The Lichenologist **48**(5): 545–557 (2016) © British Lichen Society, 2016
doi:10.1017/S0024282916000232

Photobiont associations in co-occurring umbilicate lichens with contrasting modes of reproduction in coastal Norway

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Abstract: The identity and phylogenetic placement of photobionts associated with two lichen-forming fungi, *Umbilicaria spodochroa* and *Lasallia pustulata* were examined. These lichens commonly grow together in high abundance on coastal cliffs in Norway, Sweden and Finland. The mycobiont of *U. spodochroa* reproduces sexually through ascospores, and must find a suitable algal partner in the environment to re-establish the lichen symbiosis. *Lasallia pustulata* reproduces mainly vegetatively using symbiotic propagules (isidia) containing both symbiotic partners (photobiont and mycobiont). Based on DNA sequences of the internal transcribed spacer region (ITS) we detected seven haplotypes of the green-algal genus *Trebouxia* in 19 pairs of adjacent thalli of *U. spodochroa* and *L. pustulata* from five coastal localities in Norway. As expected, *U. spodochroa* associated with a higher diversity of photobionts (seven haplotypes) than the mostly asexually reproducing *L. pustulata* (four haplotypes). The latter was associated with the same haplotype in 15 of the 19 thalli sampled. Nine of the lichen pairs examined share the same algal haplotype, supporting the hypothesis that the mycobiont of *U. spodochroa* might associate with the photobiont 'pirated' from the abundant isidia produced by *L. pustulata* that are often scattered on the cliff surfaces. Up to six haplotypes of *Trebouxia* were found within a single sampling site, indicating a low level of specificity of both mycobionts for their algal partner. Most photobiont strains associated with species of *Umbilicaria* and *Lasallia*, including samples from this study, represent phylogenetically closely related taxa of *Trebouxia* grouped within a small number of main clades (*Trebouxia* sp., *T. simplex*/*T. jamesii*, and *T. incrustata*+*T. gigantea*). Three of the photobiont haplotypes were found only in *U. spodochroa* thalli.

Key words: *Lasallia pustulata*, mutualism, photobiont guilds, phylogeny, symbiosis, *Trebouxia*, *Umbilicaria spodochroa*

Accepted for publication 15 *April* 2016

Introduction

Many lichens can establish a new thallus from small pieces (thallus fragmentation) or have evolved special reproductive structures such as soredia or isidia that allow the dispersal of both symbiotic partners simultaneously (Büdel & Scheidegger 2008). However, for numerous lichens only the fungal partner seems to disperse, either by sexually generated spores or by asexual conidia, and in order to re-establish the symbiosis these species face the challenge of finding a suitable photobiont in the environment (Bowler & Rundel 1975; Hestmark 1990, 1991*a*, *b*, *c*, 1992*a*, *b*; Nash 2008). A potential source of such suitable algae is symbiotic propagules dispersed by other lichen species (Beck *et al.* 1998; Fedrowitz *et al.* 2011). Many lichen photobionts may not occur frequently in a free-living stage due to their long co-evolution with fungi in lichen symbiosis (Ahmadjian 1988). The degree of specificity in the symbiotic relationship differs substantially between lichen taxa, from rare cases of reciprocal one-to-one specificity (e.g. Otalora *et al.* 2010) or high specificity of mycobionts for a single or a small number of photobionts (e.g., Paulsrud *et al.* 2000; Yahr *et al.* 2004; Lindgren *et al.* 2014; Muggia *et al.* 2014; Nyati *et al.* 2014; O'Brien 2014; Leavitt *et al.* 2015) to generalist mycobionts that are more flexible and may associate with multiple partners (Piercey-Normore & DePriest 2001;

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Piercey-Normore 2005; Guzow-Krzemińska 2006; Yahr *et al.* 2006; Hauk *et al.* 2007; Nelsen & Gargas 2008; Muggia *et al.* 2013). In general, one would predict that a lichen-forming fungus that needs to re-establish the symbiotic state at every reproductive cycle should be more flexible with regard to the photobionts it associates with than a lichen that propagates the intact symbiosis.

The peltate lichen-forming fungus *Umbilicaria spodochroa* Hoffm. grows in high abundance on the rocky coasts of southern Norway. It reproduces sexually through ascospores from apothecia on the upper surface, and the number of apothecia is positively correlated with thallus size (Ramstad & Hestmark 2001), a common trait also observed in several other members of the genus *Umbilicaria* Hoffm. (Hestmark *et al.* 2004; Gregersen *et al.* 2006). To form a lichen thallus, ascospores of *U. spodochroa* must re-establish symbiosis *de novo* with suitable photobionts encountered in the environment. Alternatively, new thalli could be formed through the growth of small thallus fragments containing both symbionts, but neither fragmentation nor erosion of thalli seems common in this particular species (G. Hestmark, unpublished field observations).

