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Standard Paper

The curious case of *Cladonia luteoalba*: no support for its distinction

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Abstract

Cladonia luteoalba shows a specific pattern in chemical variability. Its chemotype coincides with that of the associated *Cladonia* thalli. This has led to the formation of various hypotheses, but its true nature has never been clarified. We collected *C. luteoalba* in Central Europe and Norway. The chemotypes were detected by TLC and the mycobionts and photobionts were identified by Sanger sequencing of ITS rDNA. Mycobiont cultures were obtained and Illumina metabarcoding of the fungal ITS1 rDNA region was performed targeting minor mycobionts within the thalli. None of the methods supported *C. luteoalba* as a distinct *Cladonia* species. In phylogenetic analyses, it was placed in *C. straminea* and the *C. coccifera* agg., following the pattern in chemistry. No minor *Cladonia* were detected by metabarcoding or cultivation. Thus, *C. luteoalba* remains enigmatic as our data did not support its distinction as a separate *Cladonia* species.

Key words: *Asterochloris*, chemotypes, lichen, metabarcoding, mycobiont culture, phylogeny, Sanger sequencing

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Introduction

Cladonia luteoalba A. Wilson & Wheldon (Wheldon & Wilson 1907) is a recognizable species, characterized by large primary squamules with a yellow cottony-arachnoid lower surface, particularly conspicuous when they dry and recurve. It only rarely forms podetia, which are escyphose or very narrowly scyphose, covered with a yellow cottony hyphal layer (Stenroos 1990). It is usually found on soil or rocks, growing among other *Cladonia* species, particularly from the clade *Erythrocarpae* (former section *Cocciferae*; Stenroos *et al.* 2019), sometimes even on their podetia. *Cladonia luteoalba* is rare but distributed worldwide, from Southern Patagonia to Svalbard, mainly with an arctic boreal distribution in Eurasia and North America (e.g. Stenroos 1990; Elvebakk & Hertel 1996; Ahti *et al.* 2013).

Cladonia luteoalba was called enigmatic by Stenroos (1990) due to a peculiar pattern in its chemical variability. Remarkably, its chemotype corresponds to the chemotype of the associated *Cladonia* species. Thalli associated with *C. coccifera* (L.) Willd. and related species produce zeorin (with accessory compounds, chemotype 1), thalli associated with *C. straminea* (Sommerf.) Flörke produce squamatic acid (with accessory compounds, chemotype 2) and those associated with *C. borealis* S. Stenroos produce barbatic acid with accessory compounds (chemotype 3). Stenroos suggested three possible explanations (Stenroos 1990): mechanical hybridization, a commensalistic symbiosis system of two mycobionts with one photobiont, and a disease that induces morphological changes to other *Cladonia* species, considering the second option the most plausible. In that scenario, initially lichenicolous *C. luteoalba* parasitizes an existing *Cladonia* thallus, then acquires its photobiont and forms a symbiotic thallus of its own. Based on this hypothesis, *C. luteoalba* has been used as an example of a lichen that obtains its photobiont through theft (Nelsen & Gargas 2009; Dal Grande *et al.* 2012; Williams *et al.* 2017).

The status of *C. luteoalba* was doubted, for example by Sandstede (1938) who regarded it as a form of *C. digitata* (L.) Hoffm. The lectotype in BM was revised by Ahti who considered it a synonym of *C. sulphurina* (Michaux) Fr. (as *C. gonecha* (Ach.) Asahina) but further field collections convinced him that *C. luteoalba* was a good species (Ahti 1965). Although the species is generally accepted, the necessity of further studies has been noted (Burgaz *et al.* 2020; Pino-Bodas *et al.* 2021).

Sequences from a single specimen of *C. luteoalba* are available in GenBank. A more detailed revision using DNA sequence data could usefully resolve the unknowns. For instance, do the different chemotypes represent a single *C. luteoalba* species? Is it a well-supported *Cladonia* species (i.e. not a morphological change induced by external factors)? Is there any evidence for mechanical hybridization? What photobionts does it associate with? Is its photobiont shared with the associated *Cladonia* thalli?

The aim of this study was to address these questions using multiple approaches. First, we identified the chemotypes of the collected thalli. Second, mycobionts and photobionts of *C. luteoalba* and its associated *Cladonia* thalli were characterized by Sanger sequencing. Third, Illumina metabarcoding and mycobiont cultivation were performed in order to reveal minor mycobionts or possible mechanical hybrids.

Materials and Methods

Sampling

Altogether 38 *Cladonia luteoalba* thalli (Fig. 1) were collected at 21 sites, 29 in Norway (14 collection sites), eight in Czechia (six sites) and one in Poland. Twenty-five specimens were growing

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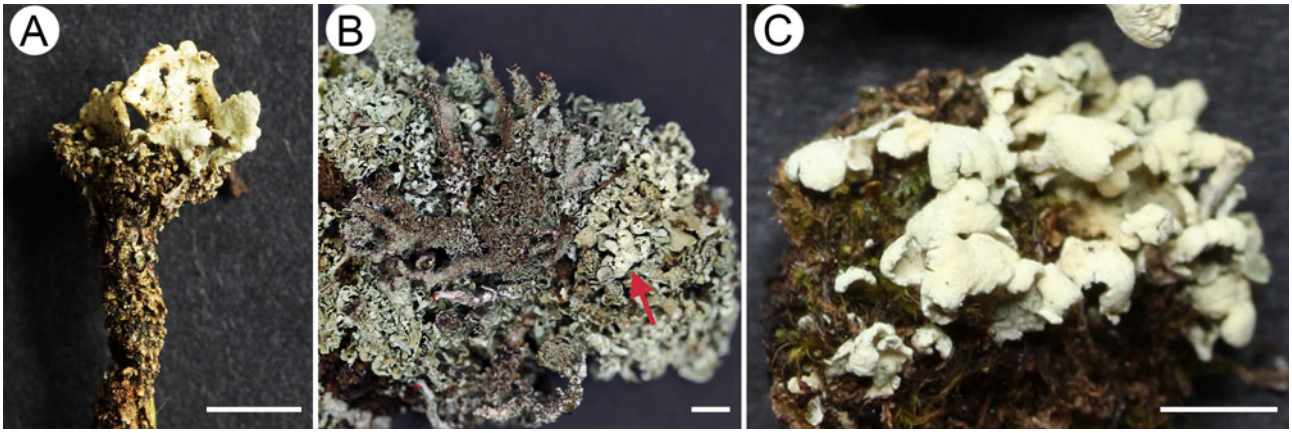

Fig. 1. *Cladonia luteoalba* thalli. A, *C. luteoalba* squamule on a podetium of *C. coccifera*. B, *C. luteoalba* (arrow) growing among other *Cladonia* species. C, *C. luteoalba* with no associated *Cladonia* thalli. Scales = 5 mm. In colour online.

in close contact with thalli of other *Cladonia* species (Fig. 1B), and two grew directly on the top of *C. coccifera* podetia (Fig. 1A). In Norway, *C. luteoalba* was found mostly on acidic soil in open habitats or on the upper horizontal surfaces of large boulders. A single epiphytic specimen was found on the trunk of a pine tree. In Central Europe, it was found exclusively in boulder screes in mountain areas. The most closely associated *Cladonia* thalli, together with other *Cladonia* species at certain localities, not in direct contact with *C. luteoalba* but at a maximum of 30 cm away, were also collected. Three of them were used as controls for metabarcoding (see below). *Cladonia luteoalba* specimens are encoded LUTxx, the associated *Cladonia* thalli LUTxx-A and control thalli LUTxx-C. All collection data are presented in Supplementary Material Table S1 (available online); specimens are deposited in PRC.

