Mycorrhiza (2002) 12:243–247
DOI 10.1007/s00572-002-0180-y

ORIGINAL PAPER

M. Glen · I.C. Tommerup · N.L. Bougher
P.A. O'Brien

Are Sebacinaceae common and widespread ectomycorrhizal associates of *Eucalyptus* species in Australian forests?

Received: 17 January 2002 / Accepted: 6 May 2002 / Published online: 29 June 2002

Abstract A molecular survey of basidiomycete ectomycorrhizal fungi colonising root tips at a site in *Eucalyptus marginata* (jarrah) forest revealed the presence of many fungal species which could not be identified from a database of ITS-PCR-RFLP profiles from morphologically identified species. Three of these unidentified taxa were among the six most frequently encountered profiles. Phylogenetic analyses of ITS and nuclear LSU sequences revealed a close relationship among the three fungi and that they belong to the family Sebacinaceae (sensu Weiß and Oberwinkler 2001). The possibility that DNA of non-ectomycorrhizal rhizosphere or endophytic fungi had been amplified selectively by the basidiomycete-specific primers was tested by amplification with fungal-specific primers. A single PCR fragment was amplified in all but two of the 24 samples tested and digestion with two restriction enzymes produced RFLP profiles which matched those from the Sebacinoid sequence. We conclude, therefore, that at least three species of Sebacinaceae are common ectomycorrhizal associates of *E. marginata.*

Keywords nLSU sequence · Phylogenetic analysis · Mycorrhiza · Orchid · Root tips

Introduction

Molecular techniques have enabled significant advances in knowledge of ectomycorrhizal ecology (Horton and Bruns 2001). Molecular studies have expanded estimates of the degree of species richness in this component of the soil nutrient-cycling system. They have also revealed the discrepancy between fungal species that fruit abundantly and those that occupy a significant proportion of root tips (Gardes and Bruns 1996; Horton and Bruns 2001; Peter et al. 2001a, b). Molecular as well as morphotyping studies have demonstrated the prevalence on root tips of species with insignificant or easily overlooked fruitbodies, such as members of the Thelephoraceae (Kõljalg et al. 2000). All molecular studies to date have uncovered at least as many unrecognised PCR-RFLP profiles as the number of fungal species found fruiting at the site(s) investigated (Gardes and Bruns 1996; Gehring et al. 1998; Jonsson et al. 1999a, b, 2000; Stendell et al. 1999; Taylor and Bruns 1999; Peter et al. 2001a, b).

A PCR-RFLP survey of basidiomycete fungi occupying root tips at a 3-ha site in a *Eucalyptus marginata* (jarrah) forest produced 135 unmatched profiles after comparison with a database of 128 local fungal species (Glen et al. 2001a, b). Many of these profiles occurred in only one soil core and at low abundance. However, three profiles, designated ERF1 (Eucalypt Root-associated Fungus), ERF2 and ERF3 were among the six most frequent and abundant basidiomycete fungi from ectomycorrhiza. Each type was found in 5–7% of soil cores. Each ectomycorrhizal root apex was pinnately branched and had an open, pyramidal form. Samples had 5–200 root tips in each cluster. Each of the three ERFs occurred at least once on the root system arising from the tap root or lignotuber of a young jarrah tree. All other samples were from roots of jarrah or marri (*Calophylla corymbia,* a co-occurring eucalypt).