On the coastal cliffs of Scandinavia, *U. spodochroa* commonly grows in mixed populations with another peltate lichen, *Lasallia pustulata* (L.) Mérat (Fig. 1), and competitive overgrowth interactions between the two species were demonstrated both *in situ* and experimentally by Hestmark (1997*a*). Although both species belong to the same family, molecular phylogenetic studies indicate that they are not closely related within the *Umbilicariaceae* (Miadlikowska *et al.* 2006, 2014). The two species have almost identical physiological niche responses to light and temperature (Kappen *et al.* 1996, 1997; Hestmark *et al.* 1997), and very similar population dynamics (Hestmark 1997*b*, *c*, 2000; Sletvold & Hestmark 1999; Ramstad & Hestmark 2000). In contrast to *U. spodochroa*, *L. pustulata* reproduces mainly through isidia, that is, packages of fungal hyphae and algae produced as coralloid or tree-like structures on the upper surface of the thallus (Fig. 2). Thalli typically start to produce isidia when they reach a minimum size of 5 cm in diameter and the isidia become more abundant as thalli grow older and larger (Hestmark 1992*b*). Very large thalli may also develop apothecia and reproduce sexually (Hestmark 1992*b*). Compared to ascospores, isidia are heavy and roll onto the rock surface adjacent to the thallus of origin. Because of the common co-occurrence of these two lichens in the same habitats, *L. pustulata* could serve as a potential 'photobiont donor' via its isidia from which newly dispersed ascospores of *U. spodochroa* could obtain a compatible photobiont. The almost entirely sympatric ranges of the two species in Norway, with that of *U. spodochroa* being slightly narrower (Fig. 3), could indicate that prior establishment of *L. pustulata* in any given habitat facilitates the successful colonization by *U. spodochroa*. The two co-occurring species seem to form an ecological guild, i.e. a group of species that exploit the same class of environmental resources in the same way (Simberloff & Dayan 1991), in this case mediated by a photobiont as has been previously proposed for other groups of lichens (Beck *et al.* 1998, 2002; Beck 1999; Rikkinen *et al.* 2002; Rikkinen 2003; Werth 2012).

Great progress in the identification of lichen photobionts has been made over the past decade due to the application of molecular methods. Large scale phylogenetic analyses of ITS sequence data of the unicellular green algal genus *Trebouxia* Puymaly, the most common lichen photobiont, have revealed at least 25 main lineages (Muggia *et al.* 2014; O'Brien 2014). Recent studies by Sadowska-Des *et al.* (2013, 2014) demonstrated that the mycobiont of *L. pustulata* associates with several haplotypes of *Trebouxia* in Europe. Comparative data for *U. spodochroa* do not exist. Studies of other *Umbilicaria* taxa indicate that, overall, members of this genus are generalists and exhibit substantial flexibility (i.e. low specificity) with regard to their choice of *Trebouxia* photobionts (Romeike *et al.* 2002; Jones *et al.* 2013). The aims of the present study were to 1) evaluate whether *U. spodochroa*

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FIG. 1. Mixed populations of *Umbilicaria spodochroa* (light grey) and *Lasallia pustulata* (olive-green) on coastal cliffs of southern Norway. Black dots on *U. spodochroa* are apothecia.

and *L. pustulata* share the same algal symbionts (forming a photobiont-mediated guild) through comparative study of ITS sequences of the photobionts associated with these two species growing side by side in several localities in Norway; 2) determine if *U. spodochroa*, which mostly propagates only the fungal partner, hosts a larger diversity of photobionts than *L. pustulata*, which propagates the intact symbiosis.

Materials and Methods

Sample collection

Individual thalli (4–5 cm in diam.) of *Lasallia pustulata* and *Umbilicaria spodochroa* growing adjacent to each other (<1 cm between the thallus margins) were sampled in pairs from five different localities on the south coast of Norway (Fig. 1 & Table 1): 1) Rogaland, Forsand kommune, Esmark-moraine, 58°54'14"N, 6°8'37"E, leg. et det. G. Hestmark 2007; 2) Vest-Agder, Flekkefjord kommune, Hidre-heiene, W of old farm Håland, 58°15'54"N, 6°34'48"E, leg. et det. G. Hestmark 2011; 3) Vestfold, Nøtterøy kommune, shore at Torød, 59°10'0"N, 10°26'26"E, leg. et det. G. Hestmark 2013; 4) Akershus, Frogn kommune, Drøbak, Torkildstranda, 59°38'46"N, 10°38'5"E, leg. et det. G. Hestmark 2013; and 5) Østfold, Hvaler, Kråkerøy, Ødegården, shore, 59°9'9"N, 10°56'53"E, leg. et det. G. Hestmark 2011. With the exception of the first locality, which is an old moraine located *c.* 60 m above sea level and 2 km from the sea, the remaining localities were all on sloping coastal cliffs, 20–30 m above sea level. Altogether, photobionts of 19 pairs (38 specimens) of *L. pustulata* and *U. spodochroa* were investigated (Table 1). The lichen material was deposited in the Oslo University Herbarium (O).