Secondary chemistry

To determine chemotypes of *C. luteoalba* thalli, standard thin-layer chromatography (TLC) in solvent systems A, B and C was performed following Orange *et al.* (2010).

Sanger sequencing: DNA extraction, amplification and sequence analyses

DNA was extracted using the CTAB protocol (Cubero *et al.* 1999), with freezing prolonged to 30 min after isopropanol precipitation and an additional washing step with 96% ethanol. A single *Cladonia* squamule was used for each extraction. Fungal nuclear ITS rDNA was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). Algal ITS rDNA was amplified using the forward primers Zeleny_F2 (Moya *et al.* 2018) or nr-SSU-1780 (Piercey-Normore & DePriest 2001) with the reverse primer ITS4. The PCR conditions were as in Škvorová *et al.* (2022). PCR products were purified with SPRI AMPure XP paramagnetic beads (Beckman Coulter) and sequenced by Macrogen Europe (Amsterdam, the Netherlands). GenBank Accession numbers of the newly obtained sequences are given in Table 1 and Supplementary Material Table S2 (available online).

Mycobiont ITS sequences newly obtained from *C. luteoalba* and their associated *Cladonia* thalli (Table 1) were aligned with the sequences of related species. These were selected based on BLAST searches and included *C. straminea* and zeorin-containing red-fruited *Cladonia* species (Steinová *et al.* 2013), viz. *C. coccifera*, *C. deformis* (L.) Hoffm., *C. diversa* Asperges. and *C. pleurota* (Flörke) Schaer., referred to here as the *C. coccifera* aggregate. The sequences were downloaded from GenBank and additional *C. straminea* sequences were produced (see Supplementary Material Table S2). The dataset was aligned with MAFFT v. 7 (Katoh *et al.* 2019), using the Q-INS-I method and manually checked. Ambiguously aligned regions were identified using the program Gblocks v. 0.91b (Castresana 2000) and eliminated. The final alignment consisted of 529 positions and 48 unique sequences, including *C. divaricata* Nyl. used as the outgroup. Substitution models estimated with jModelTest v. 2.1.4 (Darriba *et al.* 2012) using Bayesian Information Criterion were K80 for ITS1, JC for 5.8S and K80 for ITS2.

The newly obtained ITS photobiont sequences were aligned with *Asterochloris* Tschermak-Woess sequences downloaded from GenBank (Supplementary Material Table S3, available online), based on the datasets of Škaloud & Peksa (2010), Kim *et al.* (2020) and Vančurová *et al.* (2018, 2020). To increase phylogenetic resolution, actin type I sequences were also downloaded from GenBank and processed as above. The two markers gave congruent topologies so they were concatenated. *Trebouxia jamesii* (Hildreth & Ahmadjian) Gärtner was used as the outgroup. The alignment was processed as above and finally consisted of 71 unique sequences, and 498 ITS and 516 actin positions. The estimated substitution models were K80 + G for ITS1, JC for 5.8S, TrNef + G for ITS2 and K80 + I + G, TrNef + G and K80 + G for the first, second and third actin positions, respectively.

The phylogenetic trees were inferred by Bayesian inference in MrBayes v. 3.2.6 (Ronquist *et al.* 2012) using partitioned datasets. Two parallel MCMC runs, with one cold and three heated chains, were carried out, sampling the trees and parameters every 100 generations. Convergence of the chains was verified by the convergent diagnostic of the potential scale reduction factor (PSFR) using the sump option, and it approached 1 in all cases. Convergence of the two cold chains was assessed by the average standard deviation of split frequencies (SDSF). It was 0.005 and 0.001 after 15 million generations for the photobiont and mycobiont, respectively. The first 25% of the trees was discarded as burn-in in each run. A 50% majority-rule consensus tree was obtained using the sumt option. Bootstrap analyses were performed by maximum likelihood (ML) using GARLI v. 2 (Zwickl 2006) on partitioned datasets, specified as above,

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Table 1. Mycobiont and photobiont identification of *Cladonia luteoalba* and their associated *Cladonia* thalli. Samples codes are listed with GenBank Accession numbers of the newly obtained ITS sequences, and respective chemotype and locality data. For more collection data see Supplementary Material Table S1 (available online). *Cladonia luteoalba* specimens are encoded LUTxx, the associated *Cladonia* thalli LUTxx-A and control thalli LUTxx-C.

