Mycorrhiza (2010) 20:307–313
DOI 10.1007/s00572-009-0283-9

ORIGINAL PAPER

Sebacinales are associates of the leafy liverwort *Lophozia excisa* in the southern maritime Antarctic

Kevin K. Newsham · Paul D. Bridge

Received: 14 October 2009 / Accepted: 27 October 2009 / Published online: 17 November 2009
© Springer-Verlag 2009

Abstract The leafy liverwort *Lophozia excisa*, which is colonised by basidiomycete fungi in other biomes and which evidence suggests may be colonised by mycorrhizal fungi in Antarctica, was sampled from Léonie Island in the southern maritime Antarctic (67°36′ S, 68°21′ W). Microscopic examination of plants indicated that fungal hyphae colonised 78% of the rhizoids of the liverwort, apparently by entering the tips of rhizoids prior to growing into their bases, where they formed hyphal coils. Extensive colonisation of stem medullary cells by hyphae was also observed. DNA was extracted from surface-sterilised liverwort tissues and sequenced following nested PCR, using the primer set ITS1F/TW14, followed by a second round of amplification using the ITSSeb3/TW13 primer set. Neighbour-joining analyses showed that the sequences obtained nested in Sebacinales clade B as a 100% supported sister group to Sebacinales sequences from the leafy liverworts *Lophozia sudetica*, *L. incisa* and *Calypogeia muelleriana* sampled from Europe. Direct PCR using the fungal specific primer set ITS1F/ITS4 similarly identified fungi belonging to Sebacinales clade B as the principal colonists of *L. excisa* tissues. These observations indicate the presence of a second mycothallus in Antarctica and support the previous suggestion that the Sebacinales has a wide geographical distribution.

Keywords Antarctica · Jungermanniales · Mycorrhizas · Mycothalli · Sebacinales clade B

K. K. Newsham (✉) · P. D. Bridge
British Antarctic Survey, Natural Environment Research Council,
High Cross, Madingley Road,
Cambridge CB3 0ET, UK
e-mail: kne@bas.ac.uk

Introduction

Liverworts in the order Jungermanniales are frequently colonised by ascomycete or basidiomycete fungi in temperate and tropical habitats. Species in the Cephaloziaceae and Jungermanniaceae develop swollen rhizoids that are colonised by ascomycete fungi, whereas those in the Lophoziaceae, Arnelliaceae and Scapaniaceae are typically colonised by basidiomycete fungi (Read et al. 2000). Whilst bearing some similarities to mycorrhizal symbioses, such associations are usually termed 'mycothalli' to denote potentially mutualistic associations between fungi and lower plants (Boullard 1988). Although the ascomycete and basidiomycete partners of the liverworts in the above families have been identified by the presence in hyphal septa of Woronin bodies and dolipores, more recently, PCR-based molecular methods have also been applied to the study of these fungi, showing the ascomycetes typically to be *Rhizoscyphus ericae* (Pressel et al. 2008) and the basidiomycetes to be members of the Sebacinales (Kottke et al. 2003).

Mycothalli are also known to be present in Antarctica. In the first study to use molecular methods to test for the presence of these associations in the Antarctic, Chambers et al. (1999) sequenced the internal transcribed spacer (ITS) region of a fungal isolate from the leafy liverwort *Cephaloziella varians* sampled from a single location in continental Antarctica and found that the isolate was conspecific with *R. ericae*. The consistent presence of *R. ericae* in *C. varians* was later confirmed by wider sampling in the maritime and sub-Antarctic (Upson et al. 2007). However, nothing is yet known of mycothalli in other species of Antarctic hepatics or of the possible presence of basidiomycete fungi in these liverworts. Nevertheless, a monograph of the Antarctic liverwort flora notes that plants of *Lophozia excisa*, a leafy liverwort that co-occurs with

C. varians in the maritime and sub-Antarctic, are 'strongly mycorrhizal' but does not describe fungal structures or identify the fungi inhabiting the plant (Bednarek-Ochyra et al. 2000). We hence report a study that molecularly identifies the fungal associates of *L. excisa* and describes the structures formed by fungi in the liverwort at one of the southernmost locations from which the hepatic is known.