Assistance in the identification of these three ERFs was sought by DNA sequencing. Phylogenetic analysis of DNA sequences has been useful in identifying fungi to genus or family level. Bruns et al. (1998) established a database of mt rDNA sequences for the identification of unknown basidiomycetes from ectomycorrhiza. Kõljalg et al. (2000) used phylogenetic analysis of ITS sequences to identify species of resupinate fungi as widespread ectomycorrhizal symbionts in Europe. We used a fragment of nuclear LSU rDNA as there were no sufficiently similar ITS sequences publicly available. Weiß and Oberwinkler

M. Glen (✉) · P.A. O'Brien
School of Biological Sciences and Biotechnology,
Division of Science and Engineering, Murdoch University,
Murdoch, WA 6150, Australia
e-mail: m_glen@murdoch.edu.au
Fax: +61-8-93107084

I.C. Tommerup · N.L. Bougher
CSIRO Division of Forestry and Forest Products,
P.O. Box 5, Wembley, Perth, WA 6913, Australia

(2001) examined phylogenetic relationships in Auriculariales and related groups using nuclear LSU rDNA sequences. They included several species of *Sebacina* in their analysis, which produced a strongly supported Sebacinaceae clade distinct from the Tremellales or Auriculariales.

Materials and methods

Root samples

Site details are given in Glen et al. (2001b). Soil cores were taken from July to October 1996 and stored at 4°C for a maximum of 3 weeks before processing. Root samples consisting of 5–15 apices were removed under a dissecting microscope and stored at –80°C.

DNA extraction

Each sample was ground with a plastic pestle and a Kontes motorised pellet mixer in a 1.5-ml microcentrifuge tube. A total of 250 µl extraction buffer (Raeder and Broda 1985) was added and the tubes incubated at 65°C for 1 h. Tubes were centrifuged at 14,000 rpm for 15 min and the supernatant removed. DNA was purified by binding to glassmilk (http://bionet.hgmp.mrc.ac.uk/hypermail/methods/methods.199209/1493.html). Briefly, 600 µl of 100% NaI and 7 µl glass milk (silica) was added to 200 µl of the supernatant and vortexed briefly. The mixture was incubated on ice for 15 min with occasional shaking. Tubes were centrifuged for 5 s at 14,000 rpm, the supernatant removed, and the pellet resuspended in 600 µl of wash buffer (100 mM NaCl, 10 mM Tris HCl pH 7.5, 1 mM EDTA in 50% ethanol). Following centrifugation for 5 s at 14,000 rpm, the supernatant was removed, the pellet resuspended in 600 µl 100% ethanol and centrifuged as before. Finally, the supernatant was removed and the pellet dried. DNA was eluted by adding 20 µl of TE buffer (10 mM Tris HCl pH 8, 1 mM EDTA), vortexing briefly and incubating at 37°C for 5 min. Supernatant containing DNA was removed following centrifugation for 2 min at 14,000 rpm and stored at –20°C.

PCR and PCR-RFLP

DNA was diluted 1/10 in TE and 1 or 2.5 µl of diluted DNA was used as template in a 25-µl PCR. The rDNA ITS region was amplified with the primer pair ITSF/ITSR as described in Glen et al. (2001a) with the addition of 200 ng/µl BSA to reduce enzyme inhibition by plant or humic substances (Kreader 1996). Amplification with ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) was also performed under the same thermocycler conditions and reagent concentrations. A fragment of the nuclear rDNA LSU was amplified with the primer pair NL5mun/NL6Bmun according to Egger (1995), again with the addition of BSA. The ITS PCR product was digested and electrophoresed as described in Glen et al. (2001a).

DNA sequencing

PCR products were purified with a Wizard PCR DNA Purification System (Promega). Sequences were determined with an ABI Prism Dye Terminator Cycle Sequencing kit on a Bio-Rad 373 XL sequencer with stretch upgrade. Sequences were obtained in both directions using primers ITS1-F and ITS4-B (Gardes and Bruns 1993) for the ITS region and NL5mun/NL6Bmun (Egger 1995) for the LSU. Where necessary, the internal primers ITS2 and ITS3 (White et al. 1990) were also used. Sequences overlapped between the ITS and LSU fragments and confirmed that the two fragments had been amplified from the same organism.

Phylogenetic analyses

Maximum likelihood and maximum parsimony analyses were performed with DNAML and DNAPARS of the PHYLIP package and SEQBOOT and CONSENSE were used to determine bootstrap support for parsimony trees (Felsenstein 1993).