Sample codes	Mycobiont lineage[a]	GB[b] Acc. no.	Chemotype[c]	Photobiont species	GB[b] Acc. no.	Locality (site code)
LUT19/1	*C. coccifera* agg. lin. 1	OM914247	1 (C-P)	*A.* aff. *italiana*	OM914199	Norway, Østfold, Lilleby (NO-01)
LUT19/1-A	*C. coccifera* agg. lin. 1	OM914248	1 (C-P)	*A.* aff. *italiana*	OM914200	Norway, Østfold, Lilleby (NO-01)
LUT19/2	*C. coccifera* agg. lin. 1	OM914249	1 (C-P)	*A. leprarii*	OM914201	Norway, Østfold, Lilleby (NO-01)
LUT19/2-A	*C. coccifera* agg. lin. 1	OM914250	1 (C-P)	n/a		Norway, Østfold, Lilleby (NO-01)
LUT19/3	*C. straminea*	OM914251	2	*A. irregularis*	OM914202	Norway, Østfold, Lilleby (NO-02)
LUT19/4	*C. straminea*	OM914252	2	*A. glomerata*	OM914203	Norway, Østfold, Lilleby (NO-02)
LUT19/5	*C. coccifera* agg. lin. 1	OM914253	1 (P)	*A. italiana*	OM914204	Norway, Østfold, Lilleby (NO-01)
LUT19/6	*C. coccifera* agg. lin. 1	OM914254	1 (C-P)	*A. stereocaulonicola*	OM914205	Norway, Rogaland, Vikeså (NO-03)
LUT19/7	*C. coccifera* agg. lin. 1	OM914255	1 (P)	*A. italiana*	OM914206	Norway, Rogaland, Vikeså (NO-04)
LUT19/8	*C. coccifera* agg. lin. 1	OM914256	1 (C-P)	*A. italiana*	OM914207	Norway, Rogaland, Vikeså (NO-04)
LUT19/8-A	*C. coccifera* agg. lin. 1	OM914257	1 (C-P)	*A. italiana*	OM914208	Norway, Rogaland, Vikeså (NO-04)
LUT19/9	*C. coccifera* agg. lin. 1	OM914258	1 (C-P)	*A. italiana*	OM914209	Norway, Rogaland, Vikeså (NO-04)
LUT19/10	*C. coccifera* agg. lin. 1	OM914259	1 (C-P)	*A. italiana*	OM914210	Norway, Rogaland, Vikeså (NO-04)
LUT19/10-A	*C. coccifera* agg. lin. 1	OM914260	1 (P)	*A. italiana*	OM914211	Norway, Rogaland, Vikeså (NO-04)
LUT19/11	*C. coccifera* agg. lin. 1	OM914261	1 (C-P)	*Asterochloris* sp. StA3	OM914212	Norway, Rogaland, Vikeså (NO-05)
LUT19/12	*C. coccifera* agg. lin. 1	OM914262	1 (C-P)	*A. italiana*	OM914213	Norway, Rogaland, Lyngaland (NO-06)
LUT19/12-C	*C. coccifera* agg. lin. 2	OM914263	1	n/a		Norway, Rogaland, Lyngaland (NO-06)
LUT19/14	*C. coccifera* agg. lin. 2	OM914264	1 (P)	*Asterochloris* sp. StA3	OM914214	Norway, Rogaland, Paddevatnet (NO-07)
LUT19/15	*C. coccifera* agg. lin. 2	OM914265	1 (C-P)	*Asterochloris* sp. StA3	OM914215	Norway, Rogaland, Paddevatnet (NO-07)
LUT19/16	*C. coccifera* agg. lin. 2	OM914266	1 (C-P)	*A. italiana*	OM914216	Norway, Rogaland, Paddevatnet (NO-07)
LUT19/17	*C. coccifera* agg. lin. 2	OM914267	1	*A. irregularis*	OM914217	Norway, Rogaland, Blåsjø (NO-08)
LUT19/17-C1	*C. borealis*	OM914268	3	*A. irregularis*	OM914218	Norway, Rogaland, Blåsjø (NO-08)
LUT19/17-C2	*C. coccifera* agg. lin. 2	OM914269	1	*A. irregularis*	OM914219	Norway, Rogaland, Blåsjø (NO-08)
LUT19/19	*C. coccifera* agg. lin. 2	OM914270	1 (P)	*A. glomerata*	OM914220	Norway, Rogaland, Blåsjø (NO-09)
LUT19/19-A	*C. coccifera* agg. lin. 2	OM914271	1 (P)	*A. glomerata*	OM914221	Norway, Rogaland, Blåsjø (NO-09)
LUT19/20	*C. coccifera* agg. lin. 2	OM914272	1 (P)	*A. irregularis*	OM914222	Norway, Rogaland, Blåsjø (NO-09)
LUT19/20-A	*C. borealis*	OM914273	3	*A. irregularis*	OM914223	Norway, Rogaland, Blåsjø (NO-09)
LUT19/21	*C. coccifera* agg. lin. 2	OM914274	1 (P)	*A. irregularis*	OM914224	Norway, Rogaland, Blåsjø (NO-10)
LUT19/21-A	*C. coccifera* agg. lin. 2	OM914275	1	*A. irregularis*	OM914225	Norway, Rogaland, Blåsjø (NO-10)
LUT19/22	*C. coccifera* agg. lin. 2	OM914276	1	*A. glomerata*	OM914226	Norway, Rogaland, Blåsjø (NO-10)
LUT19/23	*C. coccifera* agg. lin. 2	OM914277	1	*A. irregularis*	OM914227	Norway, Rogaland, Birkelandsvegen (NO-11)
LUT19/24	*C. coccifera* agg. lin. 2	OM914278	1	*A. irregularis*	OM914228	Norway, Rogaland, Birkelandsvegen (NO-11)
LUT19/24-A	*C. coccifera* agg. lin. 2	OM914279	1	*A. irregularis*	OM914229	Norway, Rogaland, Birkelandsvegen (NO-11)
LUT19/25	*C. coccifera* agg. lin. 2	OM914280	1	*A. irregularis*	OM914230	Norway, Rogaland, Birkelandsvegen (NO-11)
LUT19/26	*C. coccifera* agg. lin. 2	OM914281	1	*A. irregularis*	OM914231	Norway, Rogaland, Birkelandsvegen (NO-11)
LUT19/28	*C. coccifera* agg. lin. 1	OM914282	1	*A. irregularis*	OM914232	Norway, Hordaland, Dyrskar (NO-12)
LUT19/29	*C. coccifera* agg. lin. 1	OM914283	1	*A. irregularis*	OM914233	Norway, Hordaland, Dyrskar (NO-12)
LUT19/30	*C. coccifera* agg. lin. 2	OM914284	1	*A. irregularis*	OM914234	Norway, Hordaland, Dyrskar (NO-12)
LUT19/31	*C. coccifera* agg. lin. 2	OM914285	1	*A. irregularis*	OM914235	Norway, Rogaland, Øvre Moen (NO-13)
LUT19/33	*C. coccifera* agg. lin. 1	OM914286	1 (P)	*A. irregularis*	OM914236	Norway, Telemark, Froland-Døkki (NO-14)
LUT19/34	*C. coccifera* agg. lin. 2	OM914287	1	*A. irregularis*	OM914237	Czechia, Krkonoše, Sněžka (CZ-01)

(*Continued*)

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Table 1. (*Continued*)

Sample codes	Mycobiont lineage[a]	GB[b] Acc. no.	Chemotype[c]	Photobiont species	GB[b] Acc. no.	Locality (site code)
LUT19/35	C. *coccifera* agg. lin. 2	OM914288	1	*A. irregularis*	OM914238	Czechia, Krkonoše, Sněžka (CZ-01)
LUT19/36	C. *coccifera* agg. lin. 2	OM914289	1	*A. irregularis*	OM914239	Czechia, Krkonoše, Sněžka (CZ-01)
LUT19/37	*C. straminea*	OM914290	2	*A. glomerata*	OM914240	Czechia, Šumava, Povydří (CZ-02)
LUT19/37-A	*C. straminea*	OM914291	2	*A. irregularis*	OM914241	Czechia, Šumava, Povydří (CZ-02)
LUT20/1	*C. straminea*	OM914292	2	*A. glomerata*	OM914242	Czechia, Šumava, Povydří (CZ-03)
LUT21/1	*C. straminea*	OM914293	2	*A. irregularis*	OM914243	Czechia, Šumava, Obří hrad (CZ-04)
LUT21/2	*C. straminea*	OM914294	2	*A. irregularis*	OM914244	Czechia, Šumava, Buzošná (CZ-05)
LUT-JS863	C. *coccifera* agg. lin. 2	OM914295	1	*A. irregularis*	OM914245	Czechia, Krkonoše, Luční hora (CZ-06)
LUT-JS864	C. *coccifera* agg. lin. 2	OM914296	1	*A. irregularis*	OM914246	Poland, Karkonosze, Mały Szyszak (PL-01)

[a]see Fig. 2; [b]GenBank; [c]chemotype 1 = zeorin with accessory porphyrillic (P) and conporpyrillic (C) acid, chemotype 2 = squamatic and didymic acid, chemotype 3 = barbatic acid.

consisting of 500 rapid bootstrap inferences with automatic termination. Other GARLI parameters were set to default.

Interaction networks were created using the package *bipartite* (Dormann *et al.* 2009) in the free software R v. 4.1.0 (R Core Team 2021).

Illumina metabarcoding and bioinformatics

To reveal possible multiple *Cladonia* mycobionts in *C. luteoalba* thalli, Illumina metabarcoding of the fungal ITS1 rDNA region was carried out. Six *C. luteoalba* samples and three control *Cladonia* samples of various chemotypes and from different localities (Supplementary Material Tables S1 & S4) were included. Amplicons for Illumina MiSeq sequencing were generated using the newly designed barcoded primers ITS1_NGS_Cladonia_forward (5′-barcode-TGC GGA AGG ATC ATT AAT GAG-3′) and ITS1_NGS_Cladonia_reverse (5′-barcode-AGA TCC GTT GAA AGT TTT-3′). These fungal primers were primarily designed to discriminate in favour of *Cladonia*. In a pilot study (data not shown), they did not amplify *Cladonia* exclusively but they effectively increased the ratio of *Cladonia* sequences compared to other fungi. Therefore, the composition of the fungal community obtained using these primers is highly biased and the results obtained mainly serve the purpose of seeking *Cladonia* sequences.

