

Chapter 4

Photobiont Diversity in Indian *Cladonia* Lichens, with Special Emphasis on the Geographical Patterns

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1 Introduction

Lichens show distinctive patterns of distribution at both micro and macro levels (Galloway 2008). Sixteen major biogeographical patterns have been distinguished in lichens, including cosmopolitan taxa, bipolar taxa, taxa specific for particular continents or areas, and endemic taxa (Galloway 2008). However, these patterns are applicable to lichen-forming fungi only. Till date, we have almost no idea about the biogeography of lichenized algae and cyanobacteria—the photobionts.

During the last 20 years, molecular phylogenetic studies dramatically changed our views regarding coevolution of lichen partners. Supposed cospeciation and parallel cladogenesis of mycobionts and photobionts has been generally rejected (Kroken and Taylor 2000; Piercey-Normore and DePriest 2001), and replaced with the domestication model, in which the fungal partner could select the best available photobiont (DePriest 2004). In general, the mycobionts are able to cooperate with several algal species and to switch them (Muggia et al. 2008; Nelsen and Gargas 2009; Nyati 2007; Piercey-Normore 2006; Wornik and Grube 2010), simultaneously, several mycobionts can share single algal partner (Beck 1999; Doering and Piercey-Normore 2009; Hauck et al. 2007; Piercey-Normore 2009; Rikkinen et al. 2002). Moreover, lichen algae and cyanobacteria could exhibit their own environmental requirements, which seem to be independent of particular mycobionts to a large extent (Cordeiro et al. 2005; Fernandez-Mendoza et al. 2011;

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Helms 2003; Muggia et al. 2008; Peksa and Škaloud 2011). Naturally, since the environmental preferences of an organism could be narrowly linked to its distribution, the geographical pattern of photobionts could be markedly different from that of their fungal partners.

Both cyanobacteria and algae are microscopic organisms. Moreover, the lichen vegetative propagules containing both symbionts (soredia, isidia, etc.) are mostly not much larger than particular algal cells (20–50 μm , Büdel and Scheidegger 2008) and they are capable of being dispersed over large distances as well (Bailey 1976). The well-known theory of ubiquitous dispersal of microbial species (Finlay and Clarke 1999) presumed that most organisms smaller than ca 1 mm should occur worldwide (in a niche-based context only).

Indeed, some photobiont lineages are apparently widely distributed. For example, *Asterochloris* clade A7 (sensu Peksa and Škaloud 2011) has been found in lichens collected from Europe, USA, and China, indicating its ubiquitous dispersal. On the other hand, many photobiont lineages have been reported only from specific continents or regions. However, because of very uneven distribution of lichen collections, it is premature to classify them as species with narrow distribution patterns. For the most studied photobiont genera (*Asterochloris*, *Trebouxia*, *Nostoc*), majority of reports have been published from Europe and North America (e.g., Bačkor et al. 2010; Blaha et al. 2006; Guzow-Krzemińska 2006; Nelsen and Gargas 2008; O'Brien et al. 2005; Paulsrud et al. 2000; Peksa and Škaloud 2011; Piercey-Normore 2004, 2006, 2009; Yahr et al. 2004), slightly less from Central and South America (Cordeiro et al. 2005; Helms 2003; Reis 2005) and Antarctica (Aoki et al. 1998; Engelen et al. 2010; Nyati 2007; Otálora et al. 2010; Romeike et al. 2002; Wirtz et al. 2003). However, only few or no data have been reported from Africa, Asia, Australia, and close islands (Helms 2003; Nelsen and Gargas 2008, 2009; Nyati 2007; Piercey-Normore and DePriest 2001). Therefore, further exploration of photobionts in these areas is necessary to obtain relevant information about biogeographical patterns in lichenized algae and cyanobacteria.

2 Objectives

In this study, we investigated *Asterochloris* photobionts from terricolous lichens (*Cladonia* spp.) collected in India and Nepal using DNA sequencing. Traditionally, *Asterochloris* (incl. former *Trebouxia*) species have been determined according to the morphological features such as cell shape, chloroplast structure, and pyrenoid ultrastructure. However, a large cryptic variability recently discovered within the genus (Piercey-Normore and DePriest 2001; Yahr et al. 2004; Škaloud and Peksa 2010) clearly points out the deficiency of morphological features to delimit real species entities within *Asterochloris*. Therefore, we sequenced the internal transcribed spacer (ITS) ribosomal DNA (rDNA) marker to genetically investigate the diversity of photobionts in *Cladonia* lichens. The newly obtained ITS rDNA sequences were added to the dataset of all sequences deposited in GenBank database to analyze the phylogenetic position of Indian photobionts and biogeographic patterns of selected *Asterochloris* lineages.