Materials and methods

Two samples of healthy *L. excisa* plants were collected from a mixed bryophyte community, including *C. varians*, on the northwestern shore of Léonie Island (67°36′ S, 68° 21′ W), located in Ryder Bay, southwestern Antarctic Peninsula (Fig. 1) on 9 December 2007. The southernmost record for *L. excisa* is from Mushroom Island (68°54′ S) off the Fallières Coast, 145 km south of Léonie Island (Bednarek-Ochyra et al. 2000). The climate of Léonie Island is classified as southern maritime Antarctic (Smith 1984), with a mean temperature of vegetation on the island during the summer of about 5°C but with daytime summer temperatures of vegetation often exceeding 20°C (Huiskes et al. 2001), owing to solar radiation reflected from the Turner glacier, about 2 km to the west of the island. Air temperatures at noon on Léonie Island vary between −30°C in winter and 30°C in summer, with photosynthetic photon flux densities often reaching 2,000 µmol photons $m^{-2}s^{-1}$ during November and December (Montiel et al. 1999). The majority of precipitation at the island falls as snow, with most available water coming from melting snow and ice during the summer.

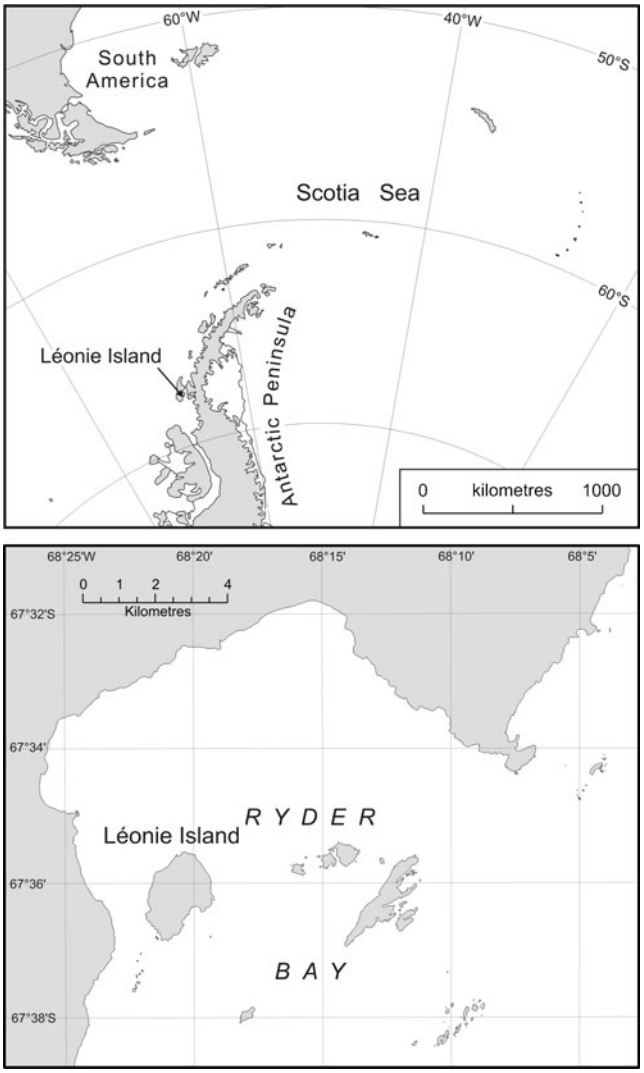

Fig. 1 Maps showing the location of Léonie Island in Ryder Bay, western Antarctic Peninsula

After collection, the plants were transferred to sterile-capped tubes and frozen at −20°C. After transfer at this temperature to the UK, the plants were defrosted at 4°C overnight, and ten were removed from each tube. They were washed in water and mounted on glass slides and images of fungal colonisation recorded using Cell^P image capture software (Olympus Soft Imaging Solutions, Olympus UK Ltd, Southend-on-Sea, UK), with a Z-sectioning facility enabling the acquisition of images at different focal positions, using differential interference contrast microscopy at ×40 and ×400 magnifications (BX50 microscope, Olympus UK Ltd). A further ten plants from each sample were washed in water and cleared in 10% KOH for 24 h at room temperature. These plants were then rinsed thoroughly with water, acidified in 5% lactic acid for 1 h, and stained with 0.01% aniline blue in lactic acid for 24 h. They were then removed from the staining solution, excess stain drawn off on absorbent paper, and were destained for at least 24 h in 80% lactic acid. The plants were then mounted in 80% lactic acid on glass slides prior to observation under UV epifluorescence, again at ×400 magnification (BX51 microscope, Olympus UK Ltd).

