242 (PRC)	Austria, Nockberge, Erlacher Bockhütte	**KT989921**	–	HE611163	HE611215
	CL143	Steinová 125 (PRC)	Czech Rep., Krkonoše, Obří důl	**KT989922**	**MK049184**	–	**MK049209**
	CL178	Steinová 332 (PRC)	Norway, Rondane, Eisenthøe	**KT989936**	**MK049188**	HE611171	HE611223
	CL179	Steinová 334 (PRC)	Finland, Heinola, Pirttijärvi lake	**KT989929**	**MK049194**	HE611172	HE611224
	CL347	Steinová 537 (PRC)	Austria, Steierische Randgebirge	**KT989911**	–	**KU053042**	**MK049210**
	CL374	Steinová 464 (PRC)	Norway, Hordaland, Bergen	**KT989904**	–	**KU053021**	**MK049211**
	CL375	Steinová 529 (PRC)	Wales, Ty Canol	**KT989896**	–	**KU053037**	**MK049212**
	CL376	Steinová 586 (PRC)	Czech Rep., Jizerské hory, Věžní skály	**KT989914**	–	**KU053038**	**MK049213**
	CL377	Steinová 528 (PRC)	Wales, St. Davids Head	**KT989897**	–	**KU053022**	**MK049213**
	CL379	Steinová 624 (PRC)	Finland, Sondby	**KT989930**	–	**KU053017**	**MK049215**
	CL381	Orange 20406 (NMW)	Wales, Anglesey, Holyhead Mountain	**KT989898**	–	**KU053011**	**MK049216**
	CL383	Steinová 639 (PRC)	Czech Rep., Krkonoše, Sněžka	**KT989905**	**MK049178**	**KU053048**	**MK049217**
	CL394[3]	Steinová 642 (PRC)	Czech Rep., Ještěd	**KT989917**	–	**KU053041**	**MK049218**
	CL395	Steinová 650 (PRC)	Czech Rep., Krkonoše, Obří sedlo	**KT989918**	–	**KU053043**	**MK049219**
	CL396	Steinová 649 (PRC)	Czech Rep., Krkonoše, Sněžka	**KT989933**	–	**KU053016**	**MK049220**
	CL398	Søchting 12153 (C)	Denmark, Zealand, Melby Ovedrev	**KT989901**	–	**KU053023**	**MK049221**
C. diversa	CL54	Bouda 777 (PRC)	Czech Rep., Českosaské Švýcarsko, Babylon	**KT989889**	–	HE611164	HE611216
	CL106	Steinová 400 (PRC)	Portugal, Beira Alta, Parque Natural de Serra de Estrela	KP318671	**MK049182**	HE611165	HE611217
	CL130	Vondrák 6242 (CBFS)	Denmark, Bornholm	**KT989891**	**MK049181**	HE611166	HE611218
	CL172	Steinová 351 (PRC)	Belgium, Kalmthout, Van Ganzenven	**KT989892**	–	HE611167	HE611219
	CL173	Steinová 352 (PRC)	Belgium, Kalmthout, Van Ganzenven	**KT989927**	–	HE611168	HE611220
	CL363	Ahti 72006 (H)	Netherlands, Gelderland, Garderen	**KT989893**	–	**KU053047**	**MK049222**
	CL364[4]	Steinová 596 (PRC)	Germany, Saxony, Oberlausitzer Heide	**KT989912**	–	**KU053013**	**MK049223**
	CL367	Steinová 635 (PRC)	Spain, Asturias, Parque Natural de Redes	**KT989913**	–	**KU053035**	**MK049224**
	CL368	Steinová 634 (PRC)	Spain, Asturias, Parque Natural de Redes	**KT989894**	–	**KU053036**	**MK049225**
	CL370	Steinová 637 (PRC)	Czech Rep., Hradiště	**KT989906**	**MK049179**	**KU053044**	**MK049226**
	CL372	Ahti 68670 (H)	Norway, Hordaland, Bergen	**KT989895**	–	**KU053039**	**MK049227**
	CL392[5]	Steinová 616 (PRC)	Czech Rep., district Tábor, Mlýny	**KT989899**	–	**KU053014**	**MK049228**
	CL397	Søchting 28. X. 2013 (C)	Denmark, Jutland, Bredevandsbakker	**KT989900**	–	**KU053040**	**MK049229**
	CL400	Søchting 12154 (C)	Denmark, Zealand, Tisvilde Hegn	**KT989902**	–	**KU053024**	**MK049230**
	CL404	MACB 97615	Spain, Riofrío de Riaza, sierra de Ayllón	**KT989903**	–	–	–
C. deformis	CLAD 08	Peksa 918 (PL)	Czech Rep., Chvaletice	FM945357	–	HE611205	HE611257
	CL175	Steinová 330 (PRC)	Finland, Suomossalmi	**KT989946**	**MK049198**	HE611190	HE611242
	CL176	Steinová 336 (PRC)	Finland, Varkaus	**KT989928**	**MK049193**	HE611186	HE611238
	CL354	Pentti Alanko 150786 (H)	Finland, Suomenlinna	**KT989947**	–	**KU053019**	**MK049231**
	CL355[5]	Steinová 617 (PRC)	Czech Rep., district Tábor, Mlýny	**KT989961**	–	**KU053029**	**MK049232**
	CL356[4]	Steinová 603 (PRC)	Germany, Saxony, Oberlausitzer Heide	**KT989962**	–	**KU053026**	**MK049233**
	CL357	Steinová 627 (PRC)	Finland, Sondby	**KT989963**	–	**KU053027**	**MK049234**
	CL359	Steinová 587 (PRC)	Czech Rep., Krkonoše, Výrovka	**KT989964**	–	**KU053020**	**MK049235**
	CL360	Palice 16632 (PRA)	Czech Rep., Šumava	**KT989948**	–	**KU053030**	**MK049236**
	CL393[3]	Steinová 644 (PRC)	Czech Rep., Ještěd	**KT989932**	–	**KU053028**	**MK049237**
	CL401	Søchting 10. IX. 2013 (C)	Denmark, Harrild Hede	**KT989966**	–	**KU053031**	**MK049238**
	CL405	MACB 97100	Spain	**KT989940**	–	–	–
C. pleurota	Backor 18	Peksa 820 (PL)	Slovakia, Veľká Fatra, Harmanec	FM945370	–	HE611191	HE611243
	CLAD 06	Peksa 588 (PL)	Czech Rep., Chvaletice	FM945351	–	HE611181	HE611233
	CL26	Palice 11305 (PRA)	Czech Rep., Dolní Loučky	**KT989951**	**MK049200**	HE611193	HE611245
	CL36	Haffelner 65635 (GZU)	Austria, Stubalpe, Lahnhofen	**KT989941**	**MK049195**	HE611194	HE611246
	CL37	Haffelner 65828 (GZU)	Austria, Stubalpe, Lahnhofen	**KT989952**	–	**KU163444**	**MK049239**
	CL43	Peksa 562 (PL)	Czech Rep., Brdy, Hřebenec	**KT989942**	**MK049196**	HE611182	HE611234
	CL44	Peksa 564 (PL)	Czech Rep., Brdy, Hřebenec	**KT989953**	**MK049201**	HE611183	HE611235
	CL45	Peksa 563 (PL)	Czech Rep., Brdy, Hřebenec	**KT989943**	**MK049202**	HE611195	HE611247
	CL64	Vondrák 3631 (CBFS)	Romania, Retezat, Cheile Butii	**KT989954**	**MK049203**	HE611187	HE611239
	CL67	Vondrák 2868 (CBFS)	Czech Rep., Křivoklátksko, Na Andělu	**KT989955**	–	HE611173	HE611225
	CL73	Peksa 574 (PL)	Czech Rep., Chvaletice	**KT989956**	–	HE611174	HE61226
	CL74	Peksa 575 (PL)	Czech Rep., Radvanice	**KT989944**	–	**KU053025**	–
	CL85	Steinová 103 (PRC)	Czech Rep., Brdy, Žďár	**KT989935**	–	HE611196	HE611248