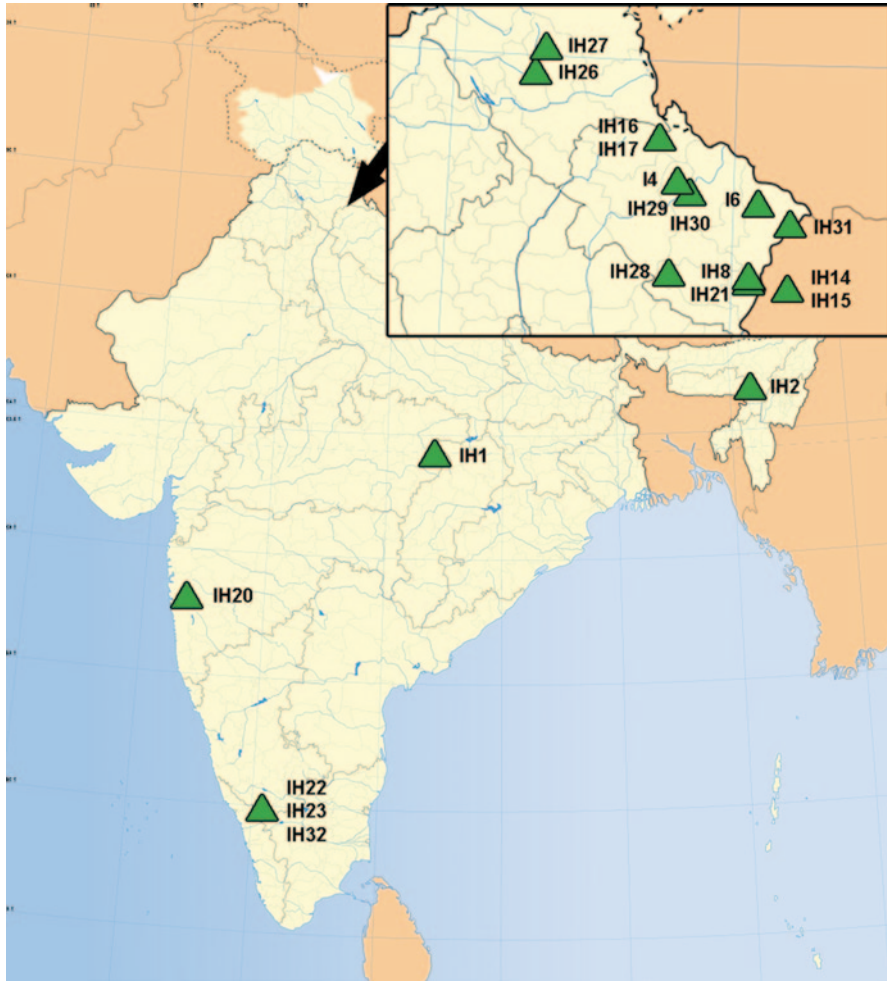


Fig. 4.1 Map showing the sampling localities of *Cladonia* lichens used in this study

3 Materials and Methods

3.1 Sample Collection

Lichen samples were collected in five different areas in India and Nepal (Fig. 4.1). Single lichen sample was collected in Maharashtra, Madhya Pradesh, and Assam states, located in west, central, and north-eastern India, respectively. Three lichen samples were obtained from collections made in Tamil Nadu state, located in South India. The majority of lichen thalli were collected in the Himalayas, Uttarakhand, and Himachal Pradesh states. Finally, two lichen thalli were collected in eastern Nepal. The collections have been made at different times during the years 2007 and 2010 (Table 4.1).

Table 4.1 List of *Cladonia* samples used for sequencing of algal internal transcribed spacer (ITS) ribosomal DNA (rDNA)

Sample No.	Taxon	Date of Collection	Altitude (m)	Country	State	District	Site	Substratum	Latitude	Longitude
I4	<i>Cladonia furcata</i> (Huds.) Schrad.	23.8.2007	3,250	India	Uttarakhand	Rudrapur	Tungnath	Soil	30°29'18.9"N	79° 12' 54.4"E
I6	<i>Cladonia rangiferina</i> (L.) Weber ex Wigg.	18.10.2007	2,553	India	Uttarakhand	Pithoragarh	Between Bogdiyar and Naher Devi	Soil	30°13'32.9"N	80°13'11.5"E
IH1	<i>Cladonia praetermissa</i> A. W. Archer	10.5.2010	1,665	India	Madhya Pradesh	Anuppur	Shambhudhara, Amarkantak Protected area	Red hard soil	22°42'48.9"N	81°44'46.3"E
IH2	<i>Cladonia scabriuscula</i> (Delise) Nyl.	18.11.2008	1,014	India	Assam	North Cachar	Haflong	Soil	25°08'01.3"N	93°00'35.5"E
IH8	<i>Cladonia verticillata</i> (Hoffm.) Schaer.	29.10.2009	1,890	India	Uttarakhand	Champawat	Lohaghat to Mayawati	Soil	29°23'54.5"N	80°05'07.1"E
IH14	<i>Cladonia coniocraea</i> (Flörke) Spreng.	18.5.2010	1,800	Nepal	Mahakali zone	Dadeldhura	Dadeldhura community forest	Soil in biological soil crust	29°18'08.5"N	80°35'32.7"E

Table 4.1 (continued)

