

The cyanomorph of *Ricasolia virens* comb. nov. (Lobariaceae, lichenized Ascomycetes)

TOR TØNSBERG¹, HANS H. BLOM², BERNARD GOFFINET³, JON HOLTAN-HARTWIG⁴ & LOUISE LINDBLOM⁵

ABSTRACT. – The cyanomorph and photosymbiodemes are here reported for the first time for *Ricasolia virens* (With.) H.H. Blom & Tønsberg comb. nov. (≡ *Lobaria virens* (With.) J.R. Laundon). The cyanomorph of *R. virens* is dendriscocauloid. The observed early developmental stages involve (1) a free-living cyanomorph and (2) a photosymbiodeme composed of the cyanomorph supporting small, foliose, chloromorphic lobes. Whereas the chloromorph continues to grow, the cyanomorph decays and disappears leading to the final stage (3), the free-living chloromorph. Secondary cyanomorphs emerging from the chloromorph are not known.

KEYWORDS. – Peltigerales, cephalodia, ascospore-to-ascospore life cycle.

INTRODUCTION

Most species of lichen-forming fungi associate with a photobiont belonging to either the green algae or cyanobacteria (e.g., Brodo et al. 2001, Henssen & Jahns 1973, Nash 2008, Schwendener 1869). Within the family Lobariaceae, however, many species associate with both photobionts (e.g., Högnabba et al. 2009, James & Henssen 1976). Such ability to establish a physiological exchange with two types of photobionts may be expressed within a single thallus, in distinct thalli or portions thereof, or in distinct developmental stages. In tripartite lichens, three partners engage in the symbiotic association, and both photobionts are present, typically with the green algae composing the main partner, and the cyanobacteria encapsulated within specialized structures called cephalodia. Some fungal species may form, in addition to the tripartite lichen, a thallus comprising solely the cyanobacterium as photosynthetic partner (Högnabba et al. 2009, Honegger 2008, James & Henssen 1976), and this thallus may subsequently incorporate green algae and then bear green lobes (Tønsberg & Goward 2001). Alternative associations by a single fungal species wherein either one of the two photobionts is the primary autotroph are called photomorphs or morphotypes (i.e., the cyanomorph or the chloromorph). Photomorphs may be physically attached (e.g., in *Ricasolia amplissima* (Scop.) De Not.), forming a composite thallus that is referred to as a photosymbiodeme, or exhibit diverging ecological preferences (e.g., *Sticta filix* (Sw.) Nyl., James & Henssen 1976) and in some case distinct geographic distributions (e.g., in *Sticta canariensis* (Bory ex Delise, see Brodo 1994). Photomorphs may have the same growth form (e.g., in *Nephroma arcticum* (L.) Torss., where they are both foliose; see Tønsberg & Holtan-Hartwig 1983), or distinct growth forms, with the fungus forming a foliose tripartite lichen and a fruticose cyanomorph (James & Henssen 1976).

¹TOR TØNSBERG – Department of Natural History, University Museum, University of Bergen, Allégaten 41, P.O. Box 7800, N-5020 Bergen, Norway. – e-mail: tor.tonsberg@uib.no

²HANS H. BLOM – Norwegian Institute of Bioeconomy Research, Fanaflaten 4, N-5244 Fana, Norway. – e-mail: hans.blom@nibio.no

³BERNARD GOFFINET – Department of Ecology and Evolutionary Biology, 75 North Eagleville road, University of Connecticut, Storrs CT, 06269-3043 U.S.A. – e-mail: bernard.goffinet@uconn.edu

⁴JON HOLTAN-HARTWIG – Rådyrveien 10 E, N-1413 Tårnåsen, Norway. – email: jon.holtan-hartwig@ski.vgs.no

⁵LOUISE LINDBLOM – Department of Natural History, University Museum, University of Bergen, Thormøhlensgate 53A, P.O. Box 7800, N-5020 Bergen, Norway. – email: louise.lindblom@uib.no

Species with fruticose, dendriscocauloid, cyanomorphs forming photosymbiodemes occur exclusively in the Lobariaceae (Högnabba et al. 2009, James & Henssen 1976, Magain et al. 2012, Moncada et al. 2013). Photosymbiodemes are particularly frequent and conspicuous in *Ricasolia amplissima* (James & Henssen 1976, Krog et al. 1994, Rose & Purvis 2009, Stenroos et al. 2003, Tønsberg & Goward 2001, Wirth et al. 2013). *Lobaria virens* (With.) J.R. Laundon also forms foliose cephalodiate chlorolichens and may be closely related to *R. amplissima* (Högnabba et al. 2009). The two species are similar but *L. virens* is not known to develop cyanomorphs and photosymbiodemes (e.g., Krog et al. 1994, Rose & Purvis 2009, Wirth et al. 2013). Here we report and characterize such associations for the first time, based on specimens from Norway, and discuss their potential significance in the development of the free-living chloromorph of the species. Furthermore, since *L. virens* was resolved within the genus *Ricasolia* in recently published phylogenetic studies (Högnabba et al. 2009, Moncada et al. 2013), we extend our discussion on the development of the lichen thallus to the genus *Ricasolia*.

MATERIALS AND METHODS

FIELDWORK. – The material of *Lobaria virens* that provided the basis for this study was primarily collected by HB, JHH, LL and TT in various parts of Western and Central Norway. All specimens are deposited in the herbarium of the University of Bergen (BG). Unless otherwise stated, the datum for localities is WGS84. The recorded altitudes (above sea-level) for the cited specimens were obtained from topographic maps with contour intervals of 20 meters.