DNA isolation, sequencing and sequence alignment

Approximately 0·5 cm^2 of each lichen thallus was homogenized with 0·7 mm zirconium beads for 8 s in a mini-beadbeater. Genomic DNA was extracted using a modified protocol from Zolan & Pukkila (1986) with 2% sodium dodecyl sulphate (SDS) as extraction buffer. Isolated DNA was resuspended in sterile water and stored at −20 °C. The ITS region of the photobionts was amplified from the lichen total genomic DNA using the following algal-specific and/or general primer pairs (including newly developed primers shown in

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TABLE 1. *Photobiont diversity found in adjacent specimens (19 pairs) of* Lasallia pustulata *and* Umbilicaria spodochroa *in five localities in Norway. GenBank accession numbers are shown in parentheses. Asterisks indicate ITS sequences representing each haplotype in the phylogenetic analysis as shown in Fig. 4.*

Collection location	Pair number	Photobiont diversity of adjacent lichen species with GenBank accession numbers†	
		Lasallia pustulata	*Umbilicaria spodochroa*
Forsand	1	H1.1.1 (KU900251)	H1.1.2 (KU900266)
Drøbak	2	H1.2.3 (KU900252)*	H1.2.4 (KU900267)
	3	H1.3.5 (KU900253)	H1.3.6 (KU900268)
	4	H1.4.7 (KU900254)	H2.4.8 (KU900275)*
Hvaler	5	H3.5.9 (KU900276)	H3.5.10 (KU900277)
	6	H1.6.11 (KU900255)	H1.6.12 (KU900269)
	7	H1.7.13 (KU900256)	H1.7.14 (KU900270)
	8	H1.8.15 (KU900257)	H4.8.16 (KU900279)
	9	H1.9.17 (KU900258)	H4.9.18 (KU900280)
Flekkefjord	10	H5.10.19 (KU900281)*	H5.10.20 (KU900282)*
	11	H2.11.21 (KU900273)	H6.11.22 (KU900283)*
	12	H1.12.23 (KU900259)	H3.12.24 (KU900278)*
	13	H1.13.25 (KU900260)	H6.13.26 (KU900284)
	14	H2.14.27 (KU900274)	H7.14.28 (KU900288)*
Nøtterøy	15	H1.15.29 (KU900261)	H6.15.30 (KU900285)
	16	H1.16.31 (KU900262)	H1.16.32 (KU900271)
	17	H1.17.33 (KU900263)	H6.17.34 (KU900286)
	18	H1.18.35 (KU900264)	H1.18.36 (KU900272)
	19	H1.19.37 (KU900265)	H6.19.38 (KU900287)

†Key to information given for each photobiont. Three numbers are given for each haplotype; the first = the unique haplotype number also indicated in different colours (H1 -7), the second = the pair number (1 -19), the third = the number of the individual thallus (1 -38). For instance H1.1.1 = Haplotype 1, pair number 1, thallus number 1.

parentheses): nrITSaJOFOR2 and nrITSaJOREV2 (Sadowska-Des *et al.* 2013); nrITSSaJOFOR2 and nrITS4T (Kroken & Taylor 2000); nrITSSaJOFOR2 and nrITS4 (White *et al.* 1990); nrITSSaJOFOR2 and Treb_1R (5'ACCTCAGGTCGAAAGCCAAA3'); nrITS1T (Kroken & Taylor 2000) and Treb_3R (5'CTGACCTCAGGTCGAAAGCCAA3'). All PCR products were cleaned with ExoSAP (Affymetrix Inc., CA, USA) following the manufacturer's protocol. Sequencing was carried out in 10 µl reactions using: 1 µl primer (10 µM), 1 µl purified PCR product, 0·75 µl BigDye (BigDye Terminator Cycle sequencing kit, ABIPRISM version 3.1; PerkinElmer, Applied Biosystems, Foster City, CA), 3·25 µl BigDye buffer, and 4 µl double-distilled water. Clean up reactions were performed over Sephadex G-50 DNA grade columns, eluting in water. Samples were then injected directly in an ABI 3730xl DNA analyzer (PE Applied Biosystems, Foster City, CA) utilizing a 22 s injection time and a 50 cm capillary array, at the Sequencing and Genomic Technologies Shared Resource core facility, part of the Duke Center for Genomic and Computational Biology.