Sample No.	Taxon	Date of Collection	Altitude (m)	Country	State	District	Site	Substratum	Latitude	Longitude
IH15	<i>Cladonia coniocraea</i> (Flörke) Spreng.	17.5.2010	1,716	Nepal	Mahakali zone	Dadeldhura	Dadeldhura community forest	Bark of Rhododendron arboreum	29°18'07.1"N	80°35'31.4"E
IH16	<i>Cladonia pyxidata</i> (L.) Hoffm.	29.10.2010	3,118	India	Uttarakhand	Uttarkashi	Gangotri	Soil	30°59'34.3"N	78°56'21.2"E
IH17	<i>Cladonia fruticulosa</i> Kremp.	29.10.2010	3,100	India	Uttarakhand	Uttarkashi	Gangotri	Soil	30°59'35.3"N	78°56'20.5"E
IH20	<i>Cladonia scabriuscula</i> (Delise) Nyl.	26.3.2010	1,410	India	Maharashtra	Satara	Mahabaleshwar, Wilson Point	Rocks with mosses	17°55'17.6"N	73°40'23.4"E
IH21	<i>Cladonia delavayi</i> Abbayes	27.11.2010	1,618	India	Uttarakhand	Champawat	Marodkhan on way to Ghat	Rock	29°19'57.7"N	80°05'27.8"E
IH22	<i>Cladonia fruticulosa</i> Kremp.	12.1.2008	2,607	India	Tamil Nadu	Nilgiri	Dodabetta, trails from Samer to Tiger Hills	Soil in coniferous forest	11°23'45.4"N	76°43'36.6"E
IH23	<i>Cladonia furcata</i> (Huds.) Schrad.	12.1.2008	2,607	India	Tamil Nadu	Nilgiri	Dodabetta, trails from Samer to Tiger Hills	Soil in coniferous forest	11°23'45.4"N	76°43'36.6"E
IH26	<i>Cladonia furcata</i> (Huds.) Schrad.	5.6.2008	3,078	India	Himachal Pradesh	Kullu	On route to Dhela	Soil	31°42'17.2"N	77°16'14.6"E

Table 4.1 (continued)

Sample No.	Taxon	Date of Collection	Altitude (m)	Country	State	District	Site	Substratum	Latitude	Longitude
IH27	<i>Cladonia furcata</i> (Huds.) Schrad.	4.5.2008	2,300	India	Himachal Pradesh	Kullu	7 km before Pulga	Soil among mosses	31°59'53.3"N	77°24'47.4"E
IH28	<i>Cladonia cariosa</i> (Ach.) Spreng.	29.10.2009	1,745	India	Uttarakhand	Champawat	Mayawati to Lohaghat	Rock	29°25'22.6"N	79°04'29.7"E
IH29	<i>Cladonia pyxidata</i> (L.) Hoffm.	8.6.2008	3,550	India	Uttarakhand	Chamoli	Kothidhar	Soil	30°23'34.9"N	79°19'08.7"E
IH30	<i>Cladonia furcata</i> (Huds.) Schrad.	8.6.2008	3,700	India	Uttarakhand	Chamoli	Srenikhal	Soil	30°22'27.5"N	79°19'10.3"E
IH31	<i>Cladonia corymbescens</i> Nyl. ex Leight	2.11.2009	2,743	India	Uttarakhand	Pithoragarh	Narain Swami Ashram	Soil	29°58'15.3"N	80°39'19.7"E
IH32	<i>Cladonia fruticulosa</i> Krempf.	12.1.2008	2,607	India	Tamil Nadu	Nilgiri	Dodabetta, trails from Samer to Tiger Hills	Soil in coniferous forest	11°24'00.9"N	76°44'06.2"E

3.2 DNA Isolation, Polymerase Chain Reaction (PCR), and Sequencing

Total genomic DNA was extracted following the standard CTAB protocol (Doyle and Doyle 1987), with minor modifications. The total genomic DNA was dissolved in sterile dH₂O and amplified by polymerase chain reaction (PCR). The ITS1-5.8S-ITS2 rDNA region was amplified using universal primer ITS4-3' (5'-TCCTCCGCT-TATTGATATGC-3'; White et al. 1990) and the algal-specific primer nr-SSU-1780-5' (5'-CTGCGGAAGGATCATTGATTC-3'; Piercey-Normore and DePriest 2001). All PCR reactions were performed in total volume of 20 µl contained 12.4 µl of sterile Mili-Q water, 2 µl of AmpliTaq Gold® 360 Buffer 10× (Applied Biosystems, Life technologies, Carlsbad, CA, USA), 1.5 µl of MgCl₂ (25 mM), 0.4 µl of dNTP mix (10 mM), 0.25 µl of each primer (25 nM), 2 µl of 360 GC Enhancer, 0.2 µl of AmpliTaq Gold® 360 DNA Polymerase and 1 µl of DNA (10 ng·l⁻¹). PCR and cycle-sequencing reactions were performed in a Touchgene Gradient cycler (Krackeler Scientific, Albany, NY, USA). PCR amplification of the algal ITS rDNA began with an initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were quantified on a 1% agarose gel stained with ethidium bromide and purified using the JetQuick PCR Purification kit (Genomed, Löhne, Germany), according to the manufacturer's protocols. The purified amplification products were sequenced with PCR primers using an Applied Biosystems (Seoul, Korea) automated sequencer (ABI 3730xl) at Macrogen Corp. in Seoul, Korea. Sequencing reads were assembled and edited using the SeqAssem programme (Hepperle 2004).

4 Phylogenetic Analyses

The newly obtained ITS rDNA sequences were added to the concatenated (ITS rDNA, actin I locus) alignment analyzed in Škaloud and Peksá (2010). Then, we added several additional ITS rDNA sequences obtained from GenBank to cover all *Asterochloris* diversity. The final concatenated matrix containing 69 taxa, was 1137 bp long, and was 100% filled for the ITS data and 67% filled for the actin data (Table 4.2). The matrix is available from Pavel Škaloud. The phylogenetic tree was inferred with Bayesian inference (BI) using MrBayes version 3.1 (Ronquist and Huelsenbeck 2003). The analysis was carried out on the partitioned dataset using the strategy described in Peksá and Škaloud (2011). Bootstrap analyses were performed by maximum likelihood (ML) and weighted parsimony (wMP) criteria using GARLI, version 0.951 (Zwickl, 2006) and PAUP version 4.0b10 (Swofford 2002), respectively. ML analysis consisted of rapid heuristic searches (100 pseudo-replicates) using automatic termination (genthreshforto-term command set to 100,000). The wMP bootstrapping (1,000 replications)