PCRs were performed in a volume of 20 µl, each reaction containing 10 µl of Q5 High-Fidelity DNA polymerase (BioLabs Inc.), 5 µl of sterile water, 1.5 µl of each primer and 2 µl of DNA. Each sample was run in three replicates and three PCR negative controls (PNC) were included. PCR conditions were as follows: initial denaturation at 98 °C for 30 s, 35 cycles of 98 °C denaturation for 10 s, 52° C amplification for 45 s and 72 °C elongation for 1 min, with a final 72 °C extension for 2 min. The PCR products were purified with SPRI AMPure XP paramagnetic beads (Beckman Coulter), pooled equimolarly and sent for library preparation and sequencing to Fasteris (Plan-les-Ouates, Switzerland). Sequencing was performed on the Illumina MiSeq platform with paired end mode (2 × 300 bp).

Quality control analysis of the Illumina MiSeq paired-end reads was performed using FastQC v. 0.11.8 (Andrews 2010). Raw reads were processed according to the pipeline published by Báilint *et al.* (2014), including quality filtering, paired-end assembly, removing primer artifacts, extracting reads by barcodes, reorienting reads to 5′-3′, demultiplexing, dereplicating, OTU clustering (this step carried out using Swarm v. 2 (Mahé *et al.* 2015), with denoising set to d = 3) and chimera filtering. Only the OTUs that had more than 100 reads in at least two of the three replicates were considered further. Fungal OTUs were identified by BLAST searches (excluding uncultured/environmental sample sequences) in SEED2 (Větrovský *et al.* 2018).

Mycobiont culturing

In order to capture possible multiple mycobionts in *C. luteoalba* thalli, isolates for culturing were prepared from selected specimens of both chemotypes. Under a stereomicroscope, tiny pieces of either medulla or the arachnoid lower surface were extracted with a sterile needle and placed onto cultivation media. Sabouraud 2% medium (SAB), malt-yeast extract medium (MYA) and Bold's Basal Medium (BBM) with 1% glucose were prepared following the instructions in Stocker-Wörgötter & Hager (2008). Fifty plates were inoculated per thallus and were incubated at 16.5 °C with a 12 h of light/dark regime. After six weeks, the plates were checked and morphologically identified mycobiont isolates were reinoculated onto fresh media. Their identity was subsequently confirmed by obtaining ITS rDNA sequences as described above. Three to ten isolates were obtained per thallus, with the exception of LUT-JS863 from which we obtained 21 mycobiont isolates. Twelve of the LUT-JS863 cultures were selected for sequencing, while all the cultures were sequenced from the other specimens.

Results

Chemistry

The *Cladonia luteoalba* specimens belonged to two chemotypes (Table 1): chemotype 1 containing zeorin was found in 32 specimens, 17 of which contained porphyrillic acid and 10 also conporphyrillic acid, with the majority also containing an unidentified accessory compound; six specimens were of chemotype 2 containing squamatic and didymic acids. All samples contained usnic acid. The chemotype of the most closely associated *Cladonia* thallus was the same in all cases examined, with one exception (LUT19/20 contained zeorin and the associated thallus barbatic acid, i.e.

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chemotype 3). No geographical pattern in chemotype occurrence was observed (longitude, latitude or altitude; data not shown).

Mycobionts

No unique sequence belonging to *C. luteoalba* that would distinguish it from related *Cladonia* species was obtained. The ITS rDNA sequences obtained by Sanger sequencing were identical to and grouped with the corresponding *Cladonia* species or species complex defined by the chemotypes (Fig. 2); specifically, squamatic acid-containing specimens belonged to *C. straminea*, and zeorin-containing specimens were placed in various lineages of the *Cladonia coccifera* agg., which includes the morphospecies *C. coccifera*, *C. deformis*, *C. diversa* and *C. pleurota* that are indistinguishable based on DNA sequence data, as shown previously (Steinová *et al.* 2013).

Culturing of mycobionts did not result in unique *C. luteoalba* cultures either. We successfully obtained *Cladonia* cultures from six zeorin-containing specimens (LUT19/1, 12, 19, 24, 30 and JS863). Multiple mycobiont cultures obtained from one thallus

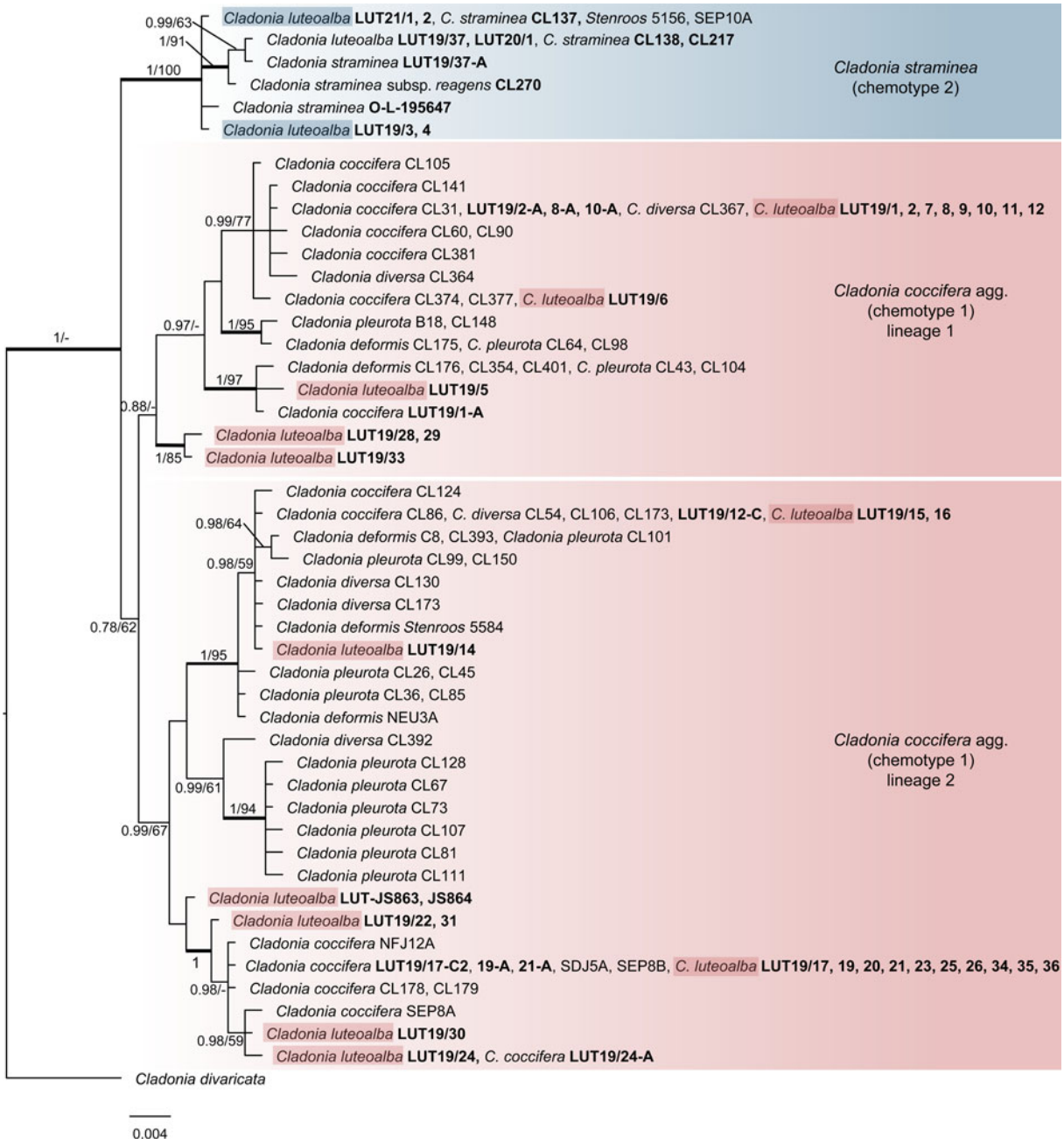

Fig. 2. Phylogenetic relationship of *Cladonia luteoalba* (highlighted) and related taxa obtained by Bayesian inference of ITS rDNA. Values at nodes show statistical support calculated by MrBayes posterior-node probability (PP)/maximum likelihood bootstrap. Only statistical supports with PP > 0.75 are shown. Thick branches represent nodes with full PP support. Newly obtained sequences are in bold. Shaded areas indicate chemotype and lineage information. *Cladonia divaricata* is the outgroup. *Cladonia luteoalba* specimens are encoded LUTxx, the associated *Cladonia* thalli LUTxx-A and control thalli LUTxx-C. For GenBank Accession numbers see Table 1 and Supplementary Material Table S2 (available online). In colour online.