Results

PCR-RFLP

To verify that the ITSF/ITSR PCR (basidiomycete) product was from the ectomycorrhizal fungus and not a contaminant, restriction digestion was carried out on the product of the fungal-specific primers ITS1-F/ITS4. One sample from each soil core (24 samples) was tested and all samples produced a profile consistent with the ITSF/ITSR profile and DNA sequence data. Two samples produced two fragments in the PCR, with an RFLP profile consisting of the expected profile plus additional fainter fragments, which were the same in the two samples.

DNA sequencing

One root sample representative of each of the three ERFs was amplified with ITSF/ITSR and the PCR product sequenced (GenBank accession numbers AY093436-AY093438). The three ITS sequences had a high degree of similarity (86–89%) to each other, but a search of GenBank and EMBL as well as private (U Kõljalg, Estonian Agricultural University, personal communication) databases revealed no close matches. A fragment of rDNA LSU was, therefore, sequenced. Database searches revealed high similarity to *Sebacina* spp. and some unidentified fungi from the roots of an ericoid plant, *Gaultheria shallon*. GenBank accession numbers for the LSU sequences are given in Fig. 1.

Phylogenetic analyses

Phylogenetic analyses were performed using the LSU sequences, first with a broad selection of basidiomycete taxa to confirm the basic grouping, then with a more targeted selection of *Sebacina* species, Auriculariales and Tremellales. Most of the sequences, apart from those determined here, are from Weiß and Oberwinkler (2001). In addition, three sequences from salal root-associated fungi (Berch et al. 2001) were retrieved from GenBank and included in the analysis. The initial analysis confirmed that the unknown fungi grouped with *Sebacina* spp. (results available on request). The result of the second analysis, including a higher number of Tremellales and Auriculariales and fewer Agaricales is shown in Fig. 1. This confirmed the *Sebacina* affinity and demonstrated intrageneric groupings. The three ERF-derived sequences are in the *Sebacina* clade, which also includes other genera placed in Sebacinaceae by Weiß and Ober-