Table 4.2 List of all samples used in the study, including GenBank accession numbers for photobiont sequences. The samples are ordered with respect to their position in the Bayesian phylogenetic tree (Fig. 4.2)

Clade No.	Fungal taxon	Origin	Collection number	GenBank accession	
				ITS	Actin
1	<i>Cladonia squamosa</i> (Scop.) Hoffm.	USA, MA	CAUP H1006	AF345406	AM906025
1	<i>Stereocaulon pileatum</i> Ach.	USA, MA	UTEX 896	AF345404	-a
1	<i>Stereocaulon pileatum</i> Ach.	USA, MA	UTEX 897	AF345405	-a
1	<i>Stereocaulon pileatum</i> Ach.	USA, MA	UTEX 1713	AF345407	-a
1	<i>Stereocaulon</i> sp.	Slovakia	Peksa 801	FM945392	-a
1	<i>Stereocaulon evolutoides</i> (H. Magn.) Frey	USA, MA	UTEX 895	AF345382	AM906024
1	<i>Cladonia coniocraea</i> (Flörke) Spreng.	Nepal	IH15	HE803028b	-
1	<i>Stereocaulon vestinianum</i> Pers.	USA, Alaska	Talbot 281	DQ229885	DQ229888
2	<i>Stereocaulon botryosum</i> Ach.	USA, Alaska	Talbot 153	DQ229880	DQ229889
2	<i>Stereocaulon pileatum</i> Ach.	Czech Republic	Peksa 999	AM905999	AM906028
2	<i>Stereocaulon subcoralloides</i> (Nyl.) Nyl.	USA, Alaska	Talbot 167	DQ229881	DQ229890a
2	<i>Stereocaulon</i> sp.	Iceland	UTEX 2236	AF345411	AM906027a
3	<i>Pilophorus aciculare</i> (Ach.) Th. Fr.	USA, WA	CAUP H1004	AM906012	AM906041
4	<i>Cladonia cristatella</i> Tuck.	USA, MA	CAUP H1005	AF345440	AM906018
II	<i>Cladonia rangiferina</i> (L.) Weber ex F.H. Wigg.	India	I6	HE803029b	-
II	<i>Cladonia furcata</i> (Huds.) Schrad.	India	IH27	HE803030b	-a
II	<i>Cladonia pyxidata</i> (L.) Hoffm.	India	IH29	HE803031b	-
II	<i>Cladonia furcata</i> (Huds.) Schrad.	India	IH30	HE803032b	-a
II	<i>Cladonia corymbescens</i> Nyl. ex Leight	India	IH31	HE803033b	-a
II	<i>Cladonia corymbescens</i> Nyl. ex Leight	India	IH31a	HE803034b	-a

Table 4.2 (continued)

Clade No.	Fungal taxon	Origin	Collection number	GenBank accession	
				ITS	Actin
I2	<i>Cladonia furcata</i> (Huds.) Schrad.	India	I4	HE803035b	–
I2	<i>Cladonia furcata</i> (Huds.) Schrad.	India	IH23	HE803036b	–a
I2	<i>Cladonia furcata</i> (Huds.) Schrad.	India	IH26	HE803037b	–
5	<i>Stereocaulon dactylophyllum</i> Flörke	USA, VT	UTEX 1714	AM905993	AM906019
6	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 173	AM906003	AM906032
6	<i>Lepraria neglecta</i> (Nyl.) Lettau	Czech Republic	Peksa 183	AM906002	AM906031a
6	<i>Lepraria neglecta</i> (Nyl.) Lettau	Czech Republic	Peksa 207	AM906005	AM906034a
A9	<i>Lepraria alpina</i> (de Lesd.) Tretiach & Baruffo	Spain	Peksa 860	FN556035	FN556048
7	<i>Lepraria rigidula</i> (de Lesd.) Tonsberg	Czech Republic	Peksa 236	AM905997	AM906023
7	<i>Lepraria rigidula</i> (de Lesd.) Tonsberg	Czech Republic	Peksa 900	FM955669	FM955673
7	<i>Lepraria borealis</i> Lohtander & Tonsberg	USA, CA	Peksa 869	FN556039	FN556049
7	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	USA, CA	Peksa 873	FN556042	FN556051
7	<i>Lepraria</i> sp.	USA, CA	Peksa 870	FN556043	FN556052
7	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	USA, CA	Peksa 872	FN556041	FN556050a
9	<i>Cladonia evansii</i> Abbayes	USA, FL	RY798	AY712691	–
9	<i>Cladonia subtenuis</i> (Abbayes) Mattick	USA, FL	RY999	AY712698	–
9	<i>Cladonia subtenuis</i> (Abbayes) Mattick	USA, FL	RY941	AY712690	–
9	<i>Cladonia scabriuscula</i> (Delise) Nyl.	India	IH20	HE803038b	–
9	<i>Cladonia comiocraea</i> (Flörke) Spreng.	Nepal	IH14	HE803039b	–

Table 4.2 (continued)