HERBARIUM STUDIES. – All specimens of *Lobaria virens* at BG were studied, and critically examined for the presence of cyanomorphs. Macroscopic descriptions of the cyanomorphs were based on composite thalli (i.e., thalli composed of a cyanomorph with an attached chloromorph) when possible. The extremely fragile and hence easily damaged cyanomorphs had to be subjected to destructive sampling for microscopic examination. To diminish or avoid damage to the composite thalli, samples were taken preferentially from free-living cyanomorphs adjacent or close to a composite thallus on the same small piece of bark. The description of the chloromorph was based on the recent collections and about 170 specimens held in BG. The cephalodia were studied on fresh collections of the chloromorphs. North American specimens identified as *Sticta herbacea* (Huds.) Ach. and filed under *L. virens* were obtained on loan from F.

CHEMISTRY. – Thin-layer chromatography (TLC) was carried out on cyanomorphs and chloromorphs according to Culberson & Kristinsson (1970) and later modifications. All three solvents (A, B' and C) were used and glass plates were used in solvent C to allow for the detection of fatty acids.

MOLECULAR METHODS. – To preliminarily test whether the cyanomorph and chloromorph were formed by the same lichen-forming ascomycete (i.e., *L. virens*), we compared the ITS sequences of seven mycobionts from separate photomorphs as well as of photosymbiodemes (see Table 1 in the Appendix). The DNA extraction, amplification, and sequencing followed Lindblom & Ekman (2005) and Lendemer & Goffinet (2015). Sequences were aligned using ClustalW in BioEdit ver. 7.2.3 (Hall 1999), and manually adjusted. To reflect the variation of ITS sequences within *Ricasolia* and the segregation of species, and hence the power of ITS in discriminating among species, a Maximum Likelihood (ML) analysis was conducted with Garli v. 2.0 (Zwickl 2006) and branch support estimated from 200 bootstrap pseudoreplicates. The sequences were partitioned into ITS1, 5.8S and ITS2 and substitution models estimated and selected using PartitionFinder (Lanfear et al. 2012) based on the AIC, with HKY+G applied to ITS 1 and ITS2 and K80+I to the 5.8S partition. No characters were excluded. The matrix of ITS sequences representing species of *Ricasolia*, including *R. virens*, and of the outgroup *Lobaria scrobiculata* (Scop.) DC., was deposited in TreeBase as study #19339.

RESULTS AND DISCUSSION

Phylogenetic inferences from variation in the mitochondrial SSU and the nuclear LSU have previously robustly resolved *Lobaria virens* as a member of *Ricasolia* (Högnabba et al. 2009, Moncada et al. 2013), yet the species has not been formally transferred to this genus. Hence we propose the new combination below. The species is endemic to Western Europe and Macaronesia and differs from the sym-

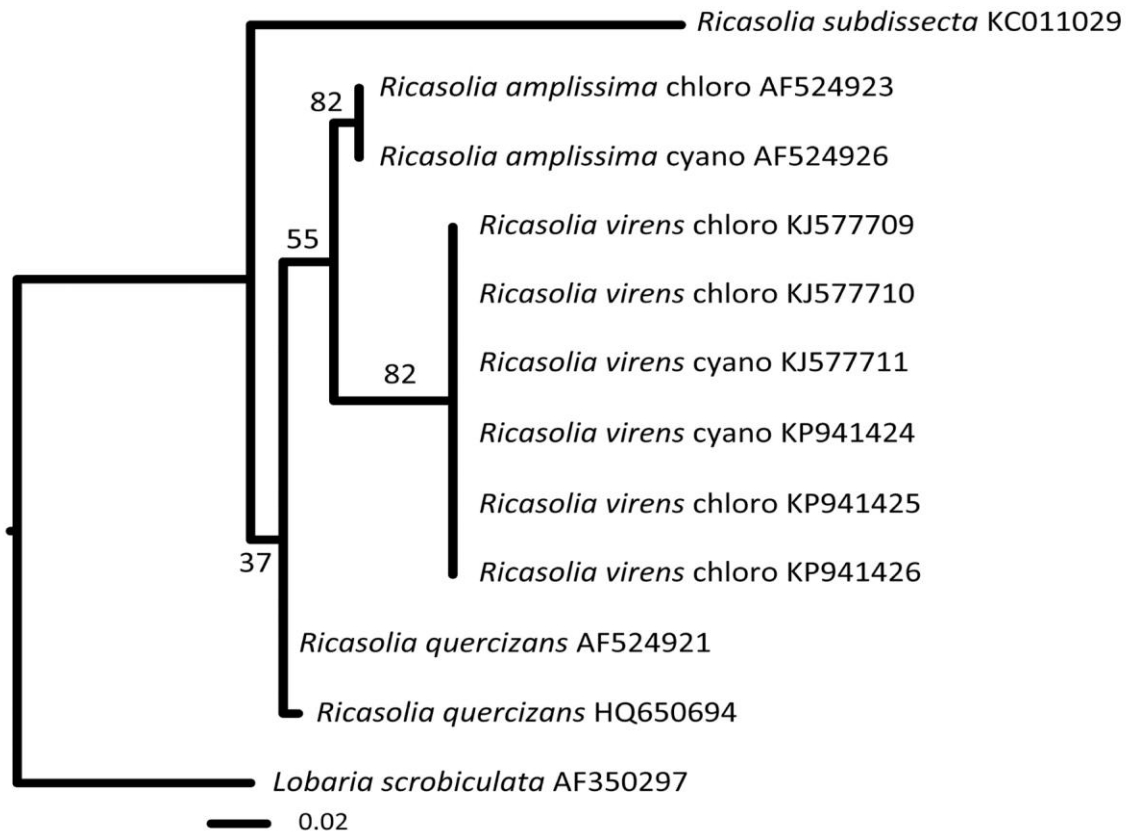


Figure 1. Most likely phylogenetic tree inferred from ITS sequences for accessions of *Ricasolia*. Values above branches refer to bootstrap support.