In order to remove adhering organic matter and microbes, the remainder of each defrosted sample (about 1 g fresh weight) was placed in a 30-ml capacity sterile Falcon tube, and the plants were washed 30 times in 10 ml of chilled (4°C) sterile water on a vortexer set to maximum speed (50 rev. s^{-1}). Between washes, each of which lasted for 5 min, the plants were drained on sterile 1 mm^2 mesh plastic nets under a sterile hood, when other bryophyte species were removed with sterile forceps. After the final wash, the plants were examined in a lidded sterile Petri dish under a dissecting microscope to ensure that the only bryophyte species remaining was *L. excisa*.

Plants were transferred to two 1.5-ml sterile Eppendorf tubes, the bases of which were immersed in liquid nitrogen. They were then ground for about 1 min with a sterile

microcentrifuge tube pestle. Elution buffer and RNase from a DNeasy Plant Mini Kit (Qiagen, Crawley, UK) were added immediately to the ground tissues and the DNA extracted, following the manufacturer's instructions, under a sterile hood. The DNAs from each sample were then purified using GFX-DNA purification kits (Qiagen), and PCR amplifications were carried out in 20 μl volumes, with concentrations of 200 μM of each deoxyribonucleotide triphosphate, 1.5 mM of $MgCl_2$, 0.5 U of Taq polymerase, and 0.5 μM of each of the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′; Gardes and Bruns 1993)/ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; White et al. 1990) and ITS1F/TW14 (5′-GCTATCCTGAGGGAAACTTC-3′; Cullings 1994). The former primer set amplifies the ITS1-5.8S-ITS2 region and the latter the ITS1-5.8S-ITS2 and part of the 28S region of fungal rDNA. The PCR amplification programme for both primer sets consisted of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min and elongation at 72°C for 3 min, followed by a final elongation step at 68°C for 10 min (PTC-225 Peltier thermal cycler, MJ Research Inc., Watertown, MA, USA). Negative controls consisted of 1 μl of sterile water in place of template DNA. The amplicons from the ITS1F/TW14 reaction were then subjected to a second round of amplification, using the primer set ITS3Seb (5′-TGAGTGTCATTGTAATCTCAC-3′/TW13, 5′-GGTCCGTGTTTCAAGACG-3′), which selectively amplifies the 5.8S-ITS2 and part of the 28S region of rDNA of fungi in the Sebacinales (Selosse et al. 2007). The PCR amplification programme for this primer set consisted of denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and elongation at 72°C for 30 s, followed by a final elongation step at 72°C for 10 min.

Sub-samples of the PCR products from all reactions were visualised on 1.5% agarose gels (agarose multi-purpose, Bioline, London, UK) containing GelRed nucleic acid stain (Biotum, Hayward, CA, USA) and were re-dissolved in 30 μl sterile water prior to ligation for 3 days at 4°C into the pGEM-T Easy Vector (Promega, Madison, WI, USA). The ligation products were transformed into XL2-Blue MRF' ultracompetent cells (Stratagene, La Jolla, CA, USA), picked by hand, amplified using the primers M13R short (5′-AACAGCTATGACCATGAT-3′)/M13F (-20) short (5′-GTAAAACGACGGCCAG-3′), and sent to a commercial sequencing facility for purification and sequencing with M13 primers. A total of 96 products, 24 derived from the amplification using the ITS3Seb/TW13 primer set (with 12 from each of the two samples) and 72 from that using ITS1F/ITS4 (with 36 from each sample), were sequenced.