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Table 1 (*continued*)

Taxon name	DNA extraction No.	Collection No. (herbarium)	Locality	GenBank No. Photobiont ITS	Photobiont actin	Mycobiont ITS	Mycobiont β-tubulin
	CL98	Steinová 45 (PRC)	Czech Rep., Krkonoše, Kotel	KT989957	–	HE611188	HE611240
	CL99	Steinová 99 (PRC)	Czech Rep., Brdy, Žďár	KT989958	–	HE611202	HE611254
	CL100	Steinová 65 (PRC)	Czech Rep., Slavkovský Les, Křížky	KT989967	MK049199	HE611176	HE611228
	CL101	Steinová 108 (PRC)	Czech Rep., Brdy, Žďár	KT989923	MK049189	HE611203	HE611255
	CL104	Steinová 126 (PRC)	Czech Rep., Brdy, Hřebenec	KT989924	MK049190	HE611185	HE611237
	CL125	Steinová 161 (PRC)	Czech Rep., Sedlčansko, Husova kazatelna	KT989959	–	KU053032	–
	CL128[2]	Steinová 164 (PRC)	Czech Rep., Sedlčansko, Drbákov-Albertovy skály	KT989960	MK049204	HE611180	HE611232
	CL136	Steinová 215 (PRC)	Finland, Helsinki, Rastila	KT989925	MK049191	HE611200	HE611252
	CL148	Steinová 241 (PRC)	Austria, Gurktaler Alpen, Nassbodensee	KT989945	MK049197	HE611189	HE611241
	CL150	Steinová 187 (PRC)	Finland, Vantaa, Fagersta	KT989926	MK049192	HE611204	HE611256
	CL350	GZU 000303377	Monte Negro, Prokletije Mountain Range, Krš Bogićevica	KT989937	–	KU053018	MK049240
	CL385	Peksa 1722 (PL)	Czech Rep., Ledce, Krkavec	KT989965	–	–	–
	CL386	Steinová 551 (PRC)	Austria, Gurktaler Alpen, Hochrindl	KT989938	–	KU053033	MK049241
	CL388	Steinová 176 (PRC)	Austria, Koralpe, Weinebene	KT989949	–	KU053045	MK049242
	CL389	Steinová 312 (PRC)	Czech Rep., Slavkovský les, Dominova skalka	KT989950	–	–	MK049243
	CL390	Steinová 339 (PRC)	Norway, Rondane, Einsethøe	KT989939	–	–	–
	CL391	Steinová 341 (PRC)	Norway, Rondane	KT989931	–	–	–
	CL403	Tønsberg 42460 (BG)	Norway, Oppland, Lom, Breidsæterdalen	KT989934	–	–	MK049244

(wMP) approaches. Models of molecular evolution were selected independently for the two photobiont loci, ITS rRNA and actin genes, according to the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.4 (Darriba et al., 2012). The models applied were the TIM2ef + G for the photobiont ITS rRNA gene partition, and the TrNef + G for the actin partition. A Bayesian analysis was implemented using MrBayes version 3.2.1 (Ronquist et al., 2012) and was used for the phylogenetic tree construction. Two parallel MCMC runs were carried out for five million generations, each with four chains. Trees and parameters were sampled every 100 generations. The convergence of the chains was assessed during the run by calculating the average standard deviation of split frequencies (SDSF). Further, the log-likelihood scores were plotted against generation time using Tracer 1.4 (Rambaut and Drummond, 2007) to determine when the stationarity of likelihood values have been reached (e.g., the burn-in stage; Ronquist et al., 2012). Burn-in was set at one million generations and the majority rule consensus trees were calculated from the posterior samples of 40,000 trees. The SDSF value between simultaneous runs was 0.006174 in the concatenated dataset. ML and MP phylograms were used for bootstrapping and they were obtained using Garli version 2.0, and PAUP version 4.0b10 (Swofford, 2002), respectively. ML analyses consisted of rapid heuristic searches (100 pseudo-replicates) by using automatic termination (the genthreshfortopoterm command set to 100,000). The weighted parsimony (wMP) bootstrapping (1000 replications) was performed using heuristic searches with 100 random sequence addition replicates, tree bisection reconnection swapping, random addition of sequences (the number limited to 10,000 for each replicate), and gap characters treated as a fifth character state. The weight to the characters was assigned using the rescaled consistency index on a scale of 0–1000. New weights were based on the mean of the fit values or each character over all of the trees in memory. The phylogenetic trees were visualized in TreeView (Page, 1996).

