Genetic diversity in cyanobacterial symbionts of thalloid bryophytes

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Abstract

Two species of thalloid liverworts, Blasia pusilla and Cavicularia densa, form stable symbioses with nitrogen-fixing cyanobacteria. Both bryophytes promote the persistence of their cyanobacterial associations by producing specialized gemmae, which facilitate the simultaneous dispersal of the host and its nitrogen-fixing symbionts. Here the genetic diversity of cyanobacterial symbionts of Blasia and Cavicularia is examined. The results indicate that the primary symbionts of both bryophytes are closely related and belong to a specific group of symbiotic Nostoc strains. Related strains have previously been reported from hornworts and cycads, and from many terricolous cyanolichens. The evolutionary origins of all these symbioses may trace back to pre-Permian times. While the laboratory strain Nostoc punctiforme PCC 73102 has been widely used in experimental studies of bryophyte–Nostoc associations, sequence-identical cyanobionts have not yet been identified from thalloid liverworts in the field.

Key words: Blasia pusilla, bryophyte, Cavicularia densa, Nostoc, tRNALeu(UAA) intron, specificity, symbiosis.

Introduction

The thalloid gametophytes of two liverwort species, Blasia pusilla L. and Cavicularia densa Steph. (Blasiales, Marchantiophyta), and all hornworts (Anthocerotophyta) form endosymbiotic associations with nitrogen-fixing cyanobacteria of the genus Nostoc (Fig. 1A). The cyanobacterial symbionts are housed in specialized auricles (Blasiales) and slime cavities (Anthocerotophyta) on the ventral surfaces of the thalloid bryophytes (Fig. 1B, D).

During bryophyte growth the symbiosis is continuously re-established as young bryophyte structures are individually infected by motile Nostoc hormogonia (Adams, 2002).

Cavicularia densa is a rare plant endemic to Japan. It resembles the more widely distributed B. pusilla in having a thickened middle region gradually tapering to delicate monostromatic wings (Fig. 1B). Both bryophyte species undergo similar apical development, have two rows of scales along the ventral mid-line of the thallus, and produce structurally complex, multicellular gemmae (Renzaglia, 1982).

Cavicularia densa produces gemmae in crescent-shaped receptacles, which form near the tips of mature thallus lobes (Fig. 1C). Two different types of gemmae are produced: small, pale gemmae and larger, stellate gemmae. The small gemmae are infected by symbiotic cyanobacteria after dispersal, during development into thallus primordia (Figs 1C, 2A). Conversely, the stellate gemmae essentially represent miniature thalli, equipped with two symbiotic auricles. The auricles are usually infected by symbiotic Nostoc while the gemma still remains attached to the receptacle of the parent gametophyte (Figs 1C, 2B, C).

The receptacles of C. densa are often situated very close to senescing auricles (Fig. 1E). It is possible that Nostoc hormogonia from such auricles can reach the receptacles directly through thin gametophyte tissue. The semi-closed receptacles themselves are lined with slime papillae and provide an ideal microenvironment for Nostoc infection. In the closely related B. pusilla, slime papillae are thought to release compounds that stimulate the formation of and/or attract Nostoc hormogonia (Campbell and Meeks, 1989; Adams, 2002).

Conserved elements in the tRNA^Leu(UAA) intron have been widely used to identify cyanobacteria at the genus...
level, while more variable regions (stem–loops P6b, P9a, and parts of P5) have been used to identify specific *Nostoc* strains. Previous studies of cyanolichens in Europe and North America (Paulsrud and Lindblad, 1998; Paulsrud et al., 1998, 2000, 2001; Oksanen et al., 2002; Rikkinen et al., 2002; Linke et al., 2003; O’Brien et al. 2005; Piercey-Normore et al., 2007), East Asia (Rikkinen et al., 2002; unpublished results), South-America (Stenroos et al., 2006), Australasia (Summerfield et al., 2002), and Antarctica (Wirtz et al., 2003) have shown that lichen-symbiotic *Nostoc* strains are a rich source of different tRNA\(^{\text{Leu}}\)(UAA) intron sequences. Additional diversity has been found from symbioses of hornworts and liverworts (Costa et al., 1999, 2004), and from non-symbiotic cyanobacteria (Xu et al., 1990; Rudi and Jakobsen, 1997, 1999; Wright et al., 2001; Fewer, 2003). All these investigations have contributed to the accumulation of an extensive data set on the distribution of different tRNA\(^{\text{Leu}}\)(UAA) intron genotypes in different cyanobacteria, geographical regions, and ecological settings (Rikkinen, 2004).