Sequences were assembled and edited using the software package Sequencher™ 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and subjected to BLAST searches (Wheeler *et al.* 2007) to confirm the algal origin of each sequence fragment. GenBank accession numbers for the 38 ITS sequences generated in this study are provided in Table 1. These ITS sequences represent seven haplotypes defined by using a 100% similarity criterion in Sequencher.

Single representative sequences for each of the seven haplotypes were added to an ITS reference data set from GenBank, that we assembled based on the *Trebouxia* phylogeny from the study by Muggia *et al.* (2014). The exception was Haplotype 5 which was represented

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FIG. 2. SEM of isidium on the thallus of *L. pustulata*: an asexually generated coralloid propagule that includes the fungus and alga in bundles. Scale = 0·5 mm.

by two sequences due to a few nucleotide uncertainties in one of them. The data matrix included multiple representatives of all major lineages and selected potential species (see also O'Brien 2014), as well as the existing sequences of photobionts from *Lasallia* and *Umbilicaria* (Romeike *et al.* 2002; Jones *et al.* 2013; Sadowska-Des *et al.* 2013, 2014) for a total of 221 OTUs (Fig. 4). Sequences were aligned manually using MacClade 4.08 (Maddison & Maddison 2005), and ambiguously aligned regions (*sensu* Lutzoni *et al.* 2000), were excluded from the first set of phylogenetic analyses. The maximum likelihood analyses on 429 characters were performed using RAxML-HPC2 version 7.2.8 (Stamatakis 2006; Stamatakis *et al.* 2008) as implemented on the CIPRES portal (Miller *et al.* 2010). Optimal tree and bootstrap searches were conducted with the rapid hill-climbing algorithm for 1000 replicates with the GTRGAMMA substitution model (Rodríguez *et al.* 1990) estimated for two partitions (ITS1+ITS2, and 5.8S). The second set of analyses was conducted on an extended data set with the re-inclusion of four ambiguous regions that were coded using PICS-Ord (Lücking *et al.* 2011), giving a total of 545 characters (426 nucleotides and 119 recoded characters). *Trebouxia galapagensis* (Hildreth & Ahmadjian) Gärtner (AJ249567) and *Trebouxia* sp. from *Ramalina peruviana* Ach. (AY842266) were used to root the phylogenies (Muggia *et al.* 2014; O'Brien 2014). We consider bootstrap values ≥70% as strong support. The NEXUS file and the resulting RAxML phylogeny were deposited in TreeBASE (accession number http://purl.org/phylo/treebase/phylows/study/TB2:S19031).

Results

The photobionts associated with 19 pairs of *Lasallia pustulata-Umbilicaria spodochroa* from five localities in Norway are represented by seven ITS haplotypes (hereafter referred to as H1–7) of *Trebouxia* algae (Table 1 & Fig. 4). Most haplotypes differed from each other substantially (i.e. by 10–37 nucleotide substitutions), except for two sets of haplotypes (H6/H7 and H2/H3) which differed only by one and two nucleotide substitutions, respectively. The most common haplotype (H1) was found in 22 of 38 thalli examined (57%), of which 15 were *L. pustulata* and seven were *U. spodochroa* (Table 1). This haplotype was present at all five study localities. Three of the remaining haplotypes (H2, H3, and H6) were less common (three to five occurrences) and appeared in two sites only, whereas three others (H4, H5 and H7) were rare and restricted to a single locality (Table 1). The highest diversity of photobionts was found in Flekkefjord, where six of the seven haplotypes were present. In the remaining sites (excluding Forsand because only one pair of lichens was sampled) up to three haplotypes of *Trebouxia* were detected. In about half of the lichen pairs examined (9 of 19), adjacent thalli of *L. pustulata* and *U. spodochroa* shared the same photobiont haplotype, which was H1 in seven of these nine pairs (Table 1). As expected, the mostly sexually reproducing *U. spodochroa* was found associated with a higher diversity of photobionts (seven haplotypes) than the mostly asexually reproducing *L. pustulata* (four haplotypes), which was found to be associated with the same haplotype (H1) in 15 of the 19 thalli sampled. Three haplotypes of *Trebouxia* (H4, H6 and H7) were found only in *U. spodochroa* thalli.

The ITS phylogeny for *Trebouxia* (Fig. 4) inferred for this study is largely in agreement with previous published phylogenies (e.g.

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FIG. 3. Distribution maps of *U. spodochroa* (A) and *L. pustulata* (B) in Norway. Stars and crosses indicate collections without exact coordinates, localized to county. Grey triangles indicate sample sites in this study, from left to right: Forsand, Flekkefjord, Torød, Drøbak, Hvaler. Maps generated from the lichen database Norsk Lavdatabase of the Natural History Museum, University of Oslo courtesy of E. Timdal.