2.4. Variance partitioning

We performed variance partitioning by multiple regression on distance matrices (MRM; Legendre et al., 1994; Lichstein, 2006; Manly, 1986; Smouse et al., 1986) to describe and to partition variance in photobiont genetic diversity. This method computes adjusted R^2 for the complete model, estimating how much of the total variability is defined by explanatory variables. It also allows estimating R^2 for each of the explanatory variables as well as their shared components. The

photobiont genetic distance matrix was used as response matrix, whereas genetic distance of the mycobionts as well as geographic, climatic and reproductive distances were used as explanatory matrices. Pairwise genetic distances of photobionts was obtained from the alignment of ITS rDNA using JC69 model of evolution (Jukes and Cantor, 1969). The JC69 model was also used for the concatenated fungal ITS rRNA and β-tubulin genes alignment. Geographic distances were calculated from longitudinal and latitudinal data of the sampling localities. Climatic variation was modelled using climatic database WorldClim (Hijmans et al., 2005) at resolution of 2.5 arc minutes. Principal component analysis of 19 bioclimatic variables was used to reduce dimensionality (for list of bioclimatic variables and PCA biplot see Supplementary material Fig. S1 and Table S1). Similarity in reproductive strategy (asexually vs. sexually) was defined as Jaccard distance (Jaccard, 1912). All the analyses of the variance partitioning were performed using R (ver. 3.0.3; R Development Core Team).

3. Results

The final dataset contained 271 sequences, 187 of which were newly obtained in this study: 80 algal ITS rRNA sequences, 28 algal actin sequences, 38 fungal ITS rRNA sequences and 41 fungal β-tubulin sequences (Table 1). Additional 84 sequences (of both fungi and algae) were retrieved from our previous datasets (Bačkor et al., 2010; Škaloud et al., 2015; Steinová et al., 2013) and from GenBank. Sequence data were unambiguous, suggesting that only single genotypes of both mycobionts and photobionts were present in the thallus. Fungal ITS rRNA and β-tubulin sequences were 547 and 673 base pairs in length, respectively, with 69 and 79 variable and 52 and 47 informative characters, respectively. For algal loci, ITS rRNA sequences were 509 base pairs long, with 76 variable characters of which 58 were parsimony-informative. Algal actin sequences were 576 base pairs long with 392 variable sites and 342 parsimony-informative sites.

3.1. Network analyses of Cladonia mycobionts

The consensus network of ITS rRNA and β-tubulin sequences (see Fig. 3) resulted in conflicting relationships among species, already found in our previous results (Steinová et al., 2013). The broad edges at the core of the network and the absence of long branches (except one subgroup of *Cladonia pleurota* containing specimens C6, CL67, CL73, CL100 and CL128) suggested incomplete lineage sorting or ongoing
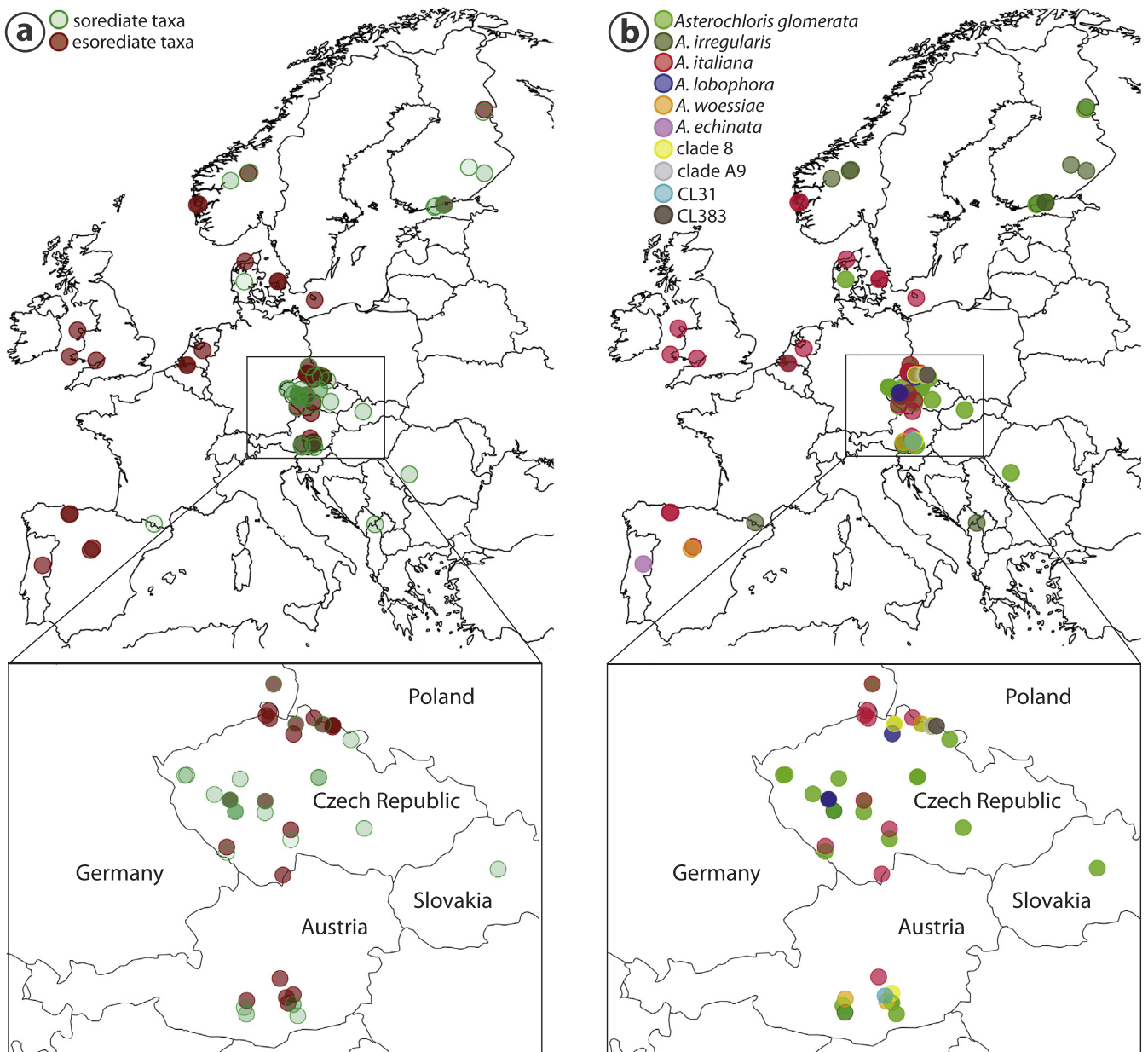
Fig. 2. Geographic maps of the collection sites across Europe with expanded maps of the Central Europe. The distribution of the *Cladonia* mycobionts (a) and the *Asterochloris* photobionts species (b) is colour coded. (a) Sorediate species with asexual reproduction and vegetative dispersal (green), esorediate species with sexual reproduction and dispersal (red). (b) Distribution of the *Asterochloris* photobionts: the eight lineages and the single *Asterochloris* sequences correspond to those recognized in the phylogenetic analysis of Fig. 4; the two *Asterochloris* lineages associated with the asexually reproducing *Cladonia* species are labelled in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison of *Asterochloris* diversity recovered in five localities in which multiple samples have been collected. Samples in grey represent sorediate collections.