-patric *R. amplissima* by the thallus color, smaller thallus, lobes and spores and the lack of erumpent dendriscocauloid cephalodia (Purvis et al. 1994, Rose & Purvis 2009, Tønnsberg & Jørgensen 2007). Based on our data the fungal ITS sequences of the chloromorph of *R. virens* are distinct from those of *R. amplissima* and *R. quercizans* (Michx.) Stizenb. deposited in GenBank (compared 12 Nov. 2015; see Figure 1), and can serve to discriminate between the species, especially when seeking to identify the mycobiont of dendriscocauloid thalli, which may not differ morphologically between the species.

We discovered multiple associations of two photomorphs in the immediate vicinity of well-developed chloromorphs of *R. virens*. We obtained six complete and one partial (KJ577709) ITS sequences for photomorphs of *R. virens* (Table 1). Phylogenetic inferences based on variation within the ITS region have been widely used to assess species boundaries among lichen-forming fungi based on the criterion of monophyly, including in the Peltigerales (e.g., Miadlikowska et al. 2003, 2014; Moncada & Lücking 2012; Moncada et al. 2013; Sérusiaux et al. 2009, 2011). Assuming the absence of hybridization, the ITS sequence may then serve to diagnose the specific identity of the mycobiont, and test the hypothesis that thalli with either prokaryotic or eukaryotic photobionts may be alternative photomorphs generated by a single fungal species (e.g., Goffinet and Bayer 1997, Lendemer and Goffinet 2015). The fungal ITS sequences we generated from each of the cyanomorphs was identical to those of the attached or independent chloromorphs of *R. virens* (Figure 1), suggesting that the same fungal species is involved in all thalli and thus that *R. virens* may compose photosymbiodemes of cyano- and chloromorphs. Unlike the photosymbiodemes of *R. amplissima* the cyanomorph does not develop secondarily on the chloromorph but in fact precedes the latter in the development of the green thallus.

TAXONOMIC SECTION

Ricasolia virens (With.) H.H. Blom & Tønsberg comb. nov.

- MycoBank #815140.
- ≡ *Lichen virens* With., A botanical arrangement of all the vegetables naturally growing in Great-Britain, p. 710. 1776. ≡ *Lobaria virens* (With.) J.R. Laundon, Lichenologist 16: 227. 1984. **TYPE:** Dillenius, J.J. 1742 [“1741”] *Historia Muscorum*. Oxford: University (Sheldonian Theatre), tab. 25, fig. 98A (lectotype [reproduction by Laundon 1984 seen; original illustration in Dillenius 1742 not seen], selected by Laundon (1984: 227)). **EPITYPE:** Dillenius herbarium of *Historia Muscorum* 98A “middle specimen” (OXF [n.v.], selected by Tønsberg & Jørgensen (2007: 145)).
 - = *Lichen laetevirens* Lightf. *nom. illeg.*, Fl. Scot. 2: 852. 1777. ≡ *Parmelia laetevirens* (Lightf.) Schaer., Lich. Helv. Spec. p. 461. 1840. ≡ *Sticta laetevirens* (Lightf.) Rabenh., Deutschl. Krypt.-Fl. 2: 64. 1845. ≡ *Ricasolia laetevirens* (Lightf.) Leight., Lich.-Fl. Great Brit. p. 121. 1871. ≡ *Lobaria laetevirens* (Lightf.) Zahlbr. in Engler & Prantl, Nat. Pflanzenfam., 1: 188. 1906.
 - = *Lichen herbaceus* Huds., Fl. Angl., ed. 2, p. 525. 1778. ≡ *Pulmonaria herbacea* (Huds.) Hoffm., Descr. Adumb. Plant. Lich. 1(2): 51. 1789. ≡ *Parmelia herbacea* (Huds.) Ach., Methodus, p. 218. 1803. ≡ *Lobaria herbacea* (Huds.) DC., in Lamarck & de Candolle, Fl. Franç., ed. 3, 2: 403. 1805. ≡ *Platysma herbaceum* (Huds.) Frege, Deutsch. Botan. Taschenb. 2: 165. 1812. ≡ *Sticta herbacea* (Huds.) Ach., Syn. Meth. Lich. p. 341. 1814. ≡ *Peltidea herbacea* (Huds.) Link, Grundr. Krauterk. 3: 176. 1833. ≡ *Ricasolia herbacea* (Huds.) De Not., G. Bot. Ital., sér. 2, 1(1): 180. 1846.