Muggia *et al.* 2014; O'Brien 2014). The addition of coded characters greatly improved the resolution and support in some parts of the tree, especially for relationships among the most similar sequences. However, as in previous phylogenies, the backbone remained poorly supported (Muggia *et al.* 2014; O'Brien 2014). No conflicting relationship was detected between the tree based on 545 characters (i.e. 426 nucleotides and 119 PICS-Ord characters derived from the recoded ambiguously aligned regions; Fig. 4) and the phylogeny based on 429 characters (nucleotides only and ambiguously aligned regions excluded; tree not shown). Thirty-four internodes weakly supported in the phylogeny based on nucleotide characters only, received bootstrap support above 70% with the addition of PICS-Ord characters, but the opposite was true for five internodes; 41 branches were highly supported by both phylogenetic analyses. Overall, bootstrap support was lower in our phylogeny compared to the results from Muggia *et al.* (2014). This might be due to the more conservative approach in delimiting ambiguously aligned regions, which were excluded from our phylogenetic analyses since only four regions were reintegrated as coded characters. In contrast to Muggia *et al.* (2014), sequences in the *T. simplex/jamesii* clade were resolved in two groups, one of which also included members of *Trebouxia* "URa2". However, none of these clades received strong bootstrap support. We also found that *T. gigantea* (Hildreth & Ahmadjian) Gärtner was nested within *T. incrustata* Ahmadjian ex Gärtner, and sequences of those two taxa were recovered within multiple well-supported monophyletic groups (Fig. 4).

Most *Trebouxia* sequences from our sampling sites are nested within two main clades corresponding to the poorly supported *T. simplex*/*T. jamesii* complex (H1, H2, and H3) and the *T. incrustata* + *T. gigantea* group (H6 and H7) that received high bootstrap support in our study (Fig. 4). Both clades contain photobionts associated with members

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of *Umbilicaria* or *Lasallia* such as *U. antarctica* Frey & I. M. Lamb in the *T. incrustata* clade and *L. pustulata* in the *T. simplex*/*T. jamesii* clade from previous studies (e.g., Sadowska-Des *et al.* 2013, 2014; Romeike *et al.* 2002). Haplotype 4, found associated only with *U. spodochroa* in our study, was recovered in a well-supported monophyletic clade of *Trebouxia* strains associated exclusively with *L. pustulata* in previous studies. This clade might represent a new *Trebouxia* species (*Trebouxia* sp. in Fig. 4). Haplotype 5 was recovered as a distinct, well-supported lineage which could also represent a new *Trebouxia* species but its relationship to other *Trebouxia* lineages is poorly supported (Fig. 4). A revised classification of the diversity that has been documented within the genus *Trebouxia* is urgently needed and has been advocated (e.g. Leavitt *et al.* 2013, 2015) in order to identify lichen photobionts and better understand patterns of associations among symbionts. Overall, the phylogenetic placements of our isolates were in agreement with nucleotide BLAST results based on the sequences deposited in GenBank. For example, H6 and H7 share an 84-nucleotide insertion (excluded from ML analyses) in ITS1 of *T. incrustata*, the highest BLAST hit (100% query cover and 99% identity) being in the sequence of a photobiont found in other lichens (e.g., *Protoparmeliopsis muralis* (Schreb.) M. Choisy and *Xanthoparmelia tinctina* (Maheu & A. Gillet) Hale).

Discussion

Our study demonstrates that the two co-occurring lichen species *Lasallia pustulata* and *Umbilicaria spodochroa* often share the same photobiont within a single site and across different localities, and might therefore form a photobiont-mediated guild *sensu* Rikkinen (2003). It is also very likely that *U. spodochroa* 'pirates' algal cells from isidia of *L. pustulata*, as corroborated by the sharing of the rare Haplotypes 3 in Hvaler and 5 in Flekkefjord. However, empirical evidence would be needed from re-lichenization where, for example, isidia of *L. pustulata* are the only available source of photobiont for *U. spodochroa*. The fact that in many cases *U. spodochroa* utilizes photobionts other than those present in adjacent *L. pustulata* (especially H6; Table 1) suggests that the former species does not entirely depend on the latter for successful colonization and establishment. The overlap in geographical range of the two species is thus less likely to be explained solely by the mutual dependence on the same photobiont, and perhaps involves other environmental and/or reproductive factors. Ecological and evolutionary theory suggests that competitive exclusion should lead to the loss of one or other of these species due to the similarity of their requirements (MacArthur & Levins 1967; Abrams 1983; Abrams & Rueffler 2009). It is therefore possible that the co-occurring *L. pustulata* and *U. spodochroa* select different photobionts to achieve some degree of niche-separation, which is driven in part by their contrasting mode of reproduction. To what degree some of the detected *Trebouxia* haplotypes occur in a free-living state or in association with other lichen species in the same habitat has yet to be examined.