| Locality ID	Geographic origin	*Cladonia* lichen species	sample ID	*Asterochloris* photobiont
1	Czech Rep., Brdy, Žďár	*C. coccifera*	CL86	*A. lobophora*
	Czech Rep., Brdy, Žďár	*C. pleurota*	CL85	*A. irregularis*
	Czech Rep., Brdy, Žďár	*C. pleurota*	CL99	*A. glomerata*
	Czech Rep., Brdy, Žďár	*C. pleurota*	CL101	*A. irregularis*
2	Czech Rep., district Tábor, Mlýny	*C. diversa*	CL392	*A. italiana*
	Czech Rep., district Tábor, Mlýny	*C. deformis*	CL355	*A. glomerata*
3	Czech Rep., Ještěd	*C. coccifera*	CL394	*clade 8*
	Czech Rep., Ještěd	*C. deformis*	CL393	*A. irregularis*
4	Czech Rep., Sedlčansko, Drbákov-Albertovy skály	*C. coccifera*	CL124	*A. italiana*
	Czech Rep., Sedlčansko, Drbákov-Albertovy skály	*C. pleurota*	CL128	*A. glomerata*
5	Germany, Saxony, Oberlausitzer Heide	*C. diversa*	CL364	*A. italiana*
	Germany, Saxony, Oberlausitzer Heide	*C. deformis*	CL356	*A. glomerata*
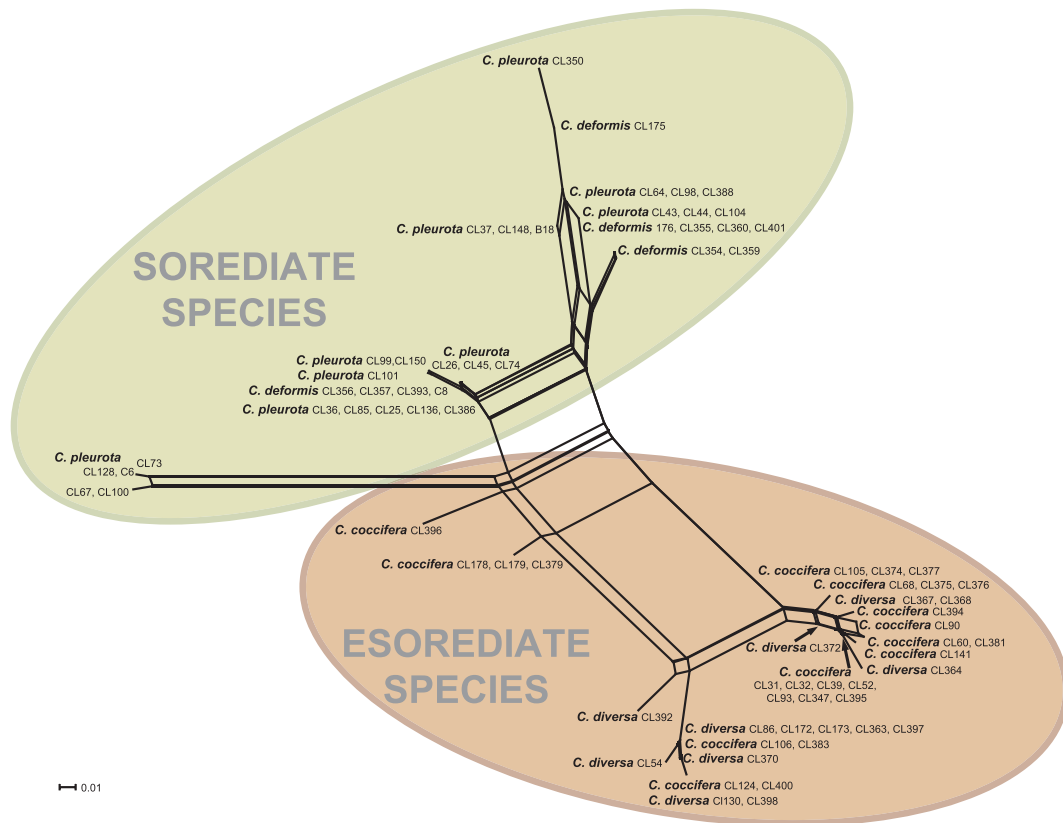
Fig. 3. Neighbor-net analysis of *Cladonia* mycobionts based on the combined fungal loci ITS and β-tubulin. Sorediate and esorediate species segregate in two defined groups joined by broad splits.