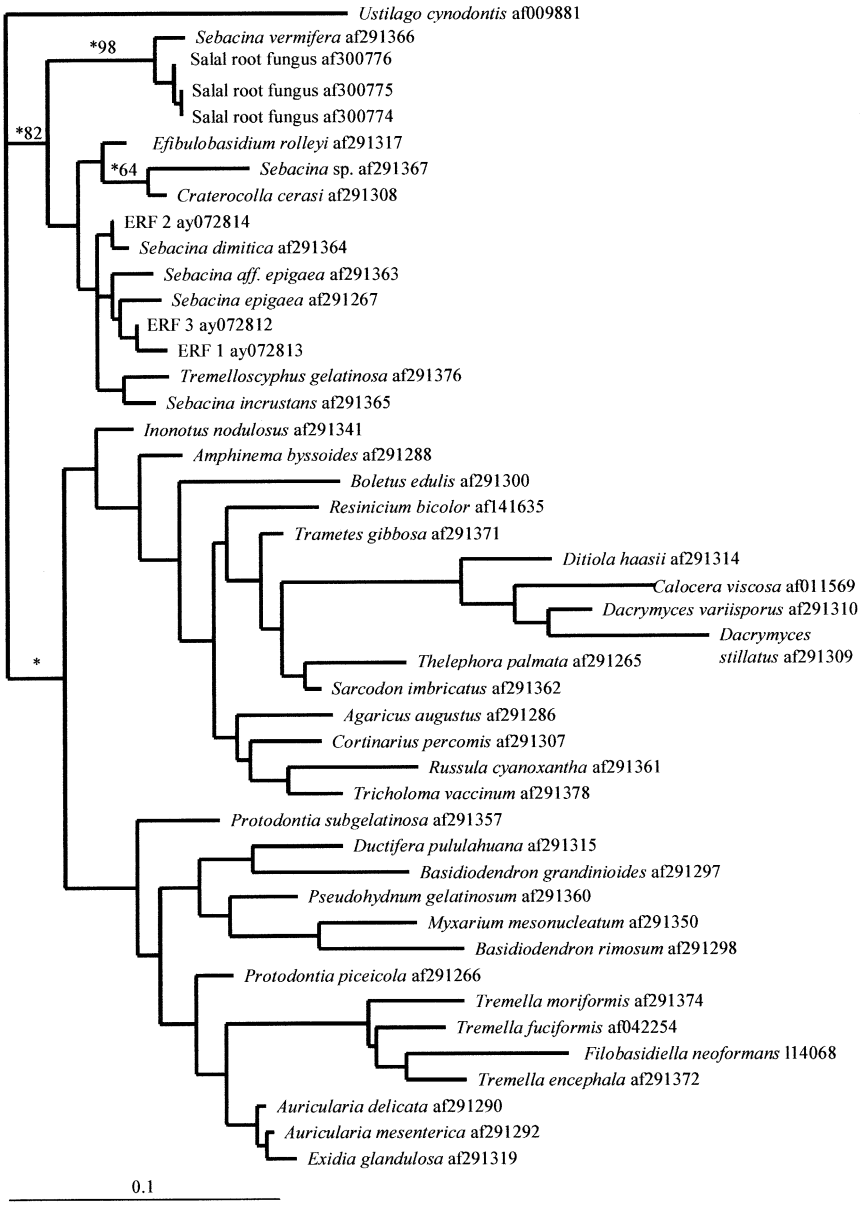

Fig. 1 Maximum likelihood analysis of nuclear rDNA LSU sequences. GenBank accession numbers are given next to the fungal species. For the Sebacinaceae clade, an asterisk above a branch signifies a branch length greater than zero ($P<0.01$), numbers above branches are the parsimony bootstrap support (100 replicates, only given if greater than 50%). Branch lengths are scaled to the expected number of nucleotide substitutions per site

winkler (2001): *Efibulobasidium*, *Craterocolla*, *Tremelloscyphus*. ERF3 and ERF1 group with *S. epigaea* and *S. aff. epigaea,* and ERF2 with *S. dimitica*, though the statistical support for these groupings is low. These three species are of European origin. Interestingly, the salal root-associated fungi from North America cluster with the *S. vermifera* isolate from Australia and this branch has strong statistical support.

In both analyses, the Sebacinaceae form a well-supported clade, including the three ERFs and the three sequences from salal root-associated fungi. This clade has 82% bootstrap support in MP analysis as well as ML significance at the P 0.01 level.

The three ERF ITS sequences were compared to ITS sequences from Weiß and Oberwinkler's Sebacinaceae collections. ERF1 and ERF3 are most similar to *Tremelloscypha gelatinosa*, with 83% similarity in ITS1 and 76–84% similarity in ITS2. ERF2 is 94% similar to *S. dimitica* in ITS1 and ITS2 (M. Weiß, University of Tübingen, personal communication); ERF2 is also 91–96% similar to *Tremellodendron pallidum* (GenBank af384862).

Discussion

That the three ectomycorrhizal fungal species belong to the Sebacinaceae (sensu Weiß and Oberwinkler 2001) is ecologically consistent with previous work in myrtaceous plant communities or soils that previously supported some of those communities (Warcup 1988). However, *Sebacina* has not been widely recognised as an ectomycorrhizal genus, despite early work demonstrating the formation of ectomycorrhiza on *Melaleuca uncinata* by isolates cultured from eucalypt ectomycorrhiza. The formation of orchid mycorrhiza and ectomycorrhiza by a