The stable secondary and tertiary structure of the tRNA\(^{\text{Leu}}\)(UAA) intron limits possibilities for random mutations in the intron sequence. The low number of informative sites, in turn, does not provide much variation for hierarchical analysis, and hence phylogenetic trees based on tRNA\(^{\text{Leu}}\)(UAA) intron sequences tend to be poorly resolved. In symbiotic *Nostoc* strains, the evolutionary history of the core sequence of the tRNA\(^{\text{Leu}}\)(UAA) intron has been found to be congruent with that of the 16S tRNA gene. However, phylogenies based on core sequences alone usually fail to reveal exact relationships among closely related symbiotic strains (Oksanen et al., 2004a, b).

The tRNA\(^{\text{Leu}}\)(UAA) intron sequences of symbiotic *Nostoc* strains tend to have relatively few substitutions except in the P6b stem–loop where both sequence and length variation is considerable. This stem–loop is built from degenerate 7mer nucleotide repeats which fold into a hairpin structure allowing the repeats to base-pair (Costa et al., 2002). Size differences between stem–loops are caused primarily by different numbers of copies of repeats and, in some cases, by insertion of additional sequences not following the repeat motif. Degenerate heptanucleotide repeats in the P6b stem–loops of all *Nostoc* tRNA\(^{\text{Leu}}\)(UAA) intron sequences can be grouped into two classes. Class 1 variable regions exhibit the consensus repeat sequence TDNGATT and its base pairing consensus sequence, while class 2 variable regions have the consensus heptanucleotide repeat sequence NNTGAGT and its base pairing consensus sequence. Differences in the P6b element of *Nostoc* tRNA\(^{\text{Leu}}\)(UAA) intron sequences can be used for distinguishing between closely related genotypes among ecologically and/or geographically delimited groups of symbiotic *Nostoc*. However, the region should not be used for phylogenetic purposes (Oksanen et al., 2004b).
Here the diversity of cyanobacteria associated with *Blasia* and *Cavicularia* is examined by using cyanobacterial tRNA\(^{Leu}(UAA)\) intron sequences as a molecular marker. Previous studies on liverwort symbioses have revealed a wide diversity of associated cyanobacteria, sometimes even within a single thallus (West and Adams, 1997; Costa *et al.*, 2001).

**Materials and methods**

Specimens of *C. densa* Steph. were collected from a deciduous broad-leaved forest in SW Honshu, Japan (Chugoku District, Tottori-ken, Daisen-cho, Kawadoko, elevation ~700 m). The bryophytes grew over a wet boulder and clayey soil on a stream bank. Under a dissecting microscope, stellate gemmae from two crescent-shaped receptacles near the tip of one mature thallus lobe were collected for culture experiments. Also some auricles from parent gametophytes and clayey soil from beneath the bryophytes were sampled. Specimens of *B. pusilla* L. were collected from a coniferous forest in central Finland (Kalmari, Saarijärvi, elevation ~150 m). The bryophytes grew on clayey soil along road banks. Stellate gemmae were picked from 12 *Blasia* thalli, many of which grew on one 20×20 cm sample plot (Fig. 3). Bryophyte growth on this sample plot was followed over a time span of 50 d. In total, 5–6 stellate gemmae were collected from 2–3 different thallus lobes.

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**Fig. 3.** Thallus growth and distribution of cyanobacterial tRNA\(^{Leu}(UAA)\) intron genotypes in symbiotic gemmae of *Blasia pusilla* on a sample plot in central Finland. Growth and proliferation of *Blasia* thalli on the sample plot (20×20 cm) are shown in different shades of grey (0 d = darkest shade, 15 d and 25 d = medium shades, 50 d = palest shade). The distribution of different cyanobacterial genotypes obtained from the stellate gemmae of different *Blasia* thalli (A–G) is shown below the sample plot. The gemmae that produced *Nostoc* colonies in culture are shown as black crosses (n=66), while gemmae that produced other types of filamentous cyanobacteria (cf. *Anabaena* sp.) are shown as black dots (n=3). The gemmae that failed to produce cyanobacterial colonies are shown as small white dots (n=8). The gemmae from which a single cyanobacterial tRNA\(^{Leu}(UAA)\) intron sequence was obtained are encircled. The other gemmae produced mixed cultures of several intron genotypes, and were not analysed further. The single gemma that produced a *Nostoc* intron genotype (Se 2) with a class 1 repetitive motif in its P6b region is boxed.
of seven different Blasia thalli. Also the soil substrate on this and similar sample plots was sampled.