speciation among the four studied taxa, and corroborate the results previously shown by Steinová et al. (2013). Four samples of *C. coccifera* (CL178, CL179, CL379 and CL396) were inferred on isolated splits and remained separated from both the sorediate and esorediate groups.

3.2. Phylogenetic analyses of the Asterochloris photobionts

The Bayesian, MP and ML phylogenies resulted in similar topologies and were congruent with the phylogenetic inference of Škaloud et al. (2015). Bayesian analysis of the concatenated ITS rDNA and actin dataset resulted in 19 well-resolved *Asterochloris* lineages (see Fig. 4). Thirteen clades represented already described *Asterochloris* species, the remaining six have not been assigned a name yet (two of which were preliminary identified by the names "clade 8" and "clade A9"). The species *A. excentrica* was represented by a single sequence and was on its own single branch. The clades were fully resolved and the majority of them were highly supported. A total of eight *Asterochloris* lineages plus two *Asterochloris* sequences, which were recovered on individual branches and did not correspond to any currently recognized lineage, were found to associate with the sampled *Cladonia* taxa. *Asterochloris glomerata*, *A. italiana* and *A. irregularis* were the most common photobionts and were recovered in 31, 24 and 18 samples, respectively.

All studied sorediate *Cladonia* specimens associated only with *A. glomerata* and *A. irregularis*. However, *A. irregularis* was also the photobiont of the esorediate mycobionts, whereas *A. glomerata* associated exclusively with the sorediate mycobionts. The esorediate *Cladonia* specimens, on the other hand, exhibited a much lower level of specificity towards the associated photobionts: seven *Asterochloris* lineages and those two *Asterochloris* sequences recovered on individual branches were found to associate with them. *C. coccifera* was found to associate with six *Asterochloris* lineages (*A. irregularis*, *A. woessiae*, *A. italiana*, *A. lobophora*, *Asterochloris* clade 8 and clade A9) and the two unique

Asterochloris sequences. *C. diversa* was found to associate with four *Asterochloris* species (*A. irregularis*, *A. echinata*, *A. italiana* and *A. lobophora*).

Asterochloris italiana was the most frequent photobiont associated with the esorediate *Cladonia* specimens (24 samples), and two subclades were recognized: the first containing samples from Belgium, Denmark, Netherlands, Spain, Wales and from lower altitudes from Czech Republic, the second comprising samples from higher elevation in Czech Republic, Austria, Germany and Spain. Specimens of sorediate (CL85, CL99, CL101, CL128, CL355, CL356, and CL393) and esorediate (CL86, CL142, CL364, CL392, and CL394) samples which were collected in the same localities were always found to associate with different photobionts.

Clear differences of photobiont distribution across Europe and in *Cladonia* thalli could be recognized (Fig. 2b). *A. italiana* was mostly recovered from localities spread in the North-Western oceanic part of Europe, including Great Britain, Denmark, Belgium, The Netherlands and the Norwegian coast, but also from Central Europe (Austria, Czech Republic and Germany), Portugal and Spain (Table 1). In the North-Eastern Fennoscandia we detected only *A. glomerata* and *A. irregularis* (associated both with sorediate and esorediate fungal species). *Cladonia* samples collected in Central and Southern Europe associated with a considerably higher number of *Asterochloris* lineages. Although we included only six *Cladonia* samples from the Iberian Peninsula, we found four *Asterochloris* lineages associated with the mycobionts in this region (*A. echinata*, *A. irregularis*, *A. italiana* and *A. woessiae*). Samples collected in Central Europe contained seven *Asterochloris* lineages and the two unique *Asterochloris* sequences. The Austrian Alps and the Krkonoše Mts. in the Czech Republic were the regions with the richest *Asterochloris* diversity detected in Central Europe.
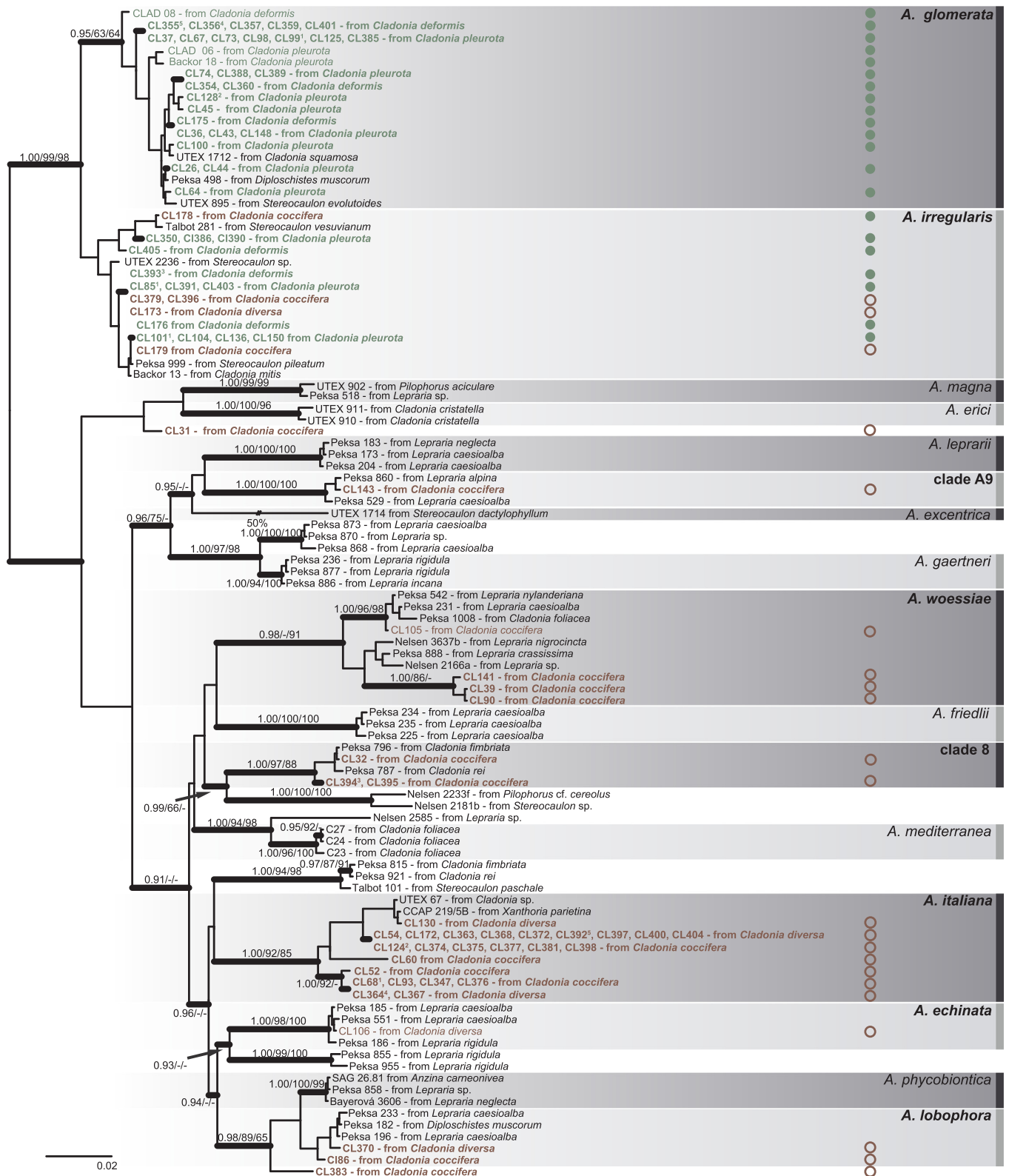
7