DESCRIPTION. – **The cyanomorph.** Thallus dendriscoauloid (Figure 2), forming fragile, convex, loose to rather compact cushions to 12 mm wide and 5 mm tall. Main branches brownish, bluish or whitish gray, terete or flattened, to 0.40 mm wide, smooth, naked to finely tomentose; terminal branchlets bluish grey or brown, terete, sometimes slightly widening towards tips; branching pattern sometimes palmate. Branches usually naked or with a few hairs evident in microscope preparations; hairs usually simple, occasionally with short side branches, moniliform, 1–5 celled, to 24 µm long; individual cells usually globose, to 7(–12) µm wide, sometimes cylindrical. Cortex brown, 1–3 cell layers thick, to 24 µm thick; cells isodiametric and 7–12 µm in diameter or elongate and 6–11(–17) × 4–8(–9.6) µm; central cord of longitudinally running hyphae 3 µm wide. Photobiont layer of uneven thickness, 29–50(–85) µm; photobiont cyanobacterial, probably *Nostoc*, bluish, sometimes pale green, single celled, irregularly rounded to irregularly ellipsoid, 5–10 × 4–7(–10) µm. Apothecia and pycnidia not observed. **The photosymbiodeme.** Composed of a primary cyanomorph and a secondary chloromorph (Figure 2). Chloromorph one (Figure 2C) to several per cyanomorph (Figures 2A and B), to 12 mm in diameter, developing from branches of the cyanomorph, evident at first as small, brownish nodules then flattened, dorsiventral, at first usually rounded to reniform, lobule-like thalli fastened to the cyanomorph by their edges (Figures 2A and B), or rarely, with a short stalk (Figure 2A, see the small lobule in the bottom left corner); cyanomorph dying and vanishing as chloromorph grows. Secondary growth of cyanomorphs from chloromorphs not seen. Apothecia and pycnidia not observed. **The chloromorph.** For complete descriptions of the mature chloromorph, see, e.g., Rose & Purvis (2009) and Tønsberg & Jørgensen (2007). Well-developed chloromorphs with spherical internal cephalodia mostly in the lower part of the medulla, visible on the underside of the thallus as brownish (contrasting with the paler surrounding cortex), ± hemispherical swellings of the lower cortex. Lobules (called *folioles* by Rose & Purvis 2009) common (i.e., in more than half of the specimens studied), mostly along damaged thallus margins and laminal cracks, varying from narrow (finger-like) and to a few mm long to more or less rounded and to 5 mm or more in diameter; rounded lobules sometimes fastened by a narrow holdfast or a stalk and thus more or less similar to the juvenile chloromorph lobules seen in the photosymbiodemes. Apothecia and pycnidia are usually frequent.

CHEMISTRY. – No substances found. Spot tests (cortex and medulla): K-, C-, KC-, P-, UV-.

ECOLOGY AND DISTRIBUTION. – *Ricasolia virens* is mainly distributed in Western Europe and Macaronesia (Degelius 1935, Rose & Purvis 2009, Tønsberg & Jørgensen 2007). In Norway, *R. virens* occurs in a broad belt along the coast from the Oslofjord area in the southeast to Nordland county in the north (*vide* The Norwegian Lichen Database; <http://nhm2.uio.no/lav/web/index.html>). *Ricasolia virens*

cyanomorphs and photosymbiodemes are known from several localities in Hordaland county in the southwest and one in Nord-Trøndelag county in Central Norway. They may be readily seen *in situ* in young populations of chloromorphs. However, some localities with large populations of well-developed and fertile *R. virens* chloromorphs on cliffs and/or tree trunks, photosymbiodemes could not be located despite extensive searches. Cyanomorphs and photosymbiodemes have been found on naked or mossy trunks of *Fraxinus excelsior* (the most common phorophyte), *Populus tremula*, and *Tilia cordata*, and on boulders in a *Corylus avellana*-*Populus tremula* stand and a *Corylus avellana* thicket.

DISCUSSION. – We were able to match the morphology of the alga-containing components of the photosymbiodemes to the chloromorphs of *Ricasolia virens* (Figure 2). These data, combined with the 100% sequence identity of the mycobionts, strongly support the hypothesis that all cyanomorphs, chloromorphs and photosymbiodemes studied involve the same fungal species. Consequently all of these morphs should be referred to as *R. virens* since the name of a lichen refers to the mycobiont.

Ricasolia virens is primarily known from Europe and Macaronesia (Rose & Purvis 2009). It is not thought to occur in North America (Esslinger 2015), but several herbaria hold specimens, mostly collected in the 1800's, that were identified as *L. virens*, *L. laetevirens* or *Sticta herbacea* (Huds.) Ach. (records viewed through <http://lichenportal.org> on 17 March 2016). We examined four specimens held in F (C0300964F, C1011324F, C1011330F, and C1011336F) and these were all conspicuously C+ (*R. virens* would be C-) and hence belong to *R. quercizans*, which is endemic and widespread in eastern North America (Brodo et al. 2001). We assume that all other North American collections filed under *R. virens* are also misidentified and likely represent *R. quercizans*.

The cyanomorph of *Ricasolia virens* is only known from Norway, and hence exhibits a much narrower geographic distribution than the chloromorph. Whether this pattern reflects significant ecological constraints on the cyanomorph or is shaped by the distribution of a specific *Nostoc* is not yet known. In the sympatric *R. amplissima*, dendriscocauloid cyanomorphs emerging from chloromorphs appear to be common throughout the range of the species in Eurasia and Africa (e.g. Degelius 1935). Whereas the geographical ranges of *R. amplissima* and *R. virens* can be defined by the ranges of their chloromorphs, the reverse is true for other species forming photosymbiodemes. A well-known example is *Sticta canariensis*, which occurs in Western Europe and Macaronesia (James & Henssen 1976, James & Purvis 2009) and in Ontario, Canada (Brodo 1994). In the Old World the chloromorph predominates in the southern and the cyanomorph in the northern part of its range (James & Henssen 1976). In the northernmost part of this range (i.e., Norway) the chloromorph is rare and present only as small lobes on well-developed cyanomorphs (Tønberg 1990). According to James & Henssen (1976), *S. canariensis* chloromorphs do not occur outside the range of the cyanomorphs. In North America, the species is only associated with *Nostoc* (Brodo 1994), strengthening the pattern of a broader geographic distribution of the cyanomorph.