In the laboratory, living cyanobacteria from liverwort gemmae and soil samples were obtained by culturing single gemmae, auricles, and minute amounts of soil on solid Zs-agarose media (Kotai, 1972) without nitrogen at room temperature. Cyanobacterial growth was examined under the microscope, and growing cyanobacterial colonies were harvested after several months.

Nested PCR was used to amplify the cyanobacterial tRNA^{Leu(UAA)} (UAA) intron directly from cultured cyanobacterial colonies. The intron was amplified with two sets of primers, i.e. the cyanobacterial-specific outer primers A and C (Paulsrud and Lindblad, 1998), and inner primers TL25 and TL23 (Biniszkwicz et al., 1994). PCR reactions were performed using Ready-To-Go™ RT-PCR Beads (Amersham Biosciences) by adding 2 µL of template and 22 µL of H₂O with outer primers, and 1 µL of template (the PCR product of outer primers) and 23 µL of H₂O with inner primers, and each primer at 1 µM. The amplification consisted of 35 cycles of denaturation (30 s with the inner primers/20 s with the outer primers at 94 °C), annealing (1 min at 60 °C), and extension (1 min at 72 °C). PCR products of inner primers were purified with the QIAquick PCR Purification Kit (QIAGEN). Between 100 ng and 200 ng of the double-stranded, amplified DNA was directly sequenced with the primers TL25 and TL23 using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequence reactions were analysed on an ABI Prism 377 automated sequencer (Applied Biosystems). The obtained sequences were deposited in GenBank (for accession numbers, see Table 1).

The obtained sequences were aligned manually on the basis of sequence similarity and a secondary structure model of the transcribed intron. The model was predicted following the conventions for cyanobacterial tRNA^{Leu(UAA)} introns. Individual stem–loop structural elements were individually folded online using the mfold version 3.1 web server (http://www.bioinfo.rpi.edu/applications/mfold/old/rna/, Zuker, 2003).

Results

A total of 100 B. pusilla gemmae and 10 C. densa gemmae were cultured. Within 3 months, 95 Blasia gemmae and 10 Cavicularia gemmae had given rise to cyanobacterial colonies. The dominant cyanobacteria in all colonies were filamentous, heterocystous, and showed no evidence of branching (Fig. 1A). They formed slowly spreading, gelatinous colonies and exhibited typical phases in the life cycle of Nostoc (minute motile hormogonia, young non-motile filaments with single heterocysts, mature non-motile filaments with multiple heterocysts). Many cultures from soil and auricles from mature bryophytes contained more than one morphologically distinct filamentous cyanobacterium. Such mixed cultures were not analysed further.

After DNA extraction, a single tRNA^{Leu(UAA)} intron sequence was amplified from 90 cyanobacterial cultures. A total of 78 sequences were obtained from cultures of Finnish B. pusilla specimens and 12 from cultures of Japanese C. densa specimens. Most of these sequences were amplified from cultures obtained from single bryophyte gemmae. Forty-nine sequences came from

**Table 1. Diversity of cyanobacterial tRNA^{Leu(UAA)} intron genotypes in bryophyte-associated cyanobacteria**

The table summarizes all intron sequences so far amplified from thalloid bryophytes (n=165). Type: Pr strains refer to Nostoc genotypes with a class 2 repeat motif in the P6b region of the intron; Se strains refer to Nostoc genotypes with a class 1 repeat motif in the P6b region; An strains refer to non-Nostoc cyanobacteria (cf. Anabaena sp.) with other repeat motifs in the P6b region. Host: Afu, Anthoceros fusiformis; Asp, Anthoceros sp.; Bla, Blasia pusilla; Cav, Cavicularia densa.

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**Blasia** gemmae collected from the most intensely studied sample plot in central Finland (Fig. 3). In addition, sequencing revealed that 20 cultures, some obtained from single bryophyte gemmae, represented mixed cultures of more than one cyanobacterial genotype. These samples were not analysed further. As each bryophyte gemma had two symbiotic auricles, some mixed cultures probably came from gemmae that had a different cyanobacterial strain in each auricle. Additional diversity may have been caused by cyanobacterial contaminants on the outer surfaces of the gemmae.
After alignment, the 90 new tRNA\textsuperscript{Leu(UAA)} intron sequences were found to represent 22 intron genotypes (Table 1). Almost all sequences were highly similar, with nucleotide differences largely restricted to the P6b stem–loop of the intron (Table 2). A search in GenBank, using the new sequences obtained, identified sequences previously amplified from various symbiotic Nostoc strains as the most similar sequences in the database.