Fig. 4. Multigene phylogenetic inference of *Asterochloris* photobionts: Bayesian hypothesis based on the combined dataset of ITS and actin I loci. Bootstrap support for the ML and MP analyses and the Bayesian posterior probability are reported at the corresponding branches. Branches have been collapsed to report multiple samples represented by the same sequence data. Upper case numbers (1–5) correspond to specimens of sorediate and esorediate species collected at the same locality, as reported in Table 2. Samples are colour coded according to the reproductive and dispersal mode of the lichens: sorediate asexual species in green and esorediate sexual species in red. *Asterochloris* lineages recovered in the studied *Cladonia* are in bold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
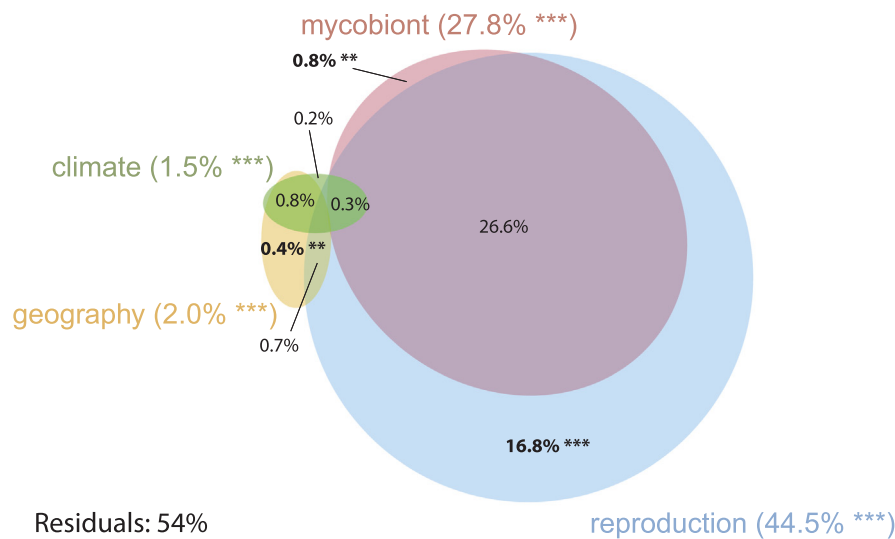
Fig. 5. Variance partitioning analysis showing the percentage of explained photobiont diversity based on the four explanatory variables of (i) the mycobiont genetic diversity, (ii) the geographic, (iii) the climatic and (iv) the reproductive distances. Values in bold show pure effects of the explanatory variables.

3.3. Variance partitioning

The PCA of bioclimatic variables revealed two main gradients. The first PC axis explained 40.39% of photobiont variability and was mainly following a precipitation gradient. The second axis corresponded to the temperature range and seasonality gradient and explained 31.52% of variability in the climatic data set (see PCA biplot in Supplementary material Fig. S1). In downstream analyses, we use scores of sites for PC1 and PC2 to represent climatic conditions.

Our linear regression model including reproductive strategies, geographical distance, climatic similarity and genetic distance of mycobiont significantly explained altogether 46.16% of variability in the genetic distance of photobionts (Fig. 5). Of these, the reproductive mode had the largest explanatory power, with 43.90% of variability explained, though a large portion was also represented by the covariance with the mycobiont genetic distance. The isolated effect of reproduction mode, when accounted for covariance with all other explanatory matrices, was associated with 16.7% of variability in genetic distance of photobiont (Fig. 5). The other explanatory variables accounted only for minor percentages of variability, although they were selected as significant for the complete linear model. A substantial percentage of variability remained unexplained by our model (53.8%) and might account for some unmeasured environmental heterogeneity, or alternatively, a degree of stochasticity in associations.