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were always identical in their ITS sequence, which was also always identical to the sequence obtained by Sanger sequencing directly from the lichen thallus.

Illumina metabarcoding did not support the hypothesis that *C. luteoalba* is the result of mechanical hybridization of more *Cladonia* species. A total of 7 582 820 reads passed demultiplexing and subsequently 5 814 251 reads passed filtering. Finally, 132 OTUs passed the criterion of occurrence of more than 100 reads in at least two of the triplicates of a sample, and they represented more than 50 genera (Supplementary Material Table S4, available online). The majority of the OTUs were found in one sample only (101 OTUs). The proportion of *Cladonia* OTUs in each sample is shown in Fig. 3. For each sample, the dominant sequence corresponded to the sequence obtained by Sanger sequencing. An additional *Cladonia* sequence was detected in seven samples. These were at least one or two orders of magnitude lower in abundance than the dominant mycobiont and they were also found in the PCR negative controls, so they should be considered cross-contaminations. Besides *Cladonia*, the most frequent OTU (OTU5, found in the three control samples and three out of six *C. luteoalba* samples) matched an unknown fungus isolated from *Quercus montana* leaf litter (KX908501, 98.7% similarity) and an uncultured fungus from alpine soil (LS958441, 100% similarity).

All the OTUs that gave relevant BLAST search results belonged to *Ascomycetes*, with two *Basidiomycete* exceptions: OTU220 (76.5% similarity to *Erythrobasidium* sp. LC272890) from a control, *C. coccifera* CLZ1; and OTU9 (93.1% similarity to *Tremella diploschistina* Millanes *et al*., JN790587), recovered from Czech *C. luteoalba* JS863, LUT19/37 and LUT20/1. Other lichenicolous taxa recovered were *Cryptodiscus galaninae* Zhurb. & Pino-Bodas (OTU68; 98.7% similarity to KY661636, in LUT20/1), *Epithamnolia xanthoriae* (Brackel) Diederich & Suija (OTU19; 99.3% similarity to MT028049, in JS863 and LUT19/17-C2) and *Lichenosticta alcicornaria* (Linds.) D. Hawksw. (OTU7; 97.1% similarity to KY661621, in LUT19/12-C). Also, sequences belonging to various lichen species commonly co-occurring in *C. luteoalba* habitats were detected (see Supplementary Material Table S4).

Photobionts

Photobionts belonging to seven lineages of *Asterochloris* were identified (Fig. 4, Table 1): *A. irregularis* (Hildreth & Ahmadjian) Skaloud & Peksa (24 samples), *A. italiana* (P. A. Archibald) Skaloud & Peksa (9 samples), *A. glomerata* (Waren) Skaloud & Peksa (6 samples), *A. leprarii* Skaloud & Peksa (1 sample), *A. stereocaulonicola* Y. J. Kim *et al.* (1 sample), and two undescribed lineages *Asterochloris* sp. StA3 (3 samples) and *Asterochloris* aff. *italiana* (1 sample), both *sensu* Vančurová *et al.* (2018).

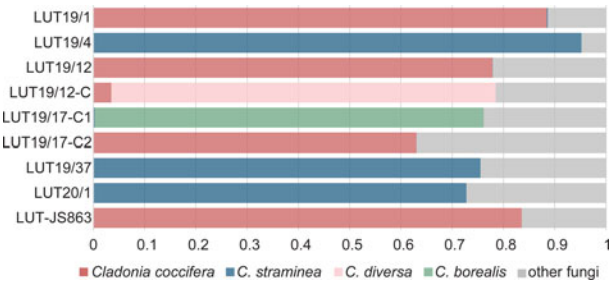

Fig. 3. Relative abundances of mycobiont sequences in *Cladonia* thalli as revealed by Illumina metabarcoding. For further details see Supplementary Material Table S4 (available online). In colour online.

Cladonia luteoalba shared its photobiont with the most closely associated *Cladonia* thalli in all cases examined, with one exception (*A. glomerata* vs *A. irregularis* in LUT19/37 and the associated *Cladonia* thallus, respectively).

Chemotype 1 of *C. luteoalba* associated with all seven *Asterochloris* species found. Chemotype 2 (*C. straminea*) associated only with *A. irregularis* and *A. glomerata*. *Asterochloris irregularis, A. italiana* and *Asterochloris* sp. StA3 were shared by both *C. coccifera* agg. lineages. Additionally, *C. coccifera* agg. lineage 1 also associated with *A.* aff. *italiana*, *A. leprarii* and *A. stereocaulonicola*; whereas *C. coccifera* agg. lineage 2 also associated with *A. glomerata* (Fig. 5). This pattern could not be explained by geography, altitude or substratum type (see Supplementary Material Table S1).

Discussion

In the genus *Cladonia*, species delimitation and taxonomy are particularly problematic. Phenotypic variability within species is wide and similarity to closely related species high, making it difficult to set boundaries. While phylogenetic studies have been beneficial in some taxa delimitations (e.g. Pino-Bodas *et al.* 2010*a*; Kanz *et al.* 2015; Stenroos *et al.* 2015) they have produced ambivalent results in others. Many taxa have proved to be polyphyletic; however, the authors often discuss the processes underlying low phylogenetic resolution and discrepancies in the molecular data, such as incomplete lineage sorting, unrecognized paralogs, introgression, homoplasy or horizontal gene transfer, and consider their data insufficient to draw taxonomic conclusions (e.g. Piercey-Normore *et al.* 2010; Steinová *et al.* 2013; Pino-Bodas *et al.* 2015). In other cases, phenotypically recognizable taxa were synonymized based on molecular revisions, and differences were attributed to effects of environmental conditions, for example, *C. pocillum* with *C. pyxidata* (L.) Hoffm. (Kotelko & Piercey-Normore 2010) and *C. convoluta* (Lamkey) Anders. with *C. foliacea* (Huds.) Willd. (Pino-Bodas *et al.* 2010*b*). The taxonomic value of lichen secondary metabolites is also inconsistent (e.g. Pino-Bodas *et al.* (2010*a*) vs Pino-Bodas *et al.* (2015)).