A majority of the bryophyte-associated Nostoc genotypes had a class 2 repeat motif in the P6b region of the tRNA\textsuperscript{Leu(UAA)} intron (Pr strains in Tables 1 and 2). Only a few of the bryophyte-associated Nostoc genotypes had a class 1 repeat motif in the P6b region (Se strains in Tables 1 and 2). In addition, three non-Nostoc tRNA\textsuperscript{Leu(UAA)} intron sequences were obtained (An strains in Tables 1 and 2). A search in GenBank identified sequences from Anabaena species as the most similar sequences in the database.

Table 1 summarizes all presently available information on cyanobacterial tRNA\textsuperscript{Leu(UAA)} intron sequences from thalloid bryophytes collected from natural habitats (n=165). Table 2 shows variation in repeat motifs, indel patterns, and individual bases in the P6b regions of the different intron genotypes.

Discussion

Many thalloid bryophytes with symbiotic Nostoc are rapidly proliferating pioneer plants. For example, in central Finland, B. pusilla mainly grows on moist loam and clay banks, where it commonly suffers a mass destruction of much of the gametophyte population during winter. Over-wintering gametophytes, even in temperate climates, tend to show a lot of die-back, their thalli becoming semi-decayed, with only the shoot apices bearing immature sporophytes surviving (Schuster, 1992). In early spring there is rapid recolonization via spores and two types of gemmae. Stellate, symbiotic gemmae are produced from epidermal cells, and ovoid, non-symbiotic gemmae are produced in long-necked flask-shaped receptacles (Renzaglia, 1982; Bartholomew, 1986; Duckett and Renzaglia, 1993). During summer, the ability of B. pusilla to colonize local temporary habitats effectively hinges on its ability to produce large numbers of symbiotic gemmae (Fig. 3).

Duckett and Renzaglia (1993) found that the two kinds of gemmae produced by B. pusilla differ in their food reserves and viability. In growth experiments, the stellate gemmae, containing starch, were relatively short-lived, while the ellipsoidal gemmae, containing protein and lipids, retained their viability for long periods. In stellate gemmae starch-packed plastids are sufficient to secure the initial development of symbiotic propagules already capable of biological nitrogen fixation. Conversely, the cells of non-symbiotic, ellipsoidal gemmae of Blasia are packed with protein and lipids as their major storage products. These reserves allow ellipsoidal gemmae to retain their viability for extended periods.

On the basis of results from culture experiments, Duckett and Renzaglia (1993) concluded that while the stellate gemmae of B. pusilla are the principal propagules of vegetative reproduction during the growing season, only the ellipsoidal gemmae would over-winter. However, the relatively poor performance of stellate gemmae observed may also have been partly due to experimental conditions. The gemmae were incubated for extended periods in a dark refrigerator at 4 °C (Duckett and Renzaglia, 1993). Under such conditions, the gemmae were metabolically active, but not able to photosynthesize.

In nature, B. pusilla grows freely under relatively low temperatures. For example, in the Arctic, it often flourishes on soils along rills from snow fields, close to permanent ice. In many such populations, which are known from as far north as West Greenland, only sterile plants with stellate gemmae occur (Schuster, 1992). Also in the boreal zones many populations of Blasia are strictly seasonal and propagate predominantly via stellate gemmae. It is possible that the winter ecology of Blasia differs over its wide distribution area. The specimens studied by Duckett and Renzaglia (1993) were collected from temperate regions (the British Isles and North Carolina).

Previous studies have documented a high diversity of cyanobacteria from thalloid bryophytes, sometimes even from a single thallus (West and Adams, 1997; Costa et al., 2001). Despite the fact that more than one Nostoc genotype is often found within individual bryophyte thalli, some symbiotic strains are dominant, very widespread, and shared both by bryophytes from different sites and by those collected from a single site during different years. For example, two Nostoc genotypes (Pr 1 and Pr 9) that were common in B. pusilla gemmae in central Finland had previously been repeatedly amplified from mature auricles of the same host bryophyte in the same area (Costa et al., 2001). Furthermore, the most dominant Nostoc genotype from C. densa in Japan (Pr 10) had previously been found from B. pusilla in central Finland. Another genotype (Pr 14) from central Finland has been previously reported from the same bryophyte in Germany (Table 1). These examples clearly indicate that there is a considerable level of spatial and temporal continuity in liverwort–Nostoc symbioses.