Sequences were aligned in ClustalW (Thompson et al. 1994) and vector sequences removed. All sequences were initially grouped according to gross similarity by aligning them in ClustalW and generating a single average distance tree based on percentage identities. Groups of sequences and any ungrouped sequences were then subjected to individual BLAST searches (Altschul et al. 1997) against the full GenBank nucleotide database. Sequences of <400 base pairs were excluded from the analyses. Further FASTA searches (Pearson 1990) were made against the EMBL fungal sequence database (http://www.ebi.ac.uk). The phylogenetic position of the sequences obtained by direct amplification with the ITS3Seb/TW13 primer set was further elucidated by alignment with 42 sequences representing the main sub-groups of Sebacinales clades A and B shown by Selosse et al. (2007). A phylogeny was constructed in MEGA4 (Tamura et al. 2007) by maximum composite likelihood to produce a consensus neighbour-joining tree. Gaps were treated as pairwise deletions, and the tree was rooted with *Auricularia auricula-judae* (DQ520099). Representative sequences have been deposited in GenBank under accession numbers FN555433–FN555435.

Results

Microscopic examination of *L. excisa* plants revealed extensive fungal colonisation of rhizoids and medullary cells of the stem. Of the 153 rhizoids that were examined, 120 (78%) were colonised by hyphae. The hyphae observed in the rhizoids were septate and hyaline and uniform in width (mean, 1.3 μm; range, 1.0–1.5 μm). Several hyphae were usually observed in the same rhizoidal cell (Fig. 2a). They apparently entered rhizoids through their tips (Fig. 2b) and rarely branched within the shaft of the cell. Clamp connections were not observed. Hyphal coils (Fig. 2c, d) were observed in the bases of all rhizoid cells that had hyphae in their shafts. Septate hyphae were also present within stem medullary cells (Fig. 2e). These hyphae, which stained deeply with aniline blue, formed continuous colonisation units throughout the stem (Fig. 2f) but were absent from epidermal and leaf cells. Occasional colonisation of epidermal cells was observed by broader (mean, 4.0 μm; range, 3.5–4.5 μm) dark septate hyphae (not shown). Necrosis of plant cells was not observed. No apparent differences were observed in the patterns of fungal colonisation between the two samples of plants.

Negative controls in PCR amplifications did not produce amplicons. PCR amplification from *L. excisa* with the ITS3Seb/TW13 primer set produced 14 amplicons with almost identical sequences. The 50 highest matches (at 87–95% similarity) for all 14 sequences were those deposited as Sebacinales. The highest matches for each sequence were at 93% similarity to a Sebacinales sequence from the leafy liverwort *Calypogeia muelleriana* from Northern