single isolate was also confirmed, raising the possibility of a tripartite mycorrhizal association, as shown for the achlorophyllous orchid *Rhizanthella gardneri* with *Melaleuca uncinata* and a *Rhizoctonia* endophyte (Warcup 1985). Germination of *Microtis unifolia* seeds and maintenance of albino *Microtis* plants by an isolate of *S. vermifera* ectomycorrhizal on *Melaleuca uncinata* supported this hypothesis (Warcup 1988). *S. vermifera* has been shown to germinate orchid seeds of a restricted range of species in the genera *Acianthus*, *Caladenia*, *Eriochilus*, *Glossodia* and *Microtis* (Warcup 1988). The species stimulated varies with the isolate. Many orchid species occur in the jarrah forest in which our ectomycorrhizal samples were taken (Marchant et al. 1987; Hoffman and Brown 1998). These include species of the genus *Caladenia*, as well as genera such as *Diuris*, for which germination by *S. vermifera* has not been shown. On the basis of LSU and ITS sequences, however, the three ERFs appear more closely related to other Sebacinaceae species and these have not been tested for orchid germination.

We have found that PCR of eucalyptus ectomycorrhizal DNA using fungal-specific or ascomycete-specific primers often results in the amplification of more than one fragment, which increases the difficulty of matching the PCR-RFLP profile. To avoid this problem, we routinely use basidiomycete-specific primers; however, this could result in false identification of a contaminating soil-inhabiting basidiomycete as the ectomycorrhizal symbiont if the root symbiont is, for example, an ascomycete. To ascertain that no other fungus was present in the root samples, we also used the fungal-specific primer pair ITS1-F/ITS4. We detected the presence of other fungi in only two of the 24 samples checked. The identity of the other fungi in these two samples was not determined and, thus, their mycorrhizal status remains unknown. PCR-RFLP profiles matching the three sebacinoid fungi discussed here were amplified from ectomycorrhizal clusters taken from naturally occurring young jarrah, which may be suppressed trees more than 6 years old (Abbott and Loneragan 1984), present in the soil cores. Thus, it is apparent, whatever other functions these fungi may perform in the roots and rhizosphere of other plants, that they are relatively common and abundant ectomycorrhizal associates of eucalypts. Sebacinoid sequences have also been amplified from ectomycorriza of *Picea* (M. Gardes, CNRS Université Toulouse, personal communication).

The mycorrhizal capacity of this genus may not be confined to orchid and ectomycorrhiza. Multinucleate *Rhizoctonia* cultures isolated from vesicles of arbuscular mycorrhizal fungi in white clover roots have also stimulated germination of *Microtis* orchids (Williams 1985). In addition, dual infections of several non-orchid plant species by *Glomus tenue* and one of these multinucleate *Rhizoctonia* isolates produced earlier and greater plant growth than colonisation by *G. tenue* alone (Williams and Thilo 1989). The teleomorph of some of these multinucleate *Rhizoctonia* was *S. vermifera* (Milligan and Williams 1987). Some of the isolates matched descriptions of *Rhizoctonia globularis*, a fungus isolated from pine seedlings (Milligan and Williams 1987).

DNA sequences similar to those of *Sebacina* spp. have been obtained from roots of the ericoid plant *Gaultheria shallon* (Berch et al. 2001). The mycorrhizal status has not been confirmed, as the fungi have proved resistant to isolation and culturing; however, close relationships between ericoid and ecto- mycorrhizal fungi have been demonstrated in other genera, for example, the ericoid mycobiont *Hymenoscyphus ericae* and fungal symbionts of the common and widespread ectomycorrhizal morphotype *Piceirhiza bicolorata* (Vrålstad et al. 2000). Sen et al. (1999) demonstrated a close genetic relationship between binucleate *Rhizoctonia* isolates endophytic in pine roots and orchid mycorrhizal isolates. In addition, non-photosynthetic orchids and monotropoid plants associate very specifically with fungal species of *Rhizopogon* and *Russula*, which are ectomycorrhizal on various hosts (Taylor and Bruns 1997; Kretzer et al. 2000). These examples all indicate that symbiotic fungi are not neatly divided among ecto-, ectendo-, ericoid and orchid mycorrhiza, and that some root-associating fungi are flexible in their associations with plants and do not conform to rigid conceptual boundaries.