The stellate gemmae of B. pusilla and C. densa promote the persistence of specific cyanobacterial associations over the dispersal phase. During subsequent thallus development, new auricles are continuously formed and infected by cyanobacteria. At this stage, many infecting cyanobacteria may represent suboptimal symbionts of even non-symbiotic cyanobacteria from the soil substrate. Usually this does not have serious consequences, as only a limited
Table 2. Variation in repeat motifs, indel patterns, and individual bases in the P6b region of tRNA\textsuperscript{Leu(UAA)} intron sequences from bryophyte-associated cyanobacteria (Costa et al., 2002)

Most bryophyte-associated 
*Nostoc* genotypes (Pr strains, presumed primary cyanobionts) had a class 2 repeat motif in the P6b region. Some bryophyte-associated 
*Nostoc* genotypes (Se strains, presumed secondary cyanobionts) had a class 1 repeat motif in the P6b region. A few cyanobacterial genotypes (cf. *Anabaena* sp., An strains, presumably non-symbiotic contaminants) from bryophyte samples had other motifs in the P6b region. Deviating bases are underlined.

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*TAGCCGTTAGCAGGGCGTTTAGCCC (24 nt inserted sequence in the third repeat of Pr21).*

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<td>Se 6</td>
<td>CGAAAAATT</td>
<td>TTAGATT</td>
<td>TGCGATT</td>
<td>TTAGATT</td>
</tr>
</tbody>
</table>

*GTTCGACTGACGCAAGCCGAAGTGC (24 nt inserted sequence in the seventh repeat of Se6).*

<table>
<thead>
<tr>
<th>5'</th>
<th>Repeats</th>
<th>Loop</th>
<th>Repeats</th>
<th>3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>An 1</td>
<td>GAAGTAGT</td>
<td>AATTAGTAAGTT</td>
<td>ACAAACAGA</td>
<td>TAACCTACAGCTAATC</td>
</tr>
<tr>
<td>An 2</td>
<td>GGAAAAATT</td>
<td>TCGACT</td>
<td>TAAGATT</td>
<td>TAATTTT</td>
</tr>
<tr>
<td>An 3</td>
<td>GGAAAAATT</td>
<td>TCGACT</td>
<td>TAAGATT</td>
<td>TAATTTT</td>
</tr>
</tbody>
</table>
number of auricles infected by symbiotically competent
*Nostoc* strains are actually needed to support vigorous
bryophyte growth. Hence, the cyanobacterial diversity
often seen in bryophyte thalli may reflect more the
difficulties in avoiding accidental infections on cyanobac-
terial-rich soil substrates, rather than the lack of symbiont
preferences in the bryophyte hosts.

The present findings indicate that the primary *Nostoc*
symbionts of thalloid bryophytes are closely related.
Similar strains have been found to live in the coralloid
roots of some cycads, in the creeping rhizomes of the
angiosperm *Gunnera*, and especially in many terricolous
cyanolichens, such as different *Peltigera* species. For
example, genotype Pr 19, which now was found from
*B. pusilla* in central Finland, has been previously reported
from *Peltigera occidentalis* in western North America.
Genotype Pr 24 has been previously reported from *Peltigera
degenii* (Canada), *P. camina* (Germany), *P. neopolydactyla*
(Oregon), *P. praetextata* (Finland), and *P. membranacea*
(Sweden, Canada, and Oregon). The same *Nostoc* genotype
has also been cultured from epiphytic mosses in Finland.
Also the *Nostoc* cyanobionts of *Peltigera didactyla*, *P.
lepidophora*, *P. pruinosa*, and *P. rufescens*, among others,
are very similar to those typically found in *B. pusilla* and
*C. densa*. All of these lichens grow on mineral soil, often
together with thalloid bryophytes.

In the laboratory, successful reconstitution of bryophyte
syymbioses has been achieved not only with the original
symbiont, but also with different *Nostoc* strains isolated
from other plants and lichens (Adams, 2002). The com-
plete genome of one symbiotic *Nostoc* strain, *Nostoc
punctiforme* [Pasteur Culture Collection (PCC) 73102,
synonym American Type Culture Collection (ATCC)
29133] has been sequenced (Meeks et al., 2001). While
this *Nostoc* strain was originally isolated from the roots
of a cycad (*Macrozamia* sp.) in Australia, it can also
establish symbioses with thalloid bryophytes in the
laboratory. Such associations have been used as model
systems to define many physiological properties of
symbiotic *Nostoc*. However, the present results indicate
that *Nostoc* PCC73102 is not a primary cyanobiont of
thalloid bryophytes in the field.