We collected two out of the four known chemotypes of *Cladonia luteoalba* (Stenroos 1990). These chemotypes correspond to the chemotypes of *C. straminea* (didymic and squamatic acids) and the *C. coccifera* agg. (zeorin and accessory (con-)porphyrillic acid). *Cladonia straminea* is a well-defined monophyletic species (see Fig. 2), while *C. coccifera* agg. includes four morphological species that are indistinguishable based on ITS rDNA and β-tubulin sequence data (Steinová *et al.* 2013). The phylogenetic placement of the *C. luteoalba* samples coincided with their chemistry. Therefore, not only is *C. luteoalba* polyphyletic, it also appears to be conspecific with *Cladonia* species of the corresponding chemotypes. It is also indistinguishable from the most closely associated *Cladonia* thallus. Commonly, the widely accepted fungal barcode marker ITS rDNA provides poor phylogenetic resolution in the genus *Cladonia* and alternative candidate markers have been suggested (Pino-Bodas *et al.* 2013). However, within the clade *Erythrocarpae* even additional markers might not resolve morphologically well-defined species (*C. coccifera* agg., two loci in Steinová *et al.* (2013), *C. coccifera* agg. and *C. macilenta-C. floerkeana* agg., five loci in Stenroos *et al.* (2019), and *C. bellidiflora-C. polydactyla-C. umbricola* complex, five loci in Steinová *et al.* (2022)), possibly due to low genetic

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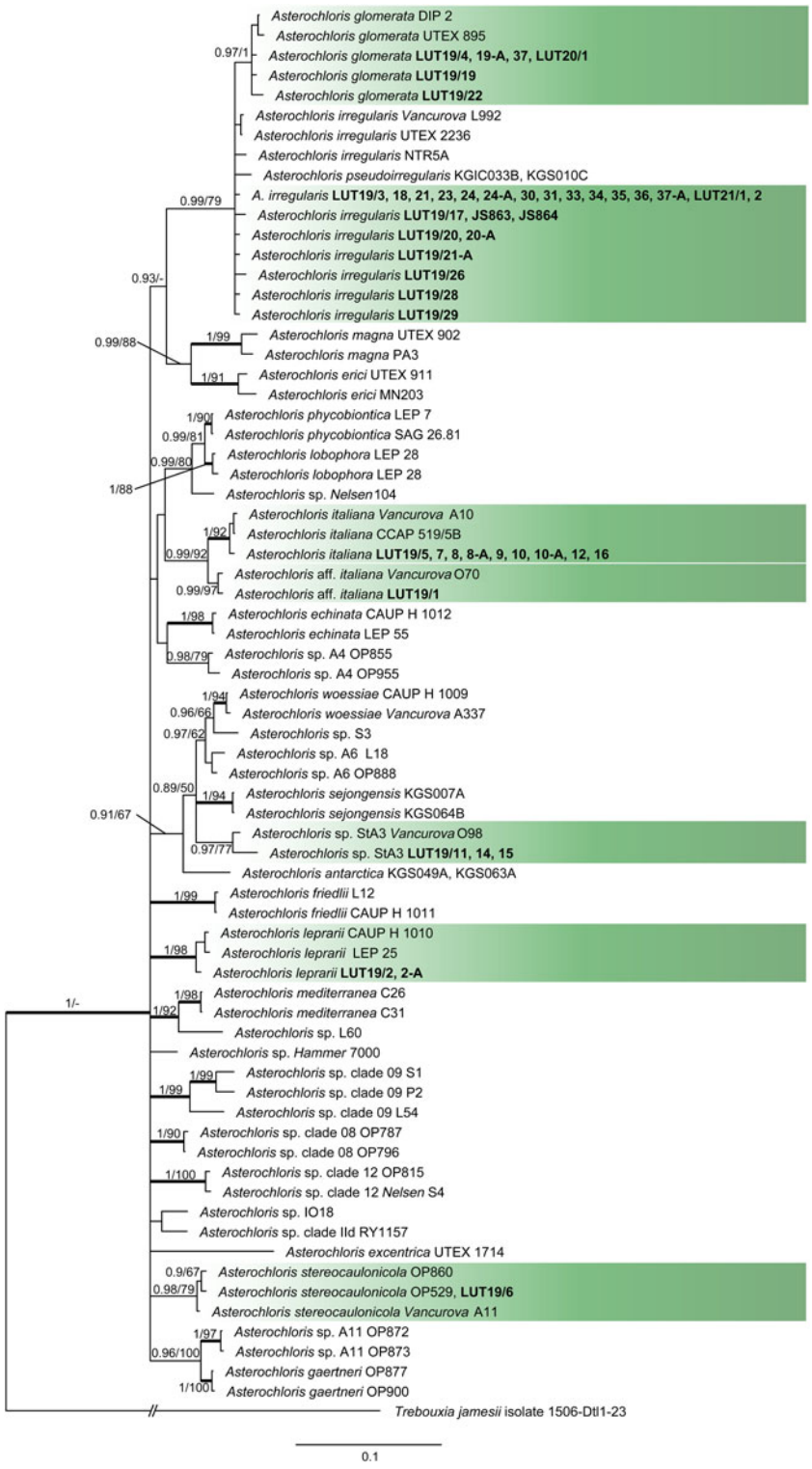

Fig. 4. Phylogeny of *Asterochloris* obtained by Bayesian inference of concatenated nuclear ITS rDNA and actin type I. Values at nodes show statistical support calculated by MrBayes posterior-node probability (PP)/maximum likelihood bootstrap. Only statistical support with PP > 0.75 is shown. Thick branches represent nodes with full PP support. Lineages with *C. luteoalba* photobionts are highlighted. Newly obtained sequences are in bold. *Trebouxia jamesii* is the outgroup. *Cladonia luteoalba* specimens are encoded LUTxx, the associated *Cladonia* thalli LUTxx-A and control thalli LUTxx-C. For GenBank Accession numbers see Table 1 and Supplementary Material Table S3 (available online). In colour online.

differentiation resulting from recent speciations (Stenroos *et al.* 2019). Advanced methods, such as microsatellite and RADseq data, are helpful in discriminating closely related species (e.g. *Usnea antarctica* Du Rietz. and *U. auratiacoatra* (Jacq.) Bory; Grewe *et al.* 2018; Lagostina *et al.* 2018) and will be essential in building a robust well-resolved phylogeny including a wide sampling of the *Erythrocarpae* clade that should be the basis for future studies. However, the fact that the *C. luteoalba* phenotype is found in different, not closely related lineages strictly following the pattern in chemotypes makes it unlikely that involvement of such methods would support its existence as a distinctive species.

Other reasons why morphologically well-distinguishable lichens are not supported by molecular data have been reported. Velmala *et al.* (2009), for example, showed that *Bryoria fremontii* (Tuck.) Brodo & D. Hawksw. and *B. tortuosa* (G. Merr.) Brodo & Hawksw., distinguished by the production of secondary metabolites

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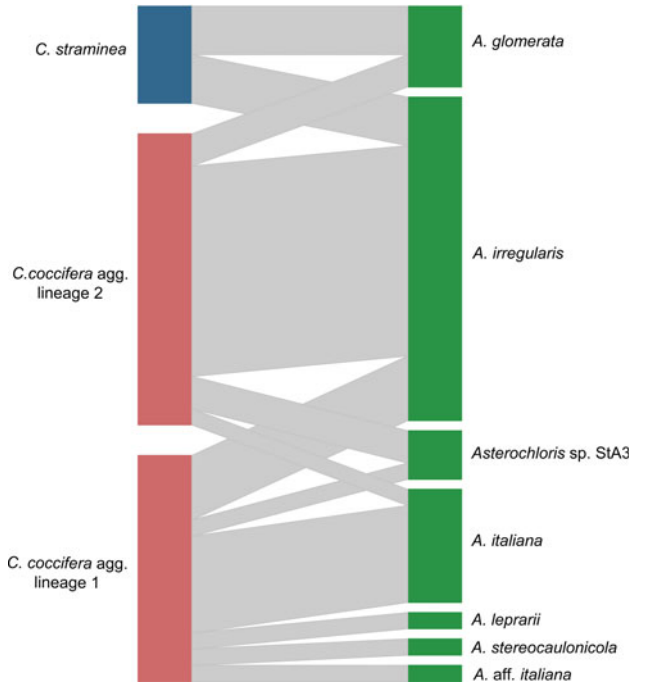

Fig. 5. Association network between *Cladonia* mycobiont lineages and *Asterochloris* photobiont species. Link widths are proportional to the number of samples in the association. In colour online.

and thus also colour, are conspecific and the difference between them was later attributed to the presence of associated fungi, specifically *Cystobasidiomycete* yeast (Spribille *et al.* 2016). Even more striking conspecificity was shown between *Lecanographa amylacea* (Ehrh. ex Pers.) Egea & Torrente (*Arthoniomycetes*) and *Buellia violaceofusca* G. Thor & Muhr (previously placed in *Lecanoromycetes*) and was explained by photobiont switching between *Trentepohlia* and *Trebouxia* (Ertz *et al.* 2018).