Ricasolia virens has probably at least two reproductive strategies, symbiotic (i.e., the simultaneous dispersal of the mycobiont and the photobiont via specialized lichenized thallus structures or fragments) and aposymbiotic (i.e., dispersal of the mycobiont by ascospores). The chloromorph often develops narrowly stalked lobules, which easily break off and hence could serve as diaspores. Such lobules may allow effective establishment on suitable substrates (i.e., rock, bark, moss), where they would grow to mature chloromorphs directly (i.e., mature chloromorph → chloromorph lobules → mature chloromorph). *Ricasolia virens* may potentially also propagate via fragments from the fragile cyanomorph. The presence of small cyanomorph fragments near the photosymbiodemes in some of the collections (see figure 4C) lends support to this hypothesis, but further study is needed to empirically test this.

At maturity *Ricasolia virens* forms a chloromorph harboring cyanobacterial colonies as internal cephalodia. The ontogeny of this tripartite association is not known. We hypothesize, given the observation of green lobules developing from the dendriscocauloid thallus, that when *R. virens* ascospores land on an appropriate substrate and germinate, they may first make contact with suitable, free-living cyanobacteria and form dendriscocauloid cyanomorphs. Free-living green algae are subsequently recruited or captured, leading to the development of chloromorphs. We have no indication that *R. virens* is capable of obtaining algae or cyanobacteria from other lichens. The juvenile life cycle stages of *R. virens* would or could thus be: Germinating ascospore (free-living) → cyanomorph → cyanomorph + chloromorph (photosymbiodeme) → chloromorph. *Nostoc* could be integrated *de novo* in the earliest stage of the chloromorph or acquired from the cyanomorph via the attachment stalk. The former is certainly possible, considering that the symbiotic lobules of the chloromorph acting as vegetative diaspores appear to lack cyanobacteria.



Figure 2. *Ricasolia virens* (all from Tønnsberg 40924, BG). A-C, dendriscoauloid cyanomorphs without or with chloromorph lobules (photosymbiodemes). Scale bars = 2 mm. Photos by E. Timdal 2013.

Whether the juvenile cyanolichen stage following ascospore dispersal is obligate in *Ricasolia virens* is not clear. Our observations may suggest that cyanomorphs are an integral part of the life cycle of the lichen association. However, large populations of *R. virens* chloromorphs have been studied in the field without any observations of cyanomorphs, which may suggest that germinating ascospores can associate with the green photobiont and form chloromorphs without a cyanomorph stage (i.e., chloromorph → germinating ascospore → chloromorph). Alternatively, the lack of observations of a cyanomorph stage in these populations could well be explained by the cyanomorph being ephemeral and thus rarely observed.

Fungi of the Peltigerales may be lichenized with either a cyanobacterium only or primarily with a green alga with subordinate associations with *Nostoc*. Neither association identifies only a single

homogenous clade, and transitions between these appear numerous during the diversification of the Peltigerales (see Moncada et al. 2013). The polarity of the shifts remains ambiguous. Miadlikowska & Lutzoni (2004) proposed that the association with *Nostoc* is ancestral in the Peltigerales and Högnabba et al. (2009) further argued that it was the ancestral type in the Lobariaceae. The latter study, however, suggested that *Ricasolia* species are primarily associated with a green alga, an interpretation that may change once the occurrence of a cyanoprothallus is integrated in the character scoring and chlorolichens are considered cephalodiate and hence tripartite.

Juvenile, lichenized stages with *Nostoc* following ascospore dispersal and germination have been observed in several species of the Peltigerineae (Holtan-Hartwig unpubl., Ott 1988, Stocker-Wörgötter & Türk 1994, Yoshimura et al. 1993). Stocker-Wörgötter & Türk (1994) were able to resynthesise *Peltigera leucophlebia* (Nyl.) Gyeln. from its three symbionts under controlled laboratory conditions. They obtained primordia arising from a cyanobacterial crust, with the primordia comprising the mycobiont and a green photobiont, a cyanobacterial photobiont, or both photobionts, but only the primordia with green photobiont developed into *P. leucophlebia*-like thalli. Yoshimura et al. (1993) were able to “reform” a cyanobacterial morphotype of *Peltigera aphthosa* (L.) Willd. by culturing the lichen in vitro from undifferentiated cell aggregates. The lobes of the juvenile cyanobacterial morphotype was sublinear and had a cortex also on the lower side and were thus anatomically and morphologically different from the lobes of the cyanomorph of *P. aphthosa* as they are in the nature. Species of *Peltigera* do not have dendriscocauloid developmental stages, but the observations by Stocker-Wörgötter & Türk (1994) and Yoshimura et al. (1993) may be consistent with a hypothesis that the association with cyanobacteria is not simply a secondary event in the life cycle of a tripartite lichen, but rather may be a critical primary ontogenetic stage in their development.