4. Discussion

4.1. Species delimitation in Cladonia lichens

In our study we tested whether reproductive mode might shape the diversity of mycobiont-photobiont association in *Cladonia* lichens. For this purpose we analyzed specimens that differed by the type of the vegetative propagules present on the podetium, which is at the same time an important morphological character used for the taxonomic assignment in this group of lichens.

As found previously, the phylogenetic analysis of the mycobionts

did not support the current delimitation of the studied taxa which is based on morphological characters, in particular the type and size of vegetative propagules. Such a discrepancy between the traditional morphologically based species delimitation and the results of phylogenetic analyses is, however, not uncommon in *Cladonia* lichens (e.g., Pino-Bodas et al., 2010, 2011, 2012) and suggests that a critical revision of characters used for species delimitation in this group of lichens is needed. Our previous study (Steinová et al., 2013) showed that some of the lineages can be characterized by subtle chemical differences.

4.2. Symbiotic diversity is shaped by host reproductive strategies

Mutualistic interactions offer suitable examples to study co-evolution, partner specificity, evolutionary responses and ecological adaptations to symbiotic lifestyles (Bronstein et al., 2004). So far only few studies have evaluated the influence of reproductive strategies of mycobionts on the specificity of photobiont associations (Cao et al., 2015; Fedrowitz et al., 2011; Otálora et al., 2010; Wornik and Grube, 2009). The selected complex of the four *Cladonia* taxa is well suited to test multiple hypotheses in this context. Because the mycobionts of sorediate species rarely build sexual reproductive structures, they have been hypothesized to reproduce and disperse asexually. The prevailing asexual dispersal mode would logically justify specific mycobiont-photobiont associations, because fungi and algae are co-dispersed. Alternatively the esorediate taxa, in which mycobionts abundantly produce apothecia, are hypothesized to reproduce mainly sexually by ascospores. By reproducing sexually, these mycobionts could show different levels of specificity towards their photobiont.

Within the broad spectrum of their geographic distribution in Europe, our results show that the two sexually reproducing *Cladonia* species adopted a generalist strategy by associating with numerous *Asterochloris* lineages. In contrast, the asexually reproducing species were associated exclusively with *A. glomerata* or *A. irregularis* even in localities where other *Asterochloris* species were detected in the sexually reproducing species (Table 2). The strict maintenance of these two *Asterochloris* lineages over large geographic distances indicates high

specificity towards its photobionts (Fedrowitz et al., 2012). A similar pattern of high mycobiont specificity towards its symbionts in asexually reproducing lichens, coupled with a low level of specificity in sexually reproducing lichens, was observed also previously in other *Cladonia* species (Yahr et al., 2004, 2006) and in *Nostoc*-associating *Nephroma* and *Degelia* species by Fedrowitz et al. (2011) and Otálora et al. (2012).

All *Cladonia* species studied here produce some kind of vegetative propagules. We hypothesize that the different specificity of the mycobiont toward its photobiont can be attributed to the differences in size and amount of vegetative propagules built on the podetium, and the ability to produce viable ascospores. The role of the relatively large vegetative propagules of esorediate *Cladonia* species in lichen dispersal is likely very limited, and the mycobiont dispersion is ensured by the ascospores produced in the always- abundant ascomata. Therefore, the low specificity towards the algal partner is advantageous for the esorediate *Cladonia* fungi, which have to find a suitable algal partner shortly after their germination. On the contrary, soredia represent abundant and light vegetative propagules that detach easily and can replace ascospores as the main dispersal propagules with all pros and cons of this strategy. Interestingly, it has been reported that ascospores of sorediate lichens can have a strongly reduced reproductive function (Molina et al., 2013). The authors compared the spore viability between the mixed species *Physconia grisea* and the related sexual species *P. distorta* and showed that mature apothecia from both species discharged meiospores capable of germination, but spores from *P. grisea* rarely (0.43%) developed, whereas those from *P. distorta* developed and germinated successfully.

The algal genetic diversity in populations of lichenized fungi having distinct propagation strategies is, however, not always necessarily different. This can be explained by the process denoted as "algal switching" (Piercey-Normore and DePriest, 2001), where a successful horizontal photobiont transmission is commonly observed in sexually reproducing lichen fungi, but it seldom takes place in asexual species. *Physconia* species, though reproducing vegetatively by soredia, presented an unexpected high photobiont diversity (Wornik and Grube, 2009). It was suggested that depending on the viability of the soredial algae, the soredial fungi could choose between establishing the new thallus with the co-propagated alga or with another photobiont, likely better adapted to the local conditions. The main role of the original photobiont would be to prolong the survival of the co-propagated fungal hyphae (Wornik and Grube, 2009).

Photobiont switching is now understood as a rather common phenomenon in lichen symbiosis (e.g., Nelsen and Gargas, 2009; Piercey-Normore, 2006) allowing the mycobiont to adapt to local environmental conditions (Werth and Sork, 2010) or to extend its geographical range or ecological niche (Fernández-Mendoza et al., 2011). The asexual *Cladonia* fungi, by having the possibility to switch algae, would be guaranteed the option of either maintaining their algal partner, or replacing it if a better-adapted one is available. Other symbiotic systems, such as corals, are well known for their *in situ* adaptation and capacity to regulate their fitness according changing ecological conditions. Studies on *Anthopleura-Symbiodinium/Elliptochloris* symbioses have shown how the presence and the identity of the photobionts in different environmental conditions balance the life and the reproductive strategies of the anemone host (Bingham et al., 2014). In lichens the maintenance of the symbiotic association would, therefore, be an option but not a strict consequence of the joint, vegetative symbiont propagation (Wornik and Grube, 2009).