Overall, the most widespread, common and shared *Trebouxia* was represented by Haplotype 1 (H1), which also associates more frequently with *L. pustulata* (79%) than with *U. spodochroa* (37%). The same *Trebouxia* haplotype was found in approximately one third of the 44 specimens of *L. pustulata* collected across Europe by Sadowska-Des *et al.* (2013). Whether an association with a particular photobiont haplotype reflects some degree of physiological adaptation of the fungus to particular local habitats or the availability/ frequency of compatible photobionts, remains unknown. In *L. pustulata*, Sadowska-Des *et al.* (2013) found hardly any genetic variation in several loci of the mycobiont sampled within a broad geographical range in Europe, suggesting that there is no correlation between mycobiont-photobiont haplotypes. *Trebouxia* Haplotype 1 has only rarely been recorded from other lichen species (e.g. *Cetraria aculeata* (Schreb.) Fr.; Fernandez-Mendoza *et al.* 2011; Domaschke *et al.* 2013). This may, however,

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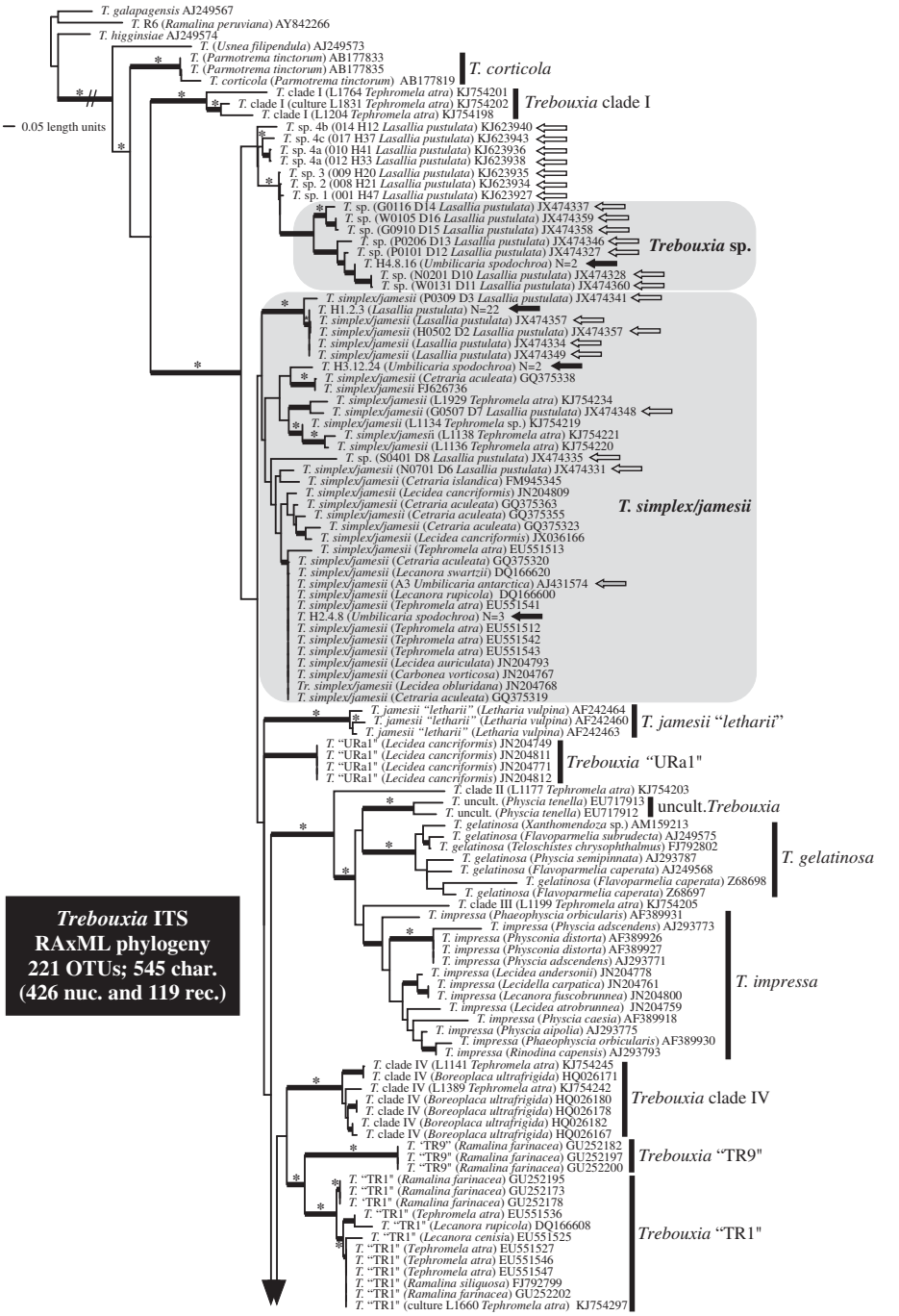