Photosymbiodemes including a foliose photomorph and dendriscocauloid cyanomorph are known also from other species of *Ricasolia*, namely *R. amplissima* (e.g., James & Henssen 1976, Tønsberg & Goward 2001, Tønsberg & Holtan-Hartwig 1983, Tønsberg & Jørgensen 2007; all as *Lobaria amplissima*), *R. ravenelii* (Tuck.) Nyl. (as *Lobaria* cf. *erosa* (Eschw.) Nyl. in Jordan 1972; as *Lobaria ravenelii* in Brodo et al. 2001) and *R. quercizans* (Parker & Goffinet unpubl.). *Ricasolia amplissima* shows the same juvenile development as described above for *R. virens* (see Tønsberg & Goward 2001, Tønsberg & Holtan-Hartwig 1983). However, unlike *R. virens*, *R. amplissima* often develops cyanomorphs laminally on the chloromorph, and composite specimens with more than two cyanomorph/chloromorph ‘generations’ are occasionally seen. We have indeed observed free-living cyanomorphs bearing the chloromorph themselves producing the cyanomorph, as well as free living chloromorphs with attached cyanomorphs bearing the chloromorph.

In conclusion, *Ricasolia virens* is widely distributed in Europe and in Macaronesia, and cyanomorphs are currently known only from Norway. If a juvenile cyanolichen stage is obligate, it may have been overlooked, as it was until recently in Norway, especially if it is ephemeral, and vanishes as the chloromorph develops. *Ricasolia virens* cyanomorphs and photosymbiodemes were indeed lacking among herbarium specimens in BG and likely elsewhere, as collectors generally seek well-developed, fertile thalli, which may lack cyanomorphs. Discovering the dendriscocauloid juvenile stage throughout the distribution range would provide, in the absence of experimental observations, evidence for the obligatory nature of the cyanolichen in the life cycle of *R. virens*. The observation of an association with *Nostoc* in a dendriscocauloid thallus preceding the development of the chloromorph in *R. virens*, combined with similar observations in *R. amplissima* and the occurrence of photosymbiodemes in other species of *Ricasolia* may lead to the hypothesis that at least for *Ricasolia* the ancestral lichenization state is one with *Nostoc*, and that species with tripartite thalli arose from such an ancestor, while maintaining the ability to establish independent cyanomorphs, which may be required when lichenization is initiated (i.e., prothallus) and provide an alternative strategy for a perennial free living lichen (e.g., typical *Dendriscocaulon*).

Specimens of photosymbiodemes with cyanomorphs examined (all BG). – NORWAY:
HORDALAND: AUSTEVOLL: island Huftarøy, the E-facing slope N of Bjelland, 60°04.69'N 5°15.65'E (ED50), alt. 0–30 m, corticolous on trunk of *Tilia cordata*, 18.ix.1985, *T. Tønsberg* 9380 (BG-L-97740).
BØMLO: island Selsøy, Kastevik, 59.8959582°N 5.099804°E (EUREF 89), alt. 15 m, on boulder in *Corylus avellana* thicket, 30.vii.2006, *H.H. Blom s.n.* (BG-L-97745).
LINDÅS: the SW-facing slope W of Storset, 60°38.439'N 5°27.116'E (Datum ED50), alt. 60–90 m, corticolous on the shaded side of trunk of *Fraxinus excelsior*, 3.iv.1984, *T. Tønsberg* 8595 & *J. Holtan-Hartwig* (BG-L-97741).
OS: Stormvågen, 60°10'N 5°24'E (ED50), alt. 5 m, corticolous on trunk of *Fraxinus excelsior*, 23.iv.1989, *T. Tønsberg* 11522 & *J. Holtan-Hartwig* (BG-L-53525).
OSTERØY: Havrå, S-facing slope, downhill from road,

60°26.214'N 5°33.907'E (ED50), alt. ca. 50 m, on old, pollarded trunk of *Fraxinus excelsior* in young deciduous forest, 13.vii.1992, A. Botnen s.n. (BG-L-14801); Havrå, downhill from road, 60°26.267'N 5°34.642'E (WGS84), alt. 20–40 m, corticolous on S-facing side of mossy trunk of *Fraxinus excelsior* in S-facing slope, 28.iv.2011, T. Tønsberg 40924 (BG-L-97742). **NORD-TRØNDELAG: FLATANGER:** Årfjordbotn, the E-facing slope W of cove Survika, 64°27.422'N 10°49.380'E, alt. 10–30 m, corticolous on mossy trunk of *Populus tremula*, 19.viii.2002, T. Tønsberg 31538 (BG-L-97743).

Specimens of chloromorphs examined for comparison. – **GEORGIA [U.R.S.S.]:** Transcaucasus: Colchis, distr. Sochi, ad corticem Aceris, 8.vi.1978, A. Vězda s.n. (BG-L-64224). **NORWAY: HORDALAND: BØMLO:** island Bømlo, E side of Grutle fjorden, S of farm Hope, Rakahopet, 59.669°N 5.169°E, alt. 0–5 m, 28.iv.2015, T. Tønsberg 44757 (BG-L-97955, BM, UPS, NY); island Spysøy, W-facing slope ca 160 m SSE (direct) from S tip of the small island Bleikja, 59°43.418'N 5°22.249'E, alt. 10–15 m, on schists on upper part of steep, seaside rock wall, 3.iv.2015, T. Tønsberg 44732 (BG-L-97760). **GRANVIN:** Nesheimlien, ad truncus vetustos Tiliae parvifoliae, mense Maio 1936, J.J. Havaas, *Lich. Norv. Occ. Exs. 128* (BG-L-59632). **OS:** Lysekloster monastery, just outside the W side of the ruin, 60°13.655'N 005°24.299'E, alt. 40–60 m, corticolous on trunk of huge *Ulmus glabra*, 10.ii.2015, T. Tønsberg 44718 (BG-L-97738).