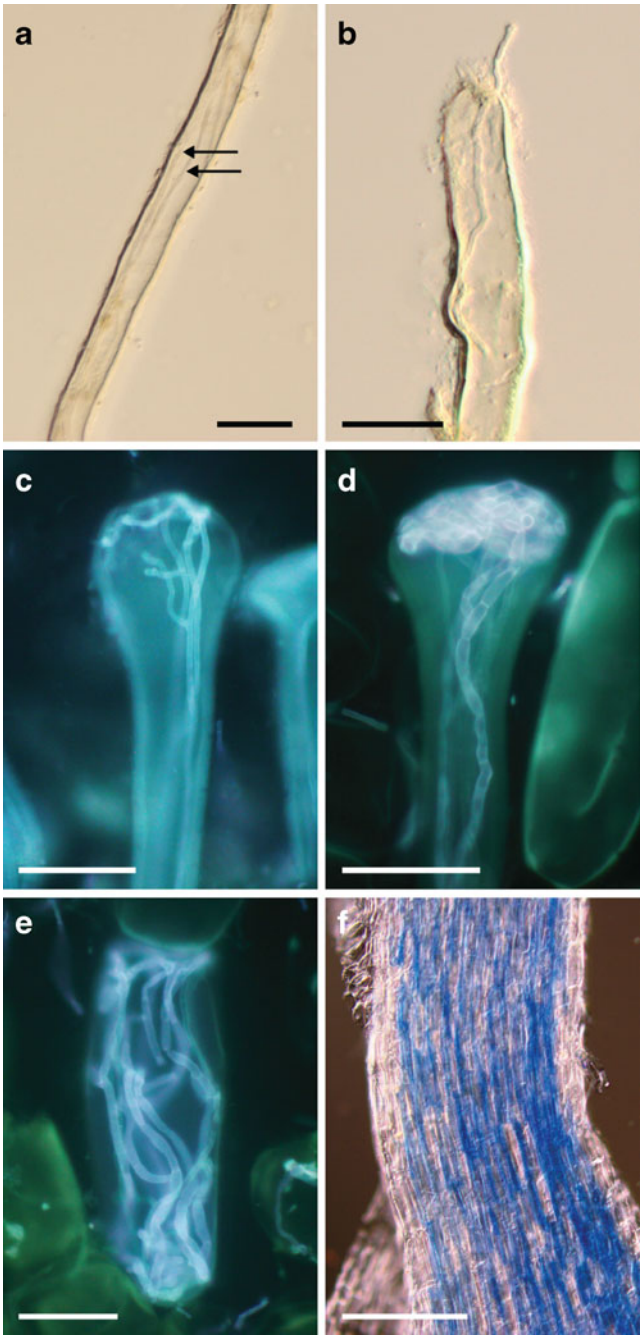

Fig. 2 Differential interference contrast (**a**, **b**, **f**) and UV epifluorescent (**c**–**e**) images of fungi in *Lophozia excisa* sampled from Léonie Island. **a** Hyphae (*arrows*) in rhizoid, **b** hypha growing through the tip of a rhizoid, **c** and **d** hyphal coils in bases of rhizoids, **e** hyphae in medullary cell and **f** stem with continuous colonisation units of fungal hyphae in medullary cells. Scale bars in **a**–**e** are 15 μm and that in **f** is 200 μm

Europe (AY298948) and at 95% similarity to a Sebacinales sequence from the ericaceous plant *Cavendishia bracteata* from Ecuador (DQ352048). The sequences from *L. excisa* sampled from Léonie Island were recovered in Sebacinales clade B as a 100% supported sister group to the Sebacinales sequence from Northern European *C. muelleriana* and two other sequences from *Lophozia sudetica* and *L. incisa* from the Pyrenees and Swedish Lapland, respectively (AY298946 and AY298947; Fig. 3).

PCR amplification with the ITS1F/ITS4 primer set produced 46 amplicons with sequences that gave initial best matches at 90–93% similarity to several unidentified soil and root-associated fungi and then at 87–90% similarity to named Sebacinales clade B sequences. A further six amplicons were obtained with the ITS1F/ITS4 primer set that gave BLAST scores of 94–99% similarity to sequences labelled as species of *Cladophialophora*. There were no differences between the sequences obtained from the two samples of plants.

Discussion

The patterns of fungal colonisation in *L. excisa* sampled from Léonie Island were the same as those observed in other members of the Lophoziaceae from lower latitudes. Hyphae apparently entered rhizoid cells at their tips, grew along the lengths of rhizoids and formed coils of hyphae in the bases of the cells, as shown for *Lophozia bicrenata*, *L. incisa*, *L. sudetica* and *L. ventricosa* from Northern Europe (Boullard 1988; Duckett and Read 1991; Kottke et al. 2003; Duckett et al. 2006). The fungi in *L. excisa* from Léonie Island also formed hyphal masses in the medullary cells of the stem, as described for each of the above species and for Northern European *Lophozia longidens*, *L. opacifolia* and *L. perssonii* (Duckett and Read 1991; Read et al. 2000; Kottke et al. 2003; Duckett et al. 2006).

The colonisation of members of the Lophoziaceae by fungi is attributable, on the basis of septal morphology and an absence of clamp connections on hyphae, to members of the heterobasidiomycetes (Read et al. 2000; Duckett et al. 2006). However, by sequencing rRNA genes amplified directly from *L. incisa* and *L. sudetica* tissues, Kottke et al. (2003) were able to place these fungi taxonomically with greater precision, showing them to group within Sebacinales clade B (sensu Weiß et al. 2004). This approach similarly identified the same fungi in the tissues of *L. excisa* sampled from Léonie Island. Although six fungal sequences were detected that were 94–99% similar to *Cladophialophora*, which was presumably the dark septate fungus observed in the epidermal cells of *L. excisa* (cf. Davey and Currah 2007), the majority of amplicons had sequences that were similar to members of Sebacinales clade B, a clade containing fungi also present in the mycorrhizas of orchidaceous and ericaceous plant species (Allen et al. 2003; Weiß et al. 2004; Setaro et al. 2006; Selosse et al. 2007). Members of Sebacinales clade B are thus associated not only with plant species in Europe (Kottke et al. 2003;