*Nostoc punctiforme* PCC73102 and the other Se strains
differ from the majority of bryophyte-associated *Nostoc*
by having a class 1 repetitive motif in the P6b region of
the tRNA\(^{Leu(UAA)}\) intron. Previous studies have shown
that the distribution of the two repeat classes does not
always follow phylogenies based on the core regions of
the intron or the 16S rRNA gene (Oksanen et al., 2004).
Evidence from these conserved elements clearly indicates
that *N. punctiforme* PCC73102 and similar strains are
more closely related to the Pr strains listed in Table 1 than
to many other *Nostoc* genotypes that have a class 1
repetitive motif in their P6b region. For example, all
lichen-symbiotic *Nostoc* strains of the well-delimited
*Nephroma* guild have a class 1 repetitive motif in their
P6b region, but they are not very closely related to the
bryophyte-associated genotypes discussed here (Rikkinen
et al., 2002, 2004; Lohtander et al., 2003; Oksanen et al.,
2004a, b; Rikkinen, 2004; O’Brien et al., 2005; Svenning
et al., 2005; Stenroos et al., 2006).

The evolutionary origin of the two complementary
repeat classes is unknown, but obviously both motifs have
been conserved and maintained. Probably they can both
fulfil the same functional role, for example, by increasing
the stability of the RNA stems during the processing of
the transcribed intron. The consequences of slipped-strand
mispairing of the two strands of the DNA double helix
provides a coherent explanation for the length variation in
both repetitive motifs (Costa et al., 2002). Slipped-strand
mispairing involves local denaturation and displacement
of the strands of a DNA duplex followed by mispairing of
complementary bases at the site of an existing short
tandem repeat. The following replication or repair may
lead to insertions or deletions of one or several repeat
units. As the regions expand, they may become predis-
posed to interhelical events, such as illegitimate recombi-
nation (Levin and Gutman, 1987).

The three non-*Nostoc* tRNA\(^{Leu(UAA)}\) intron sequences
(An 1–An 3, Tables 1, 2) were most probably amplified
from cyanobacterial contaminants. While most *Nostoc*
colonies cultured from bryophyte gemmae were morpho-
logically uniform, a very wide diversity of non-*Nostoc*
cyanobacteria grew out of soil substrate samples. Some
non-*Nostoc* filaments were also occasionally observed
among *Nostoc* colonies in cyanobacterial cultures from
*B. pusilla* gemmae. It is impossible to say whether these
contaminants originated from the surface of gemmae or
from symbiotic auricles. The tRNA\(^{Leu(UAA)}\) intron
sequence of genotype An 1 is identical to that of
*Anabaena* sp. PCC 7120. Interestingly, the same sequence
has also been reported from *Oscillatoria* sp. PCC 6506
(Oscillatoriales). The intron sequences of strains An 2 and
An 3 are almost identical to sequences from *Anabaena
cylindrica* and *A. azollae*. The taxonomic affiliations of
*Anabaena* sp. PCC 7120 and *A. azollae* remain unclear,
but neither of them belong to *Anabaena* s.str. nor *Nostoc
s.str.* (Gugger et al., 2002; Baker et al., 2003).

*Blastia pusilla* and *C. densa* (*Blastidiae*) occupy a basal
position among the complex thalloid liverworts (He-
Nyrgrén et al., 2006; Renzaglia et al., 2007). Recent
analyses have strongly supported liverworts as the sister
group to all other land plants, and hornworts seem to be
the sister group to vascular plants (Qiu et al., 2006).
Together with the cycads, these thalloid bryophytes
represent a rather conspicuous proportion of all extant
plant lineages that can be reliably traced back to pre-
Permian times (Oostendorp, 1987; Taylor and Taylor,
1994; Kugita et al., 2003; Duff et al., 2007). Also many
cyanolichens are believed to be of ancient origin (Rikkinen,
2002, 2003). Thus it may not be a mere coincidence that all these host plants and fungi associate with a specific group of closely related Nostoc symbionts (Rikkinen, 2002).

Acknowledgements

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References