Clade No.	Fungal taxon	Origin	Collection number	GenBank accession	
				ITS	Actin
9	<i>Cladonia delavayi</i> Abbayes	India	IH21A	HE803040b	-a
9	<i>Cladia aggregata</i> (Sw.) Nyl.	Costa Rica	Nelsen 2138	EU008658	-
9	<i>Cladonia verticillata</i> (Hoffm.) Schaer.	India	IH8B	HE803041b	-
9	<i>Cladonia delavayi</i> Abbayes	India	IH21B	HE803042b	-
9	<i>Cladonia scabriuscula</i> (Delise) Nyl.	India	IH2	HE803043b	-
9	<i>Cladonia spinea</i> Ahti	Guyana	MN-069	AF345418	-a
9	<i>Cladonia variegata</i> Ahti	Guyana	MN-075	AF345419	-a
9	<i>Cladonia subtenuis</i> (Abbayes) Mattick	USA, PA	RY1225	DQ482676	-
9	<i>Cladonia verticillata</i> (Hoffm.) Schaer.	India	IH8A	HE803044b	-
9	<i>Cladonia crinita</i>	Brazil	-	AY842277	-
9	<i>Cladonia fruticulosa</i> Kremp.	India	IH22	HE803045b	-
9	<i>Cladonia fruticulosa</i> Kremp.	India	IH32	HE803046b	-a
9	<i>Cladonia fruticulosa</i> Kremp.	India	IH32b	HE803047b	-a
9	<i>Cladonia praetermissa</i> A. W. Archer	India	IH1	HE803048b	-
9	<i>Cladonia cariosa</i> (Ach.) Spreng.	India	IH28	HE803049b	-
9	<i>Cladonia peltastica</i> (Nyl.) Muell. Arg.	Guyana	MN-070	AF345416	-
9	<i>Stereocaulon</i> sp.	Costa Rica	Nelsen 2181b	DQ229884	DQ229896
9	<i>Ptilophorus</i> cf. <i>cereolus</i> (Ach.) Th. Fr.	Costa Rica	Nelsen 2233f	DQ229883	DQ229895
9	<i>Lepraria</i> sp.	Costa Rica	Nelsen L54	EU008684	EU008711

Table 4.2 (continued)

Clade No.	Fungal taxon	Origin	Collection number	GenBank accession	
				ITS	Actin
8	<i>Cladonia rei</i> Schaer.	Slovakia	Peksa 787	FM945380	FM955675
8	<i>Cladonia fimbriata</i> (L.) Fr	Slovakia	Peksa 796	FM945358	FM955674
10	<i>Stereocaulon saxatile</i> H.Magn.	USA, Alaska	Talbot KIS 187	DQ229886	DQ229897
10	<i>Cladonia foliacea</i> (Huds.) Willd.	Czech Republic	Peksa 1008	AM906016	AM906049
10	<i>Lepraria borealis</i> Lohtander & Tønsberg	Bulgaria	Bayarová 3402	AM906015	AM906048
10	<i>Lepraria borealis</i> Lohtander & Tønsberg	Bulgaria	Bayarová 3401	AM900492	AM906045
10	<i>Lepraria crassissima</i> (Hue) Lettau	Czech Republic	Peksa 888	FN556033	–
10	<i>Lepraria yunnaniana</i> Diederich, Sérus. & Aptroot	Costa Rica	Nelsen 3637b	EU008681	EU008710
11	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	Romania	Peksa 225	AM905996	AM906022
11	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	Slovakia	Peksa 234	AM905994	AM906020
11	<i>Lepraria lobifcans</i> Nyl.	USA, WI	Nelsen L12	EU008675	EU008704
11	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	Slovakia	Peksa 235	AM905995	AM906021
11	<i>Lepraria lobifcans</i> Nyl.	USA, WI	Nelsen 154	DQ229877	DQ229898
11	<i>Lepraria lobifcans</i> Nyl.	USA, WI	Nelsen 153	EU008678	EU008707
11	<i>Lepraria caesioalba</i> (de Lesd.) J.R.Laundon	USA, PA	Nelsen L36	EU008664	EU008697
-	<i>Lepraria</i> sp.	China	Nelsen L60	EU008690	EU008715
12	<i>Cladonia rei</i> Schaer.	Czech Republic	Peksa 921	FM945378	FM955677
12	<i>Cladonia pocillum</i> (Ach.) Grognot	Canada	Normore4719	DQ530209	DQ530190a
12	<i>Cladonia fimbriata</i> (L.) Fr.	Slovakia	Peksa 815	FM945359	FM955676
12	<i>Cladonia pyxidata</i> (L.) Hoffm.	India	IHI16	HE803050b	–
12	<i>Stereocaulon paschale</i> (L.) Hoffm.	USA, AK	Talbot 101	DQ229887	DQ229891

Table 4.2 (continued)