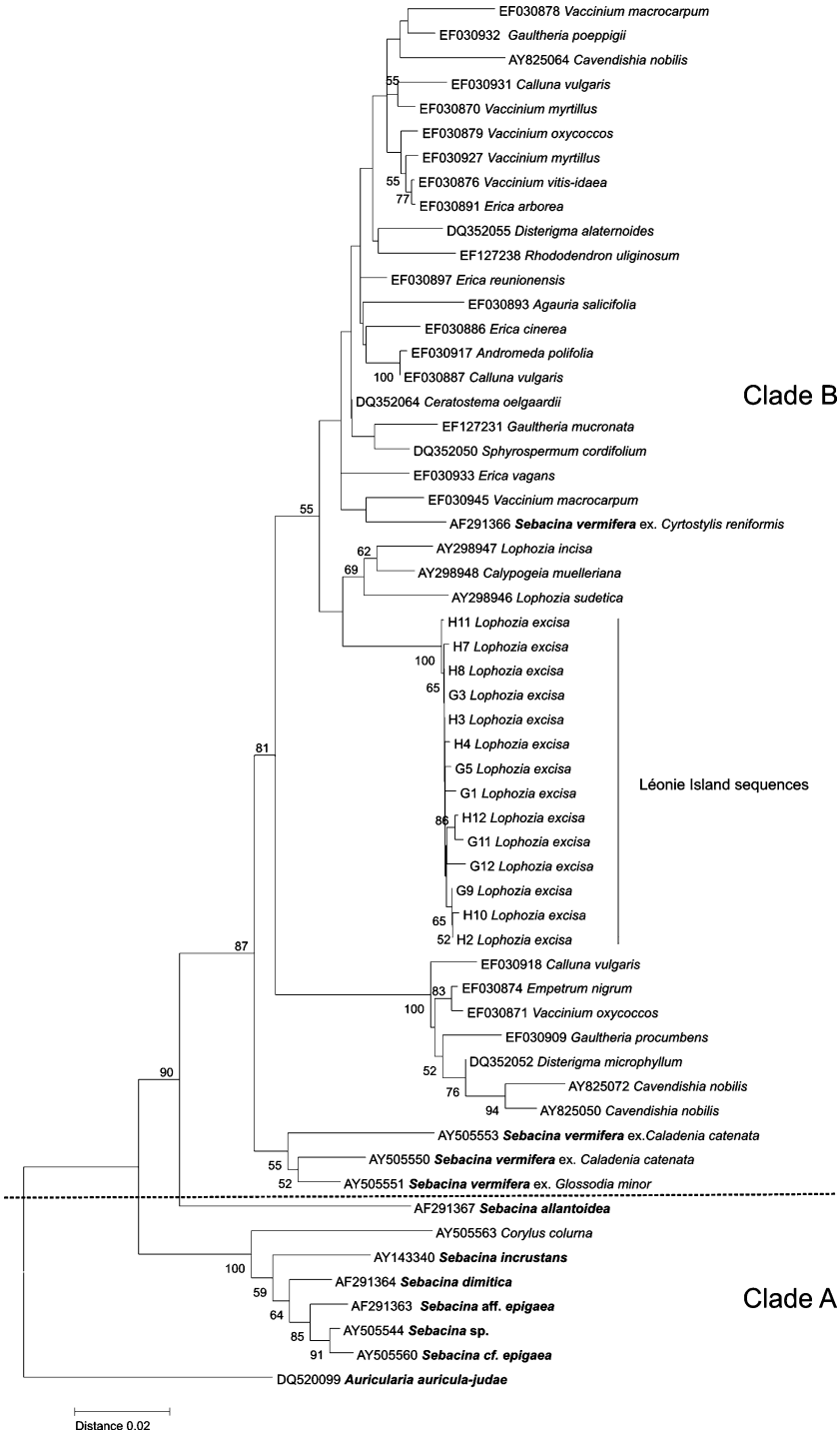

Fig. 3 Consensus neighbour-joining tree obtained from the alignment of the ITS3Seb/TW13-amplified rDNA sequences of fungi in *Lophozia excisa* sampled from Léonie Island with 42 sequences representing the main sub-groups of Sebacinales clades A and B shown by Selosse et al. (2007). Bootstrap values >50% are given at branch points. Sequences from other studies are identified by GenBank numbers and host plant species. Where assigned, fungal species names are shown in *bold*