(*Fig. continued*)

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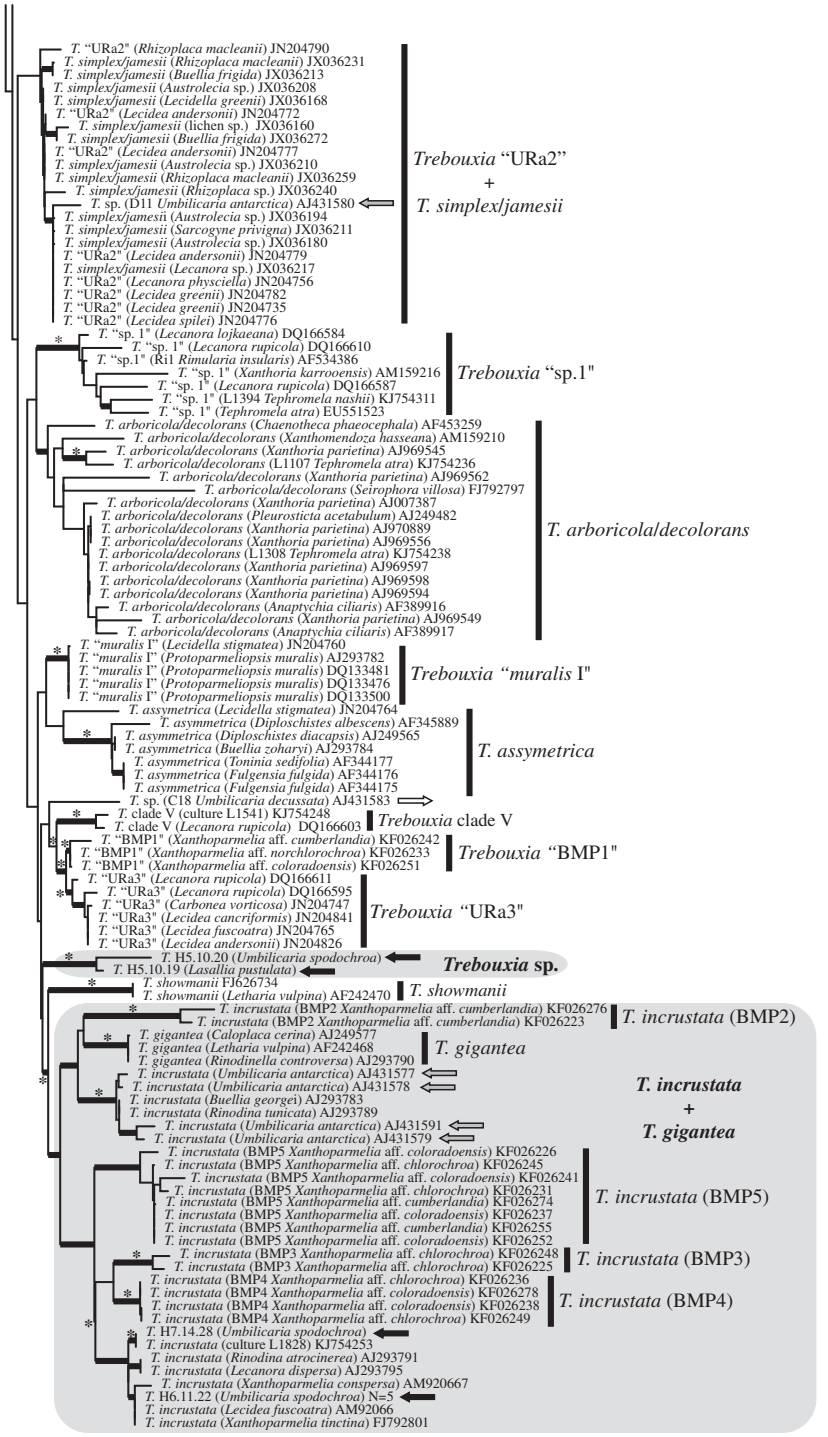

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indicate the under-sampling of certain environments, including coastal cliff habitats in Norway, rather than its uniqueness. The taxonomic identity of this strain remains unclear as two names, *T. angustilobata* (del Campo *et al.* 2010) and *T. jamesii* (e.g., Domaschke *et al.* 2013), are being used, and the phylogenetic placement of Haplotype 1 within a broadly defined *T. simplex*/*T. jamesii* complex is not conclusive as it has been placed where boundaries among species are not well defined and supported.