Specimen of Ricasolia amplissima examined for comparison. – **NORWAY. HORDALAND. OS:** Lysekloster monastery, just outside the W side of the ruin, 60°13.655'N 005°24.299'E, alt. 40–60 m, corticolous on trunk of huge *Ulmus glabra*, 10.ii.2015, T. Tønsberg 44719 (BG-L-97739).

ACKNOWLEDGEMENTS

We thank Einar Tindal for taking the photos, Beate Helle, for technical help with figures, Mats Wedin, for letting us use one of his ITS sequences, Per M. Jørgensen and Pier Luigi Nimis, for nomenclatural discussions, and the herbarium of the Field Museum of Natural History (F) for loan of specimens. Constructive comments by anonymous reviewers and James Lendemer contributed to improving and clarifying the manuscript. Tønsberg acknowledges financial support for field work from the Grolle Olsen fund, University of Bergen. Support for Goffinet was provided by US NSF grant DEB-1354631. We thank Rafael Medina (UCONN) for running the phylogenetic analysis.

LITERATURE CITED

- Brodo, I.M. 1994. *Sticta canariensis*: a new lichen for North America. *Evansia* 11: 76–77.
- Brodo, I.M., S.D. Sharnoff and S. Sharnoff. 2001. *Lichens of North America*. Yale University Press, New Haven & London.
- Culberson, C.F. and H. Kristinsson. 1970. A standardized method for the identification of lichen products. *Journal of Chromatography* 46: 85–93.
- Degelius, G. 1935. Das ozeanische Element der Strauch- und Laubflechtenflora von Skandinavien. *Acta Phytogeographica Suecica* 7: I–XII + 1–411.
- Esslinger, T.L. 2015. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. North Dakota State University: <http://www.ndsu.edu/pubweb/~esslinge/chcklst/chcklst7.htm> (First Posted 1 December 1997, Most Recent Version (#20) 19 April 2015), Fargo, North Dakota.
- Goffinet, B. and R.J. Bayer. 1997. Characterization of mycobionts of photomorph pairs in the Peltigerineae (lichenized Ascomycetes) based on ITS sequences of specifically amplified fungal ribosomal DNA. *Fungal Genetics and Biology* 21: 228–237.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Henssen, A. and H.M. Jahns. 1973 [1974]. *Lichenes*. Georg Thieme Verlag, Stuttgart.
- Högnabba, F., S. Stenroos and A. Thell. 2009. Phylogenetic relationship and evolution of photobiont associations in the Lobariaceae (Peltigerales, Lecanoromycetes, Ascomycota). *Bibliotheca Lichenologica* 100: 157–187.
- Honegger, R. 2008. Morphogenesis. In: T.H. Nash III (ed.): *Lichen biology*, pp. 69–93. Cambridge University Press.
- James, P.W. and A. Henssen. 1976. The morphological and taxonomic significance of cephalodia. In: D. H. Brown, D. L. Hawksworth & R. H. Bailey (eds.): *Lichenology: Progress and Problems*, p. 27–77. Academic Press, London.
- James, P.W. and O.W. Purvis. 2009. *Sticta* (Schreb.) Ach. (1803). In: Smith, C. W., Aptroot, A., Coppins, B. J., Fletcher, A., Gilbert, O. L., James, P. W. & Wolseley, P. A. (eds.): *The Lichens of Great Britain and Ireland*, pp. 865–867. British Lichen Society, London.