Selosse et al. 2007), North and South America (Allen et al. 2003; Setaro et al. 2006; Selosse et al. 2007) and Australia (Warcup 1988), but also with those in Antarctica, supporting the suggestion of Weiß et al. (2004) that the Sebacinales has a wide geographical distribution. The recovery of the sequences from Antarctic *L. excisa* with those from Northern European *L. sudetica*, *L. incisa* and *C. muelleriana* suggests a clade of leafy liverwort-associated Sebacinales, but this presently cannot be confirmed, as these are the only such reference sequences currently available in the GenBank nucleotide database.

The formation in the rhizoids of *L. excisa* of hyphal coils, which are sites for the transfer of nitrogen and carbon between partners in ericoid mycorrhizas (Smith and Read 2008), is suggestive of active nutrient exchange between partners. However, Read et al. (2000) point out

that the development of slow-growing poikilohydric liverworts is rarely, if ever, limited by nutrient availability. Certainly, for the substrates inhabited by *L. excisa* in the maritime Antarctic, concentrations of inorganic nitrogen are apparently ample for plant growth: for example, substrates at nine locations in the maritime Antarctic inhabited by *C. varians*, which is a frequent associate of *L. excisa*, contain moderate to high concentrations (*sensu* Allen 1989) of ammonium (1–61 mg 100 g^{-1}; Newsham 2009). It thus seems unlikely, given the availability of inorganic nitrogen in these substrates and the low nutrient demands of tiny, slow-growing liverworts such as *L. excisa*, that fungal associations supply nutritional benefits to hepatics.

The precise benefits of fungi belonging to Sebacinales clade B to leafy liverworts such as *L. excisa*—if any—remain to be defined. Efforts to discover their effects on hepatics are likely to be hampered by their poor, or lack of, growth on agar media (Allen et al. 2003; Smith and Read 2008), which in many cases will preclude Koch's postulate experiments on these fungi. Some associates, do, however, grow on artificial media (e.g. Warcup 1988), with experiments on unidentified basidiomycetes isolated from liverwort tissues and re-inoculated onto *L. excisa* and *L. ventricosa* showing increases in liverwort growth and the formation of reproductive structures (Duckett et al. 2006). One possibility is that these fungi may confer non-nutritional benefits on their hosts, as has been shown for culturable members of Sebacinales clade B, such as *Sebacina vermifera* and *Piriformospora indica*, which improve higher plant growth by inducing resistance to pathogens and abiotic stresses (e.g. Barazani et al. 2005; Deshmukh et al. 2006). A further possibility is that hepatics in the southern maritime Antarctic, which have limited opportunities for carbon fixation owing to low temperatures and the absence of solar radiation for several months every year, could derive carbon from their fungal associates.

Further surveys in the maritime and sub-Antarctic for the occurrence of Sebacinales clade B in *L. excisa* and other liverwort species should be made in order to determine the wider distribution of mycothalli on the continent. Although members of the Arnelliaceae are absent from Antarctica, surveys for Sebacinales clade B in the Scapaniaceae and other members of the Lophoziaceae in the Antarctic liverwort flora, *viz., Scapania gamundiae*, *Scapania obcordata*, *Lophozia* cf. *groenlandica*, *Barbilophozia hatcheri* and *Cryptochila grandiflora*, are now required. The presence of Sebacinales clade B in the former two species is likely, since the medullary cells of these hepatics are known to be routinely colonised by fungal hyphae in the Antarctic natural environment (Bednarek-Ochyra et al. 2000).

Acknowledgements Funding was supplied by the Natural Environment Research Council through the British Antarctic Survey's Long-Term Monitoring and Survey programme. Ryszard Ochyra identified the samples of *L. excisa*, and Katarzyna Turnau provided advice on staining procedures. The staff of the BAS Logistics Group and Rothera Research base, particularly Jim Elliot and James Wake, assisted with transport to and from Léonie Island. Peter Fretwell drew Fig. 1 and Sandra McInnes and Jamie Oliver helped with the preparation of Fig. 2. Two anonymous referees supplied helpful comments. All are gratefully acknowledged.