Clade No.	Fungal taxon	Origin	Collection number	GenBank accession	
				ITS	Actin
13	<i>Xanthoria parietina</i> (L.) Th. Fr.	Italy	CCAP 519/5	AM906001	AM906030
13	<i>Cladonia</i> sp.	The Netherlands	CAUP H1003	AF345423	DQ229894
A4	<i>Lepraria caesiocalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 526	FN556030	–
A4	<i>Lepraria rigidula</i> (de Lesd.) Tonsberg	Czech Republic	Peksa 955	FN556032	–a
A4	<i>Lepraria rigidula</i> (de Lesd.) Tonsberg	Czech Republic	Peksa 855	FN556031	FN556047
14	<i>Lepraria caesiocalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 551	FM955667	FM955671
14	<i>Lepraria caesiocalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 185	FM955666	FM955670
14	<i>Lepraria rigidula</i> (de Lesd.) Tonsberg	Czech Republic	Peksa 186	AM905992	AM906017
–	<i>Stereocaulon tomentosum</i> Fr.	USA, AK	Talbot 400	DQ229882	DQ229893
15	<i>Anzina carneonivea</i> (Anzi) Scheid.	Italy	SAG 26.81	AM900490	AM906042
15	<i>Lepraria neglecta</i> (Nyl.) Lettau	Ukraine	Bayerová 3600	AM906013	AM906044a
15	<i>Lepraria neglecta</i> (Nyl.) Lettau	Ukraine	Bayerová 3606	AM900941	AM906043a
16	<i>Cladonia fruticulosa</i> Kremp.	India	IH17	HE803051b	–
16	<i>Cladonia</i> cf. <i>bacillaris</i> (Ach.) Nyl.	USA, PA	Nelsen 3950	DQ229878	DQ229892
16	<i>Lepraria alpina</i> (de Lesd.) Tretiach & Baruffo	Czech Republic	Peksa 192	AM906010	AM906039
16	<i>Lepraria caesiocalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 194	AM906009	AM906038
16	<i>Lepraria caesiocalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 233	AM906006	AM906035
16	<i>Lepraria caesiocalba</i> (de Lesd.) J.R.Laundon	Czech Republic	Peksa 166	AM906008	AM906037

ITS internal transcribed spacers

a Identical sequences omitted in the final alignment used for the Bayesian analysis

b Newly obtained sequences

was performed using heuristic searches with 100 random sequence addition replicates, tree bisection and reconnection (TBR) swapping, and random addition of sequences (the number limited to 10,000 for each replicate). The weight to the characters has been assigned using the rescaled consistency index, in a scale from 0 to 1,000. New weights were based on the mean of the fit values for each character over all of the trees in memory.

To map the biogeographic information onto the phylogenetic tree, we prepared a dataset of 319 ITS rDNA sequences (obtained in this study and acquired from GenBank database) with known biogeographic data. The distribution of *Asterochloris* in particular continents was finally shown for those clades containing at least ten sequences with known origin.

5 Results and Discussion

5.1 Diversity of *Asterochloris* photobionts

In total, 57 natural samples of various *Cladonia* species were collected from five different areas in India and Nepal. However, the amplification of ITS rDNA region was successful in only 20 of these samples (Table 4.1). Unsuccessful amplification of more than half of the samples might have been caused by their age and storage conditions (some *Cladonia* samples were more than 4 years old) or by the presence of nonspecific inhibitors. Usually, single photobiont has been detected in each lichen sample. However, in three cases we found two different *Asterochloris* genotypes in the single lichen thallus (samples IH2, IH8, and IH21).

All *Cladonia* samples were found to be associated with green algae belonging to the genus *Asterochloris*. The Bayesian analysis of the concatenated ITS rDNA and actin type I dataset led to the recognition of 20 lineages designated as clades 1–16 (according to Škaloud and Peksa 2010), clades A4, A9 (according to Peksa and Škaloud 2011), and two novel clades I1 and I2 (Fig. 4.2). The newly obtained photobiont sequences were inferred in six clades (I1, I2, 1, 9, 12 and 16). Two novel clades I1 and I2, exclusively formed by photobionts of Indian *Cladonia* lichens, were genetically considerably different from all other known *Asterochloris* lineages. Therefore, they very probably represent new, undescribed photobiont species. The clade I1 consisted of six photobiont sequences obtained from four *Cladonia* species (*C. rangiferina*, *C. furcata*, *C. pyxidata*, and *C. corymbescens*) collected in the Himalayas at relatively high altitude (2,300–3,700 m asl; Fig. 4.3). The clade I2 comprised only three photobiont sequences obtained from *Cladonia* lichens collected in both the Himalayas (samples I4 and IH26) and South India (sample IH23). All three lichen samples were also collected at high altitudes (2,607–3,250 m asl). Interestingly, all photobionts were found in *Cladonia furcata*, suggesting their specificity for this fungal partner.