- Jordan, W.P. 1972. Erumpent cephalodia, an apparent case of phycobial influence on lichen morphology. *Journal of Phycology* 8: 112–117.
- Krog, H., H. Østhagen and T. Tønsberg. 1994. *Lavflora. Norske busk – og bladlav*. Second edition. Universitetsforlaget. Oslo.
- Lanfear, R., B. Calcott, S.Y.W. Ho and S. Guindon. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–701.
- Lendemer, J.C. and B. Goffinet. 2015. *Sticta deyana*: a new endemic photomorphic lichen from the Mid-Atlantic Coastal Plain of eastern North America. *Systematic Botany* 40: 933–941.
- Lindblom, L. and S. Ekman. 2005. Molecular evidence supports the distinction between *Xanthoria parietina* and *X. aureola* (Teloschistaceae, lichenized Ascomycota). *Mycological Research* 109: 187–199.
- Magain, N., B. Goffinet and E. Sérusiaux. 2012. Further photomorphs in the lichen family Lobariaceae from Reunion (Mascarene archipelago) with notes on the phylogeny of *Dendriscoaulon* cyanomorphs. *Bryologist* 115: 243–254.
- Miadlikowska, J. and F. Lutzoni. 2004. Phylogenetic classification of Peltigerales fungi (Peltigerales, Ascomycota) based on ribosomal small and large subunits. *American Journal of Botany* 91: 449–464.
- Miadlikowska, J., F. Lutzoni, T. Goward, S. Zoller and D. Posada. 2003. New approach to an old problem: Incorporating signal from gap-rich regions of ITS and rDNA large subunit into phylogenetic analyses to resolve the *Peltigera canina* species complex. *Mycologia* 95: 1181–1203.
- Miadlikowska, J., D. Richardson, N. Magain, B. Ball, F. Anderson, R. Cameron, J. Lendemer, C. Truong and F. Lutzoni. 2014. Phylogenetic placement, species delimitation, and cyanobiont identity of endangered aquatic *Peltigera* species (lichen-forming Ascomycota, Lecanoromycetes). *American Journal of Botany* 101: 1141–1156.
- Moncada B. and R. Lücking. 2012. Ten new species of *Sticta* and counting: Colombia as a hot spot for unrecognized diversification in a conspicuous macrolichen genus. *Phytotaxa* 74: 1–29.
- Moncada, B., R. Lücking and L. Betancourt-Macuase. 2013. Phylogeny of the Lobariaceae (lichenized Ascomycota: Peltigerales), with a reappraisal of the genus *Lobariella*. *Lichenologist* 45: 203–263.
- Nash III, T.H. 2008. Introduction. In: T.H. Nash III (ed.): *Lichen biology*, pp. 1–8. Cambridge University Press.
- Ott, S. 1988. Photosymbiodemes and their development in *Peltigera venosa*. *Lichenologist* 20: 361–368.
- Purvis, O.W., C.W. Smith, and P.W. James. 1994. Studies in the lichens of the Azores. Part 2-Lichens of the upper slopes of Pico mountain. A comparison between the lichen floras of the Azores, Madeira and the Canary Islands at high altitudes. *ARQUIPÉLAGO. Life and Marine Sciences* 12A: 35–50.
- Rose, F. and O.W. Purvis. 2009. *Lobaria* (Schreb.) Hoffm. (1796). In: C.W. Smith, A. Aptroot, B.J. Coppins, A. Fletcher, O.L. Gilbert, P.W. James and P.A. Wolseley (eds.): *The lichens of Great Britain and Ireland*, pp. 560–562. British Lichen Society, London.
- Schwendener, S. 1869. *Die Algentypen der Flechtengonidien*. Schultze, Basel.
- Sérusiaux, E., B. Goffinet, J. Miadlikowska and O. Vitikainen. 2009. The lichen-forming fungal genus *Peltigera* in Papua New Guinea: inferences from morphology and DNA sequences. *Fungal Diversity* 38: 185–224.
- Sérusiaux, E., J.C. Villarreal, T. Wheeler and B. Goffinet. 2011. Recent origin, active speciation and dispersal for the lichen genus *Nephroma* (Peltigerales) in Macaronesia. *Journal of Biogeography* 38: 1138–1151.
- Stenroos, S., E. Stocker-Wörgötter, I. Yoshimura, L. Myllys, A. Thell and J. Hyvönen. 2003. Culture experiments and DNA sequence data confirm the identity of *Lobaria* photomorphs. *Canadian Journal of Botany* 81: 232–247.
- Stocker-Wörgötter, E. and R. Türk. 1994. Artificial resynthesis of the photosymbiodeme *Peltigera leucophlebia* under laboratory conditions. *Cryptogamic Botany* 4: 300–308.
- Tønsberg, T. 1990. The green algal phototype of *Sticta canariensis* found in Norway. *Graphis Scripta* 3: 27.
- Tønsberg, T. and T. Goward. 2001. *Sticta oroborealis* sp. nov., and other Pacific North American lichens forming dendriscoauloid cyanotypes. *Bryologist* 104: 12–23.
- Tønsberg, T. and J. Holtan-Hartwig. 1983. Phycotype pairs in *Nephroma*, *Peltigera* and *Lobaria* in Norway. *Nordic Journal of Botany* 3: 681–688.
- Tønsberg, T. and P.M., Jørgensen. 2007. *Lobaria*. *Nordic Lichen Flora* 3: 77–82.
- Wirth, V., M. Hauck and M. Schultz. 2013. *Die Flechten Deutschlands. Band 2*. Eugen Ulmer KG, Stuttgart.
- Yoshimura, I., T. Kurokawa, Y. Yamamoto, Y. Kinoshita. 1993. Development of lichen thalli in vitro. *Bryologist* 96: 412–421.
- Zwickl, D.J. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Doctoral Dissertation. The University of Texas at Austin.

APPENDIX – VOUCHERS AND GENBANK DATA FOR SEQUENCES USED IN THIS STUDY

Table 1. Species, morph (phenotypic expression), and voucher information (i.e., country, collector and herbarium) sampled for the sequencing of the ITS region; GenBank accession numbers for newly generated sequences are in bold.

Species	Morph	Country	Voucher Specimen	GenBank Accession No.
<i>Lobaria scrobiculata</i>	?	?	<i>M.A. Thomas 1239</i> (OTA)	AF350297
<i>Ricasolia virens</i>	chloro-	Norway	<i>M. Wedin 6192</i> (BM)	KJ577709
<i>R. virens</i>	chloro-	Norway	<i>H.H. Blom VI</i> (BG)	KJ577710
<i>R. virens</i>	cyano-	Norway	-	KJ577711
<i>R. virens</i>	photosymb.: cyano-	Norway	<i>T. Tønsberg 40924</i> (BG)	KP941424
<i>R. virens</i>	photosymb.: chloro-	Norway	<i>T. Tønsberg 40924</i> (BG)	KP941425
<i>R. virens</i>	photosymb.: chloro-	Norway	<i>T. Tønsberg 31538</i> (BG)	KP941426
<i>R. virens</i>	chloro-	Norway	<i>T. Tønsberg 44757</i> (BG)	KR632514
<i>R. amplissima</i>	chloro-	Norway	<i>E. Stocker-Wörgötter 1717</i> (TUR)	AF524923
<i>R. amplissima</i>	cyano-	Norway	<i>H. Holien s.n.</i> (TUR)	AF524926
<i>R. quercizans</i>	?	Canada	<i>T. Ahti 57089</i> (H)	AF524921
<i>R. quercizans</i>	?	?	<i>collector unknown</i> (DUKE) [AFTOL-ID 369]	HQ650694
<i>R. subdissecta</i>	?	Colombia	<i>B. Moncada 3152</i> (UDBC)	KC011029