Our study confirms the capacity of *L. pustulata* to utilize several different strains of *Trebouxia*. This was earlier demonstrated by Sadowska-Des *et al.* (2013), but the genetic variation we detected among the photobionts was lower, perhaps due to the more limited sampling area restricted to the southern coast of Norway. *Umbilicaria spodochroa* seems to accept a wider range of algal partners (seven haplotypes) compared with *L. pustulata* (four haplotypes). This pattern may be explained partly by the greater likelihood of algal switches in *U. spodochroa* because a new thallus is often re-established *de novo* through ascospores by the mycobiont encountering a suitable algal partner in the environment, compared to *L. pustulata* which transmits its photobiont from one generation to the next using isidia. In these circumstances, it might be advantageous for a sexually reproducing species, which is required to re-associate with a new photobiont at each generation, to be a generalist when selecting a 'suitable photobiont' especially when competing with a mainly clonally reproducing species that propagates the symbiosis intact. This is the pattern we observed in the two umbilicate lichen species sampled. In the genus *Umbilicaria* this strategy is not restricted to sexually reproducing species but seems to occur also in about one third of its species that reproduce mainly by specialized asexual fungal propagules called thalloconidia seceded from the lower cortex, which are strictly fungal (Hestmark 1990, 1991*a*, *b*, *c*, 1992*a*). Numerous photobionts (based on the ITS) and diverse sharing patterns were found in four thalloconidia-producing species (*U. antarctica*, *U. decussata* (Vill.) Zahlbr., *U. kappenii* Sancho *et al.* and *U. umbilicarioides* (Stein) Krog & Swinscow) in the Antarctic (Romeike *et al.* 2002). Similarly, a number of *Trebouxia* strains associated with *U. aprina* Nyl. and *U. decussata* were reported from several localities in Antarctica (Jones *et al.* 2013). Somewhat in contrast to these results, another study of *U. aprina* in Antarctica revealed only a single algal strain in 19 thalli (Pérez-Ortega *et al.* 2012), but this may be due to the restricted geographical sampling in that particular study. The fact that different algae were detected in thalli of *L. pustulata* supports previous studies reporting that even in a mostly clonally reproducing species with propagules containing both partners, diverse symbiotic associations occur at various spatial scales (e.g. Piercey-Normore 2009; Dal Grande *et al.* 2012; Sadowska-Des *et al.* 2013).

Our results show that *U. spodochroa* associates with three *Trebouxia* haplotypes (H4, H6 and H7) not found in the thalli of the neighbouring *L. pustulata*. The H4 strain was, however, previously reported from *L. pustulata* (Sadowska-Des *et al.* 2013)

FIG. 4. Phylogenetic placement of seven haplotypes (H1–H7) of photobionts sampled from adjacent thalli of *Lasallia pustulata* and *Umbilicaria spodochroa* in Norway, in a broad phylogenetic context of the *Trebouxia* phylogeny based on ITS sequences (545 characters including 426 nucleotide sites and 119 recoded PICS-Ord characters) for 221 OTUs. See Table 1 for the sampling design and abbreviations used. Reference sequences were selected from Muggia *et al.* (2014), Sadowska-Des *et al.* (2013, 2014) and Romeike *et al.* (2002). The phylogeny was rooted with *T. galapagensis* AJ249567 and *Trebouxia* AY842266 following Muggia *et al.* (2014) and O'Brien (2014). Arrows indicate sequences of photobionts from *L. pustulata*, *U. spodochroa* (published – white arrows pointing to the left; from this study – black arrows), *U. antarctica* (grey arrows) and *U. decussata* (white arrow pointing to the right). Clades containing *Trebouxia* sequences from this study are delimited by grey boxes. Clade annotations follow Muggia *et al.* (2014). Thick branches indicate bootstrap support ≥70%. Stars indicate internodes that received high bootstrap support (>70%) in the maximum likelihood analysis based on nucleotides only (i.e. without PICS-Ord characters). Haplotype 5 (H5) is represented by two sequences due to a few nucleotide uncertainties in one of the sequences.

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and H7 is known from other saxicolous lichens such as *Lecanora dispersa* (Pers.) Sommerf. (UTEX 784), and *Tephromela atra* (DC.) Hafellner (Muggia *et al.* 2014). Overall, *Trebouxia* strains associated with *Umbilicaria*/*Lasallia* are not spread evenly across the ITS phylogeny but cluster in a few lineages (*Trebouxia* sp., *T. simplex*/*T. jamesii*, and *T. incrustata*+*T. gigantea*), and might include new *Trebouxia* species. Having a more stable phylogeny for *Trebouxia* will not only help in establishing boundaries among potential species and reveal their phylogenetic relationships, but will also facilitate the taxonomic revision of the genus and allow a more reliable identification of the most common lichen photobiont.

The authors are grateful to Brendan Hodkinson for his assistance in the installation and implementation of PICS-Ord. Funding for this study came from a research sabbatical grant from the University of Oslo to GH.

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