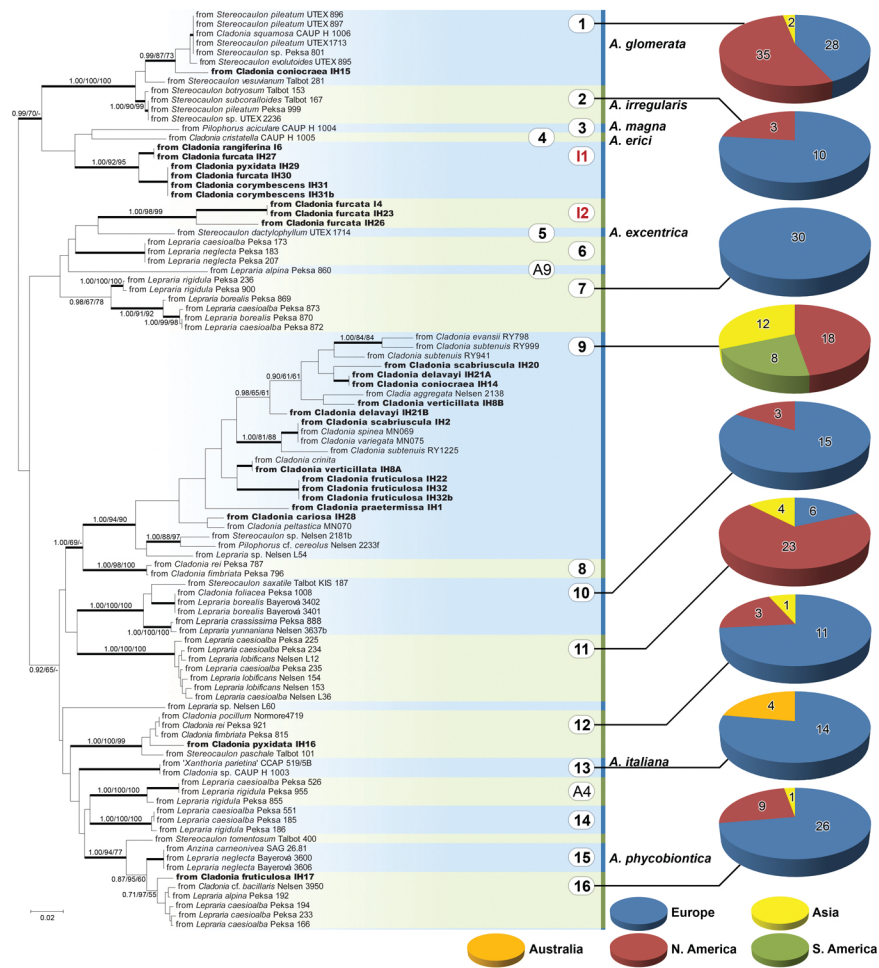


Fig. 4.2 Phylogenetic tree and biogeography of lichen photobiont *Asterochloris*. Bayesian analysis is based on the combined and partitioned internal transcribed spacer (ITS) ribosomal DNA (rDNA) and actin type I dataset using a HKY+I model for ITS1 and ITS2, F81 model for 5.8 ribosomal RNA (rRNA) (rRNA) partition, a HKY+Γ model for the actin-intron 206, GTR+Γ model for the actin intron 248 and K80+I model for the actin-exon partition. Values at the nodes indicate statistical support estimated by three methods: MrBayes posterior node probability (*left*), maximum likelihood bootstrap (in the *middle*) and maximum parsimony (right). *Thick branches* represent nodes receiving high Bayesian support (≥ 0.99) or consisting of genetically identical strains. New sequences from Indian *Cladonia* lichens are given in *bold*. Strain affiliation to 20 clades is indicated. Biogeography of selected lineages (those containing at least ten sequences with known origin) is shown next to the tree, including the total number of occurrences on each continent. Scale bar—estimated number of substitutions per site

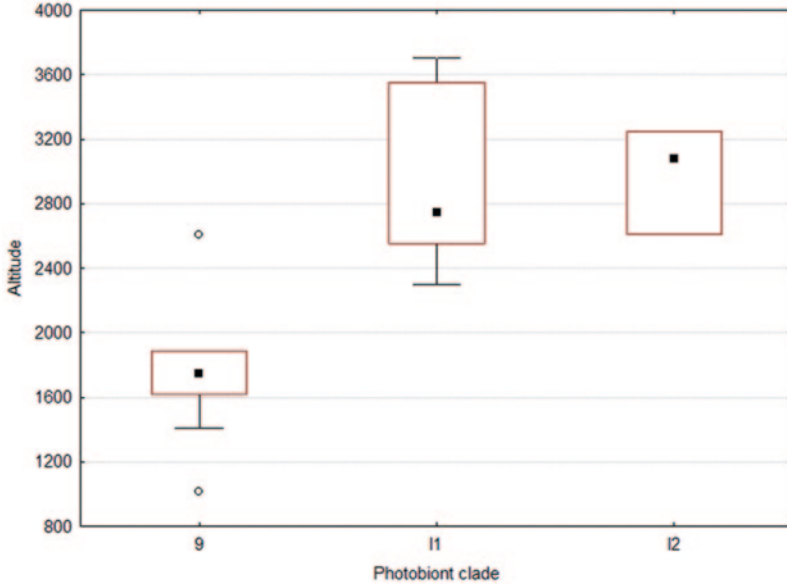


Fig. 4.3 Differences in the distribution of selected three *Asterochloris* clades along the altitudinal gradient. Box plots are based on altitudinal data of *Cladonia* samples analyzed in this study. All samples were collected in India and Nepal

The majority of investigated photobionts (found in 12 *Cladonia* samples belonging to 7 different species) were inferred in the clade 9. The clade is known as a lineage of North, Central, and South American lichen photobionts, having low specificity towards the lichen-forming fungi (it associates at least with 18 species from 5 lichen genera; Cordeiro et al. 2005; Nelsen and Gargas 2006; Piercey-Normore and DePriest 2001; Reis 2005; Yahr et al. 2004). Our lichen samples containing clade 9 photobionts were collected from various substrate types, such as bare soil, red hard soil, soil in coniferous forest, or rocks. In comparison with algal genotypes inferred in clades I1 and I2, clade 9 photobionts were found in the *Cladonia* samples collected at lower altitudes (1,014–2,607 m asl; Fig. 4.3). The remaining photobionts, found in *Cladonia* samples IH15, IH16, and IH 17 were inferred in three separate clades. The photobiont of *Cladonia coniocraea* (IH15) belongs to a very common species *Asterochloris glomerata* (clade 1). Two remaining photobionts, found in lichens *Cladonia pyxidata* (IH16) and *C. fruticulosa* (IH17) were inferred as members of clades 12 and 16, respectively.