References

Allen SE (1989) Chemical analysis of ecological materials. Blackwell, Oxford, p 368

Allen TR, Millar T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytol 160:255–272

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl Acids Res 25:3389–3402

Barazani O, Benderoth M, Groten K, Kuhlemeier C, Baldwin IT (2005) *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. Oecologia 146:234–243

Bednarek-Ochyra H, Váňa J, Ochyra JVR, Smith RIL (2000) The liverwort flora of Antarctica. Institute of Botany, Polish Academy of Sciences, Cracow, p 236

Boullard B (1988) Observations on the coevolution of fungi with hepatics. In: Pyrozynski KA (ed) Coevolution of fungi with plants and animals. Academic, London, pp 107–124

Chambers SM, Williams PG, Seppelt RD, Cairney JWG (1999) Molecular identification of *Hymenoscyphus* sp. from rhizoids of the leafy liverwort *Cephaloziella exiliflora* in Australia and Antarctica. Mycol Res 103:286–288

Cullings KW (1994) Molecular phylogeny of the Monotropoideae (Ericaceae) with a note on the placement of the Pyroloideae. J Evol Biol 7:501–516

Davey ML, Currah RS (2007) A new species of *Cladophialophora* (hyphomycetes) from boreal and montane bryophytes. Mycol Res 111:106–116

Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiß M, Waller F, Kogel K-H (2006) The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc Nat Acad Sci USA 103:18450–18457

Duckett JG, Read DJ (1991) The use of the fluorescent dye, 3, 3′ dihexyloxacarbocyanine iodide, for selective staining of ascomycete fungi associated with liverwort rhizoids and ericoid mycorrhizal roots. New Phytol 118:259–272

Duckett JG, Russell J, Ligrone R (2006) Basidiomycetous endophytes in jungermannialean (leafy) liverworts have novel cytology and species-specific host ranges: a cytological and experimental study. Can J Bot 84:1075–1093

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Huiskes AHL, Lud D, Moerdijk-Poortvliet TCW (2001) Field research on the effects of UV-B filters on terrestrial Antarctic vegetation. Plant Ecol 154:77–86

Kottke I, Beiter A, Weiß M, Haug I, Oberwinkler F, Nebel M (2003) Heterobasidiomycetes form symbiotic associations with hepatics: Jungermanniales have sebacinoid mycobionts while *Aneura pinguis* (Metzgeriales) is associated with a *Tulasnella* species. Mycol Res 107:957–968

Montiel P, Smith A, Keiller D (1999) Photosynthetic responses of selected Antarctic plants to solar radiation in the southern maritime Antarctic. Polar Res 18:229–235

Newsham KK (2009) The biology and ecology of the liverwort *Cephaloziella varians* in Antarctica. Antarct Sci 22. doi:10.1017/S0954102009990630

Pearson WR (1990) Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymol 183:63–98

Pressel S, Ligrone R, Duckett JG (2008) The ascomycete *Rhizoscyphus ericae* elicits a range of host responses in the rhizoids of leafy liverworts: an experimental and cytological analysis. Fieldiana: Botany 47:59–72

Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal associations in 'lower' land plants. Phil Trans R Soc Lond B 355:815–831

Selosse M-A, Setaro S, Glatard F, Richard F, Urcelay C, Weiß M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. New Phytol 174:864–878

Setaro S, Weiß M, Oberwinkler F, Kottke I (2006) Sebacinales form ectendomycorrhizas with *Cavendishia nobilis*, a member of the Andean clade of Ericaceae, in the mountains rain forest of southern Ecuador. New Phytol 169:355–365

Smith RIL (1984) Terrestrial plant biology of the sub-Antarctic and Antarctic. In: Laws RM (ed) Antarctic ecology vol 1. Academic, London, pp 61–162

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Upson R, Read DJ, Newsham KK (2007) Widespread association between the ericoid mycorrhizal fungus *Rhizoscyphus ericae* and a leafy liverwort in the maritime and sub-Antarctic. New Phytol 176:460–471

Warcup JH (1988) Mycorrhizal associations of isolates of *Sebacina vermifera*. New Phytol 110:227–231

Weiß M, Selosse M-A, Rexer K-H, Urban U, Oberwinkler F (2004) Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol Res 108:1003–1010

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand H, Sninsky JS, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322