The distinctiveness of *C. luteoalba* was also not supported by mycobiont culturing and DNA metabarcoding. They did not support the hypothesis that *C. luteoalba* is the result of mechanical hybridization and did not reveal any fungal taxon always associated with *C. luteoalba* and never with the other related lichens. However, the possibility that the morphotype is caused by a fungal infection still cannot be ruled out. The primers we used were designed to favour *Cladonia* sequences, thus the PCR bias here is great and the fungal spectrum we obtained cannot be considered representative. Lichenicolous fungi commonly cause morphological changes in the thallus, most conspicuously discolorations or necrotic patches formed by, for example, *Lichenoconium* species (Hawksworth 1977) or colour change of whole *Cladonia* squamules by *Arthrorhaphis aeruginosa* R. Sant. & Tønsberg (Santesson & Tønsberg 1994), and galls induced by, for example, *Tremella* species (Millanes *et al.* 2012, 2015; Zamora *et al.* 2018). However, in those cases, the parasite mycelia are visible in cross-sections of the host thalli if fruiting bodies are absent. Galls on lichens are also provoked by invertebrates such as nematodes (Siddiqi & Hawksworth 1982) or mites (Gerson 1973). The increased production of usnic acid that causes the yellow colour of the squamule underside suggests a parasite might be involved, since antibiotic, antiviral, antifungal, anti-insect, antiherbivore and other effects of usnic acid have been shown (reviewed by, e.g. Ingólfsdóttir (2002)). Therefore, DNA metabarcoding studies targeting a wide range of organisms (i.e. fungi and bacteria, but also viruses) should be the next step in resolving this enigma.

The *C. luteoalba* morphotype is obviously not linked to photobiont switching. It shares its photobiont with the closely associated *Cladonia* thalli. Our *C. luteoalba* samples can be divided into two groups based on the *Asterochloris* species they associate with (Fig. 5). The first group included the *C. straminea* genotypes and several representatives of the *C. coccifera* agg.; it associated with *A. glomerata* and *A. irregularis* which are the typical *Cladonia* photobionts of colder climates and acidic substrata, according to Škvorová *et al.* (2022: module 2 therein). All Central European samples from higher altitudes and more than half of the Norwegian samples belonged to this group (Table 1). The second group included *C. coccifera* agg. representatives, which associated with the other five *Asterochloris* species (see 'Results'). Among them, only two were included in the study of Škvorová *et al.* (2022): compared to the first group, *A. italiana* represents a photobiont of warmer, wetter and more nutrient-rich habitats, while *A.* aff. *italiana* is of warmer and drier habitats with higher substratum pH (modules 4 and 1, respectively, in Škvorová *et al.* (2022)). Given the acidic bedrocks and relative climatic uniformity of our Norwegian collection sites, we suggest that microclimatic differences or minor pH variations, caused, for example, by surrounding vegetation, may also play a role in photobiont choice. In any case, no clear patterns between the mycobiont phylogenetic lineages and their associated photobionts were observed in *C. luteoalba*.

In conclusion, our data do not support the existence of *C. luteoalba* as a separate *Cladonia* species. However, neither the lectotype (BM 000006761) nor the isolectotype (NMW 0000803) contain identifiable associated *Cladonia* species with which *C. luteoalba* could be synonymized. The lectotype contains zeorin (Østhagen 1972) but the taxonomy of the zeorin-containing species of the *C. coccifera* agg. is unclear and requires further revision. Consequently, *C. luteoalba* remains a valid name for now.

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References

Ahti T (1965) Some notes on British *Cladoniae*. *Lichenologist* **3**, 84–88.

Ahti T, Stenroos S and Moberg R (2013) *Nordic Lichen Flora Vol. 5* Cladoniaceae. Museum of Evolution, Uppsala University, 117pp.

Andrews S (2010) *FastQC: a quality control tool for high throughput sequence data.*[WWW resource] URL https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Báilint M, Schmidt P-A, Sharma R, Thines M and Schmitt I (2014) An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**, 2642–2653.

Burgaz AR, Ahti T and Pino-Bodas R (2020) *Mediterranean* Cladoniaceae. Madrid: Spanish Lichen Society.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**, 540–552.

Cubero OF, Crespo A, Fatehi J and Bridge PD (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium stored, lichenized, and other fungi. *Plant Systematics and Evolution* **216**, 243–249.

Dal Grande F, Widmer I, Wagner HH and Scheidegger C (2012) Vertical and horizontal photobiont transmission within populations of a lichen symbiosis. *Molecular Ecology* **21**, 3159–3172.

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

Dormann CF, Frueund J, Bluethgen N and Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks. *Open Ecology Journal* **2**, 7–24.

Elvebakk A and Hertel H (1996) Part 6. Lichens. In Elvebakk A and Prestrud P (eds), *A Catalogue of Svalbard Plants, Fungi, Algae and Cyanobacteria*. Oslo: Norsk Polarinstitutt Skrifter, pp. 271–359.

Ertz D, Guzow-Krzemińska B, Thor G, Łubek A and Kukwa M (2018) Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the *Arthoniomycetes*. *Scientific Reports* **8**, 4952.

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

Gerson U (1973) Lichen-arthropod associations. *Lichenologist* **5**, 434–443.

Grewe F, Lagostina E, Wu H, Printzen C and Lumbsch HT (2018) Population genomic analyses of RAD sequences resolves the phylogenetic relationship of the lichen-forming fungal species *Usnea antarctica* and *Usnea aurantiacoatra*. *MycoKeys* **43**, 91–113.

Hawksworth DL (1977) Taxonomic and biological observations on the genus *Lichenoconium* (*Sphaeropsidales*). *Persoonia* **9**, 159–198.

Ingólfsdóttir K (2002) Usnic acid. *Phytochemistry* **61**, 729–736.

Kanz B, Brackel W von, Cezanne R, Eichler M, Hohmann M-L, Teuber D and Printzen C (2015) Molekulargenetische Untersuchung zum Vorkommen der Rentierflechte *Cladonia stygia* in Hessen. *Botanik und Naturschutz in Hessen* **28**, 5–20.

Katoh K, Rozewicki J and Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**, 1160–1166.

Kim JI, Kim YJ, Nam SW, So JE, Hong SG, Choi H-G and Shin W (2020) Taxonomic study of three new Antarctic *Asterochloris* (*Trebouxiophyceae*) based on morphological and molecular data. *Algae* **35**, 17–32.

Kotelko R and Piercey-Normore MD (2010) *Cladonia pyxidata* and *C. pocillum*; genetic evidence to regard them as conspecific. *Mycologia* **102**, 534–545.

Lagostina E, Dal Grande F, Andreev M and Printzen C (2018) The use of microsatellite markers for species delimitation in Antarctic *Usnea* subgenus *Neuropogon*. *Mycologia* **110**, 1047–1057.