5.2 Biogeography of Lichen Photobionts

During the last decade, biogeography of protists has become a highly controversial topic. It has been postulated that the small size, extremely large populations, and high dispersal potential of protists result in the cosmopolitan distribution of

the vast majority of species (Finlay 2002; Finlay and Fenchel 2004). Conversely, the limited geographical distributions has been implied by Foissner (1999), based mainly on the observed restricted distribution of “flagship” species, i.e., species with easily recognizable morphologies whose presence/absence can be easily demonstrated (Foissner 2006, 2008). However, all protistan biogeographic studies have been based on the investigation of the free-living organisms.

Our study could bring valuable information about the distribution patterns of symbiotic protists. So far, the investigations on *Asterochloris* photobionts were predominantly conducted on European and American lichen samples, only a few data have been obtained from other continents (see Introduction). Therefore, addition of more than 20 newly generated *Asterochloris* sequences obtained from Indian *Cladonia* samples could improve the dataset for subsequent estimation of biogeographical patterns in lichen photobionts.

The biogeography of particular lichen photobiont clades is illustrated in Fig. 4.2. Only those clades containing at least ten sequences with known origin were analyzed. In general, the majority of clades show wide (eurychoric) distribution, i.e., they were found in two or three continents. For example, *Asterochloris glomerata*, the commonest species of the genus, display almost ubiquitous distribution. According to all published data so far, this species has been found in a number of various lichen taxa (almost 50 species from genera *Cladia*, *Cladonia*, *Stereocaulon*, *Pycnothelia*, *Diploschistes*, *Hertelidea*) collected in many different places in Europe, North America, and Asia. It has obviously wide ecological amplitude, occurring in lichens growing on a variety of different substrates and in various microclimatic conditions. Nevertheless, all records of *A. glomerata* originate from warm-temperate to (sub)arctic zones of northern hemisphere (similar to the clades 2, 10, 11, 12, and 16).

In comparison to other photobiont lineages, the clade 9 has extraordinary distribution pattern because of its absence in Europe (see Fig. 4.2). It is widely dispersed, reported from South to North America and Asia, however, all records occurred between latitudes 25°S (Brazil, Paraná) and 36°N (USA, North Carolina). Thus, the algae from clade 9 probably prefer tropical to warm-temperate climate. This fact could explain their absence in European samples (only warm Mediterranean regions of Europe can comply with such criterion, however, they have been poorly investigated for *Asterochloris* photobionts so far).

The earlier mentioned *Asterochloris* lineages exhibit wide distribution; nevertheless, their habitat area seems to be more or less restricted. Our current data, together with the results of Fernandez-Mendoza et al. (2011), Helms (2003), Kroken and Taylor (2000), Muggia et al. (2008), and Peksa and Škaloud (2011) suggest that one of the most important factors influencing the distribution of eukaryotic photobionts is climate. Such climatic preferences influence the type and size of species habitat. There are reports on lineages of *Trebouxia* photobionts occurring predominantly in tropical regions (Helms 2003), on the other hand, other clades (haplotypes) exhibit polar (bipolar) distribution pattern (Fernandez-Mendoza et al. 2011).

Thus, it is obvious that at least some clades occur only in specific biomes or latitudes in general, across different continents. It is a question whether there is any photobiont lineage living in one continent or region only (endemic species). According to our data, three clades seem to have rather restricted distribution. Photobionts of clade 7 (30 samples) have been reported so far only from Europe. Similarly, the clades I1 and I2 seem to be restricted to Asia (India). According to Foissner (2006), the restricted distribution of protist species could be caused by either historic, biological, climatic, or habitat factors. The biogeography of clades 7, I1 and I2 cannot be affected by the limited dispersal of their fungal partners. *Lep-raria caesiaolba* and *L. rigidula* (mycobionts of clade 7 algae), as well as *Cladonia furcata*, *C. rangiferina* and *C. pyxidata* (mycobionts of I1 and I2 algae) represent lichens with very wide to cosmopolitan distribution (Smith et al. 2009) and many of them disperse intensively via vegetative propagules (soredia, granules) which provide a possibility of intensive dispersal of both mycobionts and photobionts. Moreover, we cannot rule out the simple dispersal of photobionts independent of a fungus. *Asterochloris*, a unicellular green alga, asexually reproducing by high number of aplanospores (Škaloud and Peksa 2010) has virtually unlimited dispersal capacity. It is well supported by its common distribution and ubiquity of the majority of its species. Therefore, the restricted distribution of photobiont clades 7, I1, and I2 cannot be explained by either historic or biological factors. More likely, the clades are restricted in their distribution by having specific climatic or habitat preferences. The clade 7 photobionts, so far reported only from Europe, have been recently demonstrated to be significantly associated with ombrophobic lichens (i.e., growing in fully rain-sheltered sites, where the vapour is the only available source of water) growing predominantly on the bark of broadleaf trees in temperate belt. It is highly probable that further investigation of photobiont diversity in bark-associated green-algal lichens conducted in other continents than Europe would reveal much wider distribution of this clade.

6 Conclusion

This study revealed significant photobiont diversity in *Cladonia* lichens collected in India and Nepal. The discovery of two novel, not yet reported clades emphasizes the large hidden diversity of lichen photobionts. Despite the fact that we investigated symbiotic organisms, almost all *Asterochloris* lineages exhibit eurychoric distribution. We suppose that the existence of several *Asterochloris* clades so far reported from single continent is affected by limited sampling and specific climatic or habitat preferences rather than by restricted distribution patterns. It is increasingly evident that the distinct preferences for environmental factors, not the dispersal barriers, shape the global distribution patterns of lichen photobionts. Consequently, narrow ecological preferences of lichen photobionts could to a certain extent determine the distribution pattern of the entire lichen association.

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