Our results show that the studied asexual *Cladonia* species do not switch photobionts, and we suggest that this may result in severe consequences for their survival in a changing environment. The ability to switch photobionts allows a fine-tuned symbiosis to be flexible and resilient over geographic and environmental gradients in space and time, while a very specific mutualism may lead to its termination (Nelsen and Gargas, 2009). This is particularly true for asexually reproducing lichens in which a substantial proportion of the evolutionary flexibility has already been lost by the absence of sexual reproduction, thought to be beneficial to the longevity of a species (Muller, 1932). Clonal reproduction via vegetative propagules helps to overcome the problem of limited availability of symbiotic partners. However, such a tight and rigid relationship between symbiotic partners together with the loss of adaptability by strictly asexual reproduction may become an evolutionary trap in the long term.

In corals, the adaptive bleaching hypothesis (Buddemeier and Fautin, 1993) explains that process by which the animal hosts reshuffle their photosynthetic symbiont to overcome environmental changes and survive (Baker, 2003; Parkinson and Baums, 2014). The studied *Cladonia* species are found in diverse ecological conditions, and we demonstrate that the photobionts are significantly structured by both climate and geography, although the explanatory power of these is smaller compared to the mode of reproduction. In the future, however, they might face severe environmental changes. If they will not evolve the ability to switch to locally adapted photobionts, they may become rare or even go extinct (Domaschke et al., 2012), as it has already been shown in other systems (LaJeunesse et al., 2003).

4.3. Can symbionts substantially control the distribution of the hosts?

We observed two main patterns of *Asterochloris* diversity across Europe: (i) high *Asterochloris* diversity within relatively small geographic regions, and (ii) wide geographic areas dominated by only one or two *Asterochloris* species. The first group was represented in Czech Krkonoše Mts. and the Austrian Alps, where we found five named *Asterochloris* lineages plus a single *Asterochloris* type, belonging to a still unnamed taxon: these were associated with seven (from Krkonoše Mts.) and ten (from Austrian Alps) *Cladonia* specimens respectively. The Central European mountains seem therefore to represent hotspots of *Asterochloris* species richness. In contrast, the North-Western oceanic parts of Europe were dominated by *A. italiana*. In Nordic countries (Norway and Finland), *Cladonia* species were found to associate mostly with *A. glomerata* and *A. irregularis*. This can be explained either by the preference of the *Cladonia* species to associate with those *Asterochloris* lineages adapted to the local environmental conditions or by a very low *Asterochloris* diversity in these areas. The low *Asterochloris* diversity in the area of Fennoscandia may be caused by environmental filtering (Dal Grande et al., 2017) or, alternatively, can possibly be the consequence of the recolonization history after the last glacial maximum (e.g., Hoarau et al., 2007). This finding would contradict the hypothesis of ubiquitous distributions of microorganisms caused by their high dispersal rates (Fenchel and Finlay, 2004; Finlay, 2002; Ryšánek et al., 2015; but see Lowe et al., 2012). However, patterns similar to those observed here have already been reported for other symbiotic protists (Domaschke et al., 2012; LaJeunesse et al., 2010) and might well be explained by the co-propagation of both symbiotic partners constraining their distributional ranges. Further study of other potential *Asterochloris* hosts in Fennoscandia could help to test this hypothesis.

There is strong evidence that symbiotic organisms associate preferentially with locally adapted partners, both in lichens (e.g., Dal Grande et al., 2017, 2012; Muggia et al., 2014) and other mutualistic associations (e.g., Finney et al., 2010; Pánková et al., 2014; Sampayo et al., 2007; Ulstrup and Van Oppen, 2003). This implies that low specificity of the host towards its symbiotic partner(s) helps the host to take advantage of the locally adapted symbiotic partners and colonize larger geographic areas. In contrast, hosts which strictly associate with a limited number of symbiotic partners are expected to show narrower ecological width, resulting in more restricted geographical and/or ecological distribution. In symbiotic associations the generalist pattern is far more common and has been reported from coral-algae symbioses (Pochon and Pawlowski, 2006; Rowan et al., 1997), fungus-farming insects (Schlick-Steiner et al., 2008), mycorrhizae (Porras-Alfaro and Bayman, 2007), as well as lichens (Rikkinen et al., 2002). In contrast the specialist pattern is much rarer and has been reported for rare

orchid species associating with a limited number of fungal species (Graham and Dearnaley, 2011; Swarts et al., 2010) or for *Nostoc*-associating lichen fungi (Otálora et al., 2010). In our study, the sexually reproducing *Cladonia* lichens were shown to be generalists, whereas asexual *Cladonia* can be considered specialists. Also, there are clear differentiations in the geographical distributions in Europe which correlate with the degree of mycobiont specificity of the studied *Cladonia* species. The asexually reproducing *Cladonia deformis* and *C. pleurota* are common in boreal zone and in mountain regions all over Europe but are rare in North-Western oceanic part of Europe (Belgium, Denmark, Great Britain, Ireland, Netherlands). This is, at the same time, an area in which the photobiont *A. italiana* has been shown to dominate in this lichen group and where the sexually reproducing lichens showing lower level of specificity (*C. coccifera* and *C. diversa*) are common (Fig. 2). It is possible that the distribution of sorediate *Cladonia* species in this part of Europe may be limited by the local environmental conditions not suitable for the physiological optimum of their preferred photobionts *A. glomerata* and *A. irregularis*. Another possible explanation is that the performance of both interacting partners as one unit (holobiont) can be negatively affected by the local conditions (Dal Grande et al., 2017), although *A. glomerata* and *A. irregularis* may be present in the same geographical area associated with other mycobionts. This could be confirmed or ruled out by a more extensive sampling of other *Cladonia* species potentially harbouring *A. glomerata* and *A. irregularis* in W Europe. These observations would then support the hypothesis that photobiont availability and its ability to cope with local environmental conditions may play key roles in shaping the distribution of lichens which present high specificity towards their algal partner.

Acknowledgements

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