Mahé F, Rognes T, Quince C, de Vargas C and Dunthorn M (2015) Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ* **3**, e1420.

Millanes AM, Westberg M, Wedin M and Diederich P (2012) *Tremella diploschistina* (*Tremellomycetes*, *Basidiomycota*, *Fungi*), a new lichenicolous species growing on *Diploschistes*. *Lichenologist* **44**, 321–332.

Millanes AM, Diederich P, Westberg M, Pippola E and Wedin M (2015) *Tremella cetrariellae* (*Tremellales*, *Basidiomycota*, *Fungi*), a new lichenicolous fungus on *Cetrariella delisei*. *Lichenologist* **47**, 359–368.

Moya P, Chiva S, Molins A, Jadrná I, Škaloud P, Peksa O and Barreno E (2018) *Myrmecia israeliensis* as the primary symbiotic microalga in squamulose lichens growing in European and Canarian terricolous communities. *Fottea* **18**, 72–85.

Nelsen MP and Gargas A (2009) Symbiont flexibility in *Thamnolia vermicularis* (*Pertusariales*: *Icmadophilaceae*). *Bryologist* **112**, 404–417.

Orange A, James PW and White FJ (2010) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Østhagen H (1972) The chemical strains in *Cladonia luteoalba* Wils. et Wheld. and their distribution. *Norwegian Journal of Botany* **19**, 37–41.

Piercey-Normore MD and DePriest PT (2001) Algal switching among lichen symbioses. *American Journal of Botany* **88**, 1490–1498.

Piercey-Normore MD, Ahti T and Goward T (2010) Phylogenetic and haplotype analyses of four segregates within *Cladonia arbuscula* s.l. *Botany* **88**, 397–408.

Pino-Bodas R, Burgaz AR and Martín MP (2010*a*) Elucidating the taxonomic rank of *Cladonia subulata* versus *C. rei* (*Cladoniaceae*). *Mycotaxon* **113**, 311–326.

Pino-Bodas R, Martín MP and Burgaz AR (2010*b*) Insight into the *Cladonia convoluta-C. foliacea* (*Cladoniaceae*, *Ascomycota*) complex and related species, revealed through morphological, biochemical and phylogenetic analyses. *Systematics and Biodiversity* **8**, 575–586.

Pino-Bodas R, Martín MP, Burgaz AR and Lumbsch T (2013) Species delimitation in *Cladonia* (*Ascomycota*): a challenge to the DNA barcoding philosophy. *Molecular Ecology Resources* **13**, 1058–1068.

Pino-Bodas R, Burgaz AR, Martín MP, Ahti T, Stenroos S, Wedin M and Lumbsch HT (2015) The phenotypic features used for distinguishing species within the *Cladonia furcata* complex are highly homoplasious. *Lichenologist* **47**, 287–303.

Pino-Bodas R, Sanderson N, Cannon P, Aptroot A, Coppins B, Orange A and Simkin J (2021) *Lecanorales*: *Cladoniaceae*, including the genera *Cladonia*, *Pilophorus* and *Pycnothelia*. *Revisions of British and Irish Lichens* **19**, 1–45.

R Core Team (2021) *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. [WWW resource] URL https://www.R-project.org/.

Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Hohna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

Sandstede H (1938) Erganzungen zu Wainios Monographia Cladoniarum Universalis, unter besonderer Beriicksichtigung des Verhaltens zu Asahinas Diaminprobe. *Repertorium Specierum Novarum Regni Vegetabilis* **103**, 1–103.

Santesson R and Tønsberg T (1994) *Arthrorhaphis aeruginosa* and *A. olivaceae*, two new lichenicolous fungi. Lichenologist **26**, 295–299.

Siddiqi MR and Hawksworth DL (1982) Nematodes associated with galls on *Cladonia glauca*, including two new species. *Lichenologist* **14**, 175–184.

Škaloud P and Peksa O (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris* (*Trebouxiophyceae*, *Chlorophyta*). *Molecular Phylogenetics and Evolution* **54**, 36–46.

Škvorová Z, Černajová I, Steinová J, Peksa O, Moya P and Škaloud P (2022) Promiscuity in lichens follows clear rules: partner switching in *Cladonia* is regulated by climatic factors and soil chemistry. *Frontiers in Microbiology* **12**, 781585.

Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinski H, Aime MC, Schneider K, Stabentheiner E, Toome-Heller M, Thor G, *et al.* (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* **353**, 488–492.

Steinová J, Stenroos S, Grube M and Škaloud P (2013) Genetic diversity and species delimitation of the zeorin-containing red-fruited *Cladonia* species (lichenized *Ascomycota*) assessed with ITS rDNA and β-tubulin data. *Lichenologist* **45**, 665–684.

Steinová J, Holien H, Košuthová A and Škaloud P (2022) An exception to the rule? Could photobiont identity be a better predictor of lichen phenotype than mycobiont identity? *Journal of Fungi* **8**, 275.

Stenroos S (1990) *Cladonia luteoalba* – an enigmatic *Cladonia*. *Karstenia* **30**, 27–32.

Stenroos S, Pino-Bodas R, Weckman D and Ahti T (2015) Phylogeny of *Cladonia uncialis* (*Cladoniaceae*, *Lecanoromycetes*) and its allies. *Lichenologist* **47**, 215–231.

Stenroos S, Pino-Bodas R, Hyvönen J, Lumbsch HT and Ahti T (2019) Phylogeny of the family *Cladoniaceae* (*Lecanoromycetes*, *Ascomycota*) based on sequences of multiple loci. *Cladistics* **35**, 351–384.

Stocker-Wörgötter E and Hager A (2008) Appendix: culture methods for lichens and lichen symbionts. In Nash TH, III (ed.), *Lichen Biology*, 2nd Edn. New York: Cambridge University Press, pp. 353–363.

Vančurová L, Muggia L, Peksa O, Řídká T and Škaloud P (2018) The complexity of symbiotic interactions influences the ecological amplitude of the

https://doi.org/10.1017/S002428292200024X Published online by Cambridge University Press

host: a case study in *Stereocaulon* (lichenized *Ascomycota*). *Molecular Ecology* **27**, 3016–3033.

Vančurová L, Kalníková V, Peksa O, Škvorová Z, Malíček J, Moya P, Chytrý K, Černajová I and Škaloud P (2020) Symbiosis between river and dry lands: phycobiont dynamics on river gravel bars. *Algal Research* **51**, 102062.

Velmala S, Myllys L, Halonen P, Goward T and Ahti T (2009) Molecular data show that *Bryoria fremontii* and *B. tortuosa* (*Parmeliaceae*) are conspecific. *Lichenologist* **41**, 231–242.

Větrovský T, Baldrian P and Morais D (2018) SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. *Bioinformatics* **34**, 2292–2294.

Wheldon JA and Wilson A (1907) *The Flora of West Lancashire*. Liverpool: Henry Young and Sons.

White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. San Diego: Academic Press, pp. 315–322.

Williams L, Colesie C, Ullmann A, Westberg M, Wedin M and Büdel B (2017) Lichen acclimation to changing environments: photobiont switching vs. climate-specific uniqueness in *Psora decipiens*. *Ecology and Evolution* **7**, 2560–2574.

Zamora JC, Millanes AM, Etayo J and Wedin M (2018) *Tremella mayrhoferi*, a new lichenicolous species on *Lecanora allophana*. *Herzogia* **31**, 666–676.

Zwickl DJ (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. thesis, University of Texas at Austin.

https://doi.org/10.1017/S002428292200024X Published online by Cambridge University Press