The repeated evolution of the ectomycorrhizal symbiosis has been demonstrated by phylogenetic analysis (Hibbett et al. 2000). It is, therefore, possible that some species of *Sebacina* are ectomycorrhizal while others are not, or that the association is not obligatory for the fungus. Inclusion of *Craterocolla, Efibulobasidium* and *Tremelloscyphus* in the Sebacinaceae clade in our study concurs with the results of Weiß and Oberwinkler (2001) and is concordant with lack of clamp connections in these genera. These authors also discussed the discrepancy between the original description of *S. vermifera* (Oberwinkler 1964), which had sub-basidial clamp connections, and *S. vermifera* sensu Warcup and Talbot (1967), which did not. They concluded that *S. vermifera* in its original sense is not a member of the Sebacinaceae and that *S. vermifera* sensu Warcup and Talbot is.

The phylogenetic analysis of the nuclear LSU sequences appears to group the ERFs with European species *S. dimitica* and *S. epigaea*, but the statistical support for these groupings is low. *Sebacina* has not been extensively studied in Australia and herbarium collections are few. Phylogenetic analyses of ITS sequences is only possible for subgroups within Sebacinaceae because of the high sequence variation (Selosse et al 2002).

This new molecular information from a natural forest ecosystem emphasises the need to investigate further the role played by these fungi in nutrient cycling, both between plants and soil and among plant species. The occurrence of at least three species of Sebacinaceae as ectomycorrhiza in a 3-ha jarrah forest site, combined with the occurrence of related fungal species in roots from other eucalypt ecosystems from the other side of the Australian continent (Warcup 1988) and vastly different ecosystems on the other side of the planet (Sen et al. 1999; Berch et al. 2001; Selosse et al 2002), indicates that this role may be greater than previously considered.

Acknowledgements This work was funded by an APA award to M.G., the CSIRO Biodiversity Sector and Australian Research Council SPIRT grant C00002212.

References

Abbott I, Loneragan O (1984) Growth rate and long-term population dynamics of jarrah (*Eucalyptus marginata* Donn. Ex Sm.) regeneration in Western Australian forests. Aust J Bot 32:353–362

Berch SM, Allen TR, Berbee ML (2001) Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. Proceedings of the Third International Conference on Mycorrhiza

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y (1998) A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272

Egger KN (1995) Molecular analysis of ectomycorrhizal fungal communities. Can J Bot 73:S1415-S1422

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c, distributed by the author. Department of Genetics, University of Washington, Seattle

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

Gardes M, Bruns TD (1996) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Can J Bot 74:1572–1583

Gehring CA, Theimer TC, Whitham TG, Keim P (1998) Ectomycorrhizal community structure of Pinyon pines growing in two environmental extremes. Ecology 79:1562–1572

Glen M, Tommerup IC, Bougher NL, O'Brien PA (2001a) Specificity, sensitivity and discrimination of primers for PCR-RFLP of larger basidiomycetes and their applicability to identification of ectomycorrhizal fungi in *Eucalyptus* forests and plantations. Mycol Res 105:138–149

Glen M, Tommerup IC, Bougher NL, O'Brien PA (2001b) Interspecific and intraspecific variation of ectomycorrhizal fungi associated with *Eucalyptus* ecosystems as revealed by ribosomal DNA PCR-RFLP. Mycol Res 105:843–858

Hibbett DS, Gilbert LB, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbiosis in basidiomycetes. Nature 407:506–508

Hoffman N, Brown A (1998) Orchids of south-west Australia. University of Western Australia Press, Nedlands, Australia

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10:1855–1871

Jonsson L, Dahlberg A, Nilsson MC, Kårén O, Zackrisson O (1999a) Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. New Phytol 142:151–162

Jonsson L, Dahlberg A, Nilsson MC, Zackrisson O, Kårén O (1999b) Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Mol Ecol 8:205–215

Jonsson L, Dahlberg A, Tor-Erik B (2000) Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Picea abies* forest subjected to experimental nitrogen addition: above- and below-ground views. For Ecol Manage 132:143–156

Kõljalg U, Dahlberg A, Taylor AFS, Larsson E, Hallenberg N, Stenlid J, Larsson KH, Fransson PM, Karen O, Jonsson L (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Mol Ecol 9:1985–1996

Kreader CA (1996) Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. Appl Environ Microbiol 62:1102–1106

Kretzer AM, Bidartondo MI, Grubisha LC, Spatafora JW, Szaro TM, Bruns TD (2000) Regional specialization of *Sarcodes sanguinea* (Ericaceae) on a single fungal symbiont from the *Rhizopogon ellenae* (Rhizopogonaceae) species complex. Am J Bot 87:1778–1782

Marchant NG, Wheeler JR, Rye BL, Bennett EM, Lander NS, Macfarlane TD (1987) Flora of the Perth region, vol 2. West Australian Government Printers, Perth, Australia, pp 583–1080

Milligan MJ, Williams PG (1987) The mycorrhizal relationship of multinucleate rhizoctonias from non-orchids with *Microtis* (Orchidaceae) New Phytol 108:205–209

Oberwinkler F (1964) Intrahymeniale Heterobasidiomycoten. Fruchtkörperlose *Sebacina*-Sippen und ihre systematische Stellung. Nova Hedwigia Kryptogamenkd 7:489–498

Peter M, Ayer F, Egli S (2001a) Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytol 149:311–325

Peter M, Ayer F, Egli S, Honegger R (2001b) Above- and below-ground community structure of ectomycorrhizal fungi in three Norway spruce (*Picea abies*) stands in Switzerland. Can J Bot 79:1134–1151

Raeder U, Broda P (1985) Rapid preparation of DNA from filamentous fungi. Lett Appl Microbiol 1:17–20

Selosse M-A, Weiß M, Jany J-L, Tillier A (2002) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. Mol Ecol (in press)

Sen R, Hietala AM, Zelmer CD (1999) Common anastomosis and internal transcribed spacer RFLP groupings in binucleate *Rhizoctonia* isolates representing root endophytes of *Pinus sylvestris*, *Ceratorhiza* spp. from orchid mycorrhizas and a phytopathogenic anastomosis group. New Phytol 144:331–341

Stendell ER, Horton TR, Bruns TD (1999) Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycol Res 103:1353–1359

Taylor DL, Bruns TD (1997) Independent specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proc Natl Acad Sci USA 94:4510–4515

Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol 8:1837–1850

Vrålstad T, Fossheim T, Schumacher T (2000) *Piceirhiza bicolorata* – the ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? New Phytol 145:549–563

Warcup JH (1985) *Rhizanthella gardneri* (Orchidaceae), its Rhizoctonia endophyte and close association with *Melaleuca uncinata* (Myrtaceae) in Western Australia. New Phytol 99:273–280

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

Warcup JH, Talbot PHB (1967) Perfect states of Rhizoctonias associated with orchids. New Phytol 66:631–641

Weiß M, Oberwinkler F (2001) Phylogenetic relationships in *Auriculariales* and related groups – hypotheses derived from nuclear ribosomal DNA sequences. Mycol Res 105:403–415

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MD, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322

Williams PG (1985) Orchidaceous rhizoctonias in pot cultures of vesicular-arbuscular mycorrhizal fungi. Can J Bot 63:1329–1333

Williams PG, Thilo E (1989) Ultrastructural evidence for the identity of some multinucleate rhizoctonias. New Phytol 112:513–518