Mycorrhiza (2007) 17:217–222
DOI 10.1007/s00572-006-0098-x

ORIGINAL PAPER

Morphological and molecular evidence supporting an arbutoid mycorrhizal relationship in the Costa Rican páramo

Todd W. Osmundson · Roy E. Halling · Henk C. den Bakker

Received: 10 November 2006 / Accepted: 8 December 2006 / Published online: 11 January 2007

Abstract This study examines evidence for a particular arbutoid mycorrhizal interaction in páramo, a high-altitude neotropical ecosystem important in hydrological regulation but poorly known in terms of its fungal communities. *Comarostaphylis arbutoides* Lindley (Ericaceae) often forms dense thickets in Central American páramo habitats. Based on phylogenetic classification, it has been suggested that *C. arbutoides* forms arbutoid mycorrhizae with diverse Basidiomycetes and Ascomycetes; however, this assumption has not previously been confirmed. Based on field data, we hypothesized an arbutoid mycorrhizal association between *C. arbutoides* and the recently described bolete *Leccinum monticola* Halling & G.M. Mueller; in this study, we applied a rigorous approach using anatomical and molecular data to examine evidence for such an association. We examined root samples collected beneath *L. monticola* basidiomes for mycorrhizal structures, and we also compared rDNA internal transcribed spacer (ITS) sequences between mycorrhizal root tips and leaf or basidiome material of the suspected symbionts. Root cross sections showed a thin hyphal sheath and intracellular hyphal coils typical of arbutoid mycorrhizae. DNA sequence comparisons confirmed the identity of *C. arbutoides* and *L. monticola* as the mycorrhizal symbionts. In addition, this paper provides additional evidence for the widespread presence of minisatellite-like inserts in the ITS1 spacer in *Leccinum* species (including a characterization of the insert in *L. monticola*) and reports the use of an angiosperm-specific ITS primer pair useful for amplifying plant DNA from mycorrhizal roots without co-amplifying fungal DNA.

Keywords Basidiomycota · Boletaceae · Ericaceae · Arbutoid mycorrhizae · Ribosomal DNA internal transcribed spacer (ITS) sequences

T. W. Osmundson (✉) · R. E. Halling
Institute of Systematic Botany,
The New York Botanical Garden,
Bronx, NY 10458, USA
e-mail: tosmundson@nybg.org

T. W. Osmundson
The Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies,
The New York Botanical Garden,
Bronx, NY 10458, USA

T. W. Osmundson · R. E. Halling
Department of Ecology, Evolution and Environmental Biology,
Columbia University,
New York, NY 10027, USA

H. C. den Bakker
Department of Plant Pathology, Cornell University,
Ithaca, NY 14853, USA

Introduction

Páramo is a high mountain neotropical ecosystem situated between treeline and the permanent snowline at elevations of approximately 3,000–5,000 m. Distributed discontinuously between 11° N and 8° S latitude, páramo occurs predominantly in the northern Andes of Colombia and Ecuador, with scattered occurrences in Peru, Venezuela, and Costa Rica (Luteyn 1999). Páramo has the greatest degree of plant diversity of any high elevation ecosystem in the world and a high level of endemism, and plays a critical role in hydrological regulation, serving as a major water source for lower elevation habitats (Luteyn 1999). Páramo habitats face increasing degradation due to agriculture, grazing, and recreation. Like temperate high elevation (alpine) ecosystems, they are predicted to be severely threatened by global climate change (Kappelle et al. 1999;

Luteyn 1999). Mutualistic interactions in these ecosystems are poorly characterized; a better understanding of high-elevation mutualisms, including levels of host specificity and the population and source-sink dynamics of high elevation and neighboring lower elevation populations, is important for better predicting the level of threat to species in these ecosystems. The ecological and economic importance of páramo and the level of anthropogenic threat that it faces make understanding its ecology an important conservation concern.

The site of this study is located on Cerro de la Muerte (9°33′16″N″, 83°45′18″W, elevation 3,491 m), located in the Talamanca Mountains of Costa Rica and representing the westernmost and one of the northernmost extents of the páramo ecosystem type (Halling and Mueller 2006; Kappelle et al. 1999; Luteyn 1999). Among the dominant plant species in this site is *C. arbutoides* Lindley, an ericaceous shrub that forms dense thickets or semi-open stands in the páramo (Halling and Mueller 2006). Based on morphological observations of exotic material from Chiapas, Mexico transplanted in the University of California at Berkeley Botanical Garden (M. Bidartondo, personal communication) and phylogenetic analyses placing *C. arbutoides* as closely related to *Arctostaphylos* and *Arbutus* spp., Bidartondo and Bruns (2001) inferred that *C. arbutoides* forms arbutoid mycorrhizae with diverse species of Basidiomycetes and Ascomycetes. However, this inference has not been otherwise documented (Halling and Mueller 2003). *Leccinum monticola* Halling & G.M. Mueller (Basidiomycota, Boletaceae), a macrofungus recently described from type material collected on Cerro de la Muerte, occurs in *C. arbutoides* thickets and in subpáramo habitats where *C. arbutoides* co-occurs with *Quercus costaricensis* Liebm.; on the basis of this observed proximity in the field, previous workers have inferred, though not confirmed, a mycorrhizal association (den Bakker et al. 2004b; Halling and Mueller 2003, 2006). An earlier report based on an herbarium collection of *C. arbutoides* subsp. *arbutoides* (Gómez et al. 21655, CR) noted a field association with *Leccinum aurantiacum* (Diggs 1995), which may actually be *L. monticola*. Such field observations, while important for making rapid assessments of ectomycorrhizal community composition, amount to circumstantial evidence in support of specific mycorrhizal associations and have in the past led to a number of mistaken designations (Brundrett 2004). The goal of the present study is to evaluate the accuracy of these observations for an arbutoid mycorrhizal association between *C. arbutoides* and *L. monticola* using morphological and molecular evidence.

The Arbutoideae form a well-supported monophyletic group that, like *Enkianthus* and the Monotropoideae, represents one of the basal lineages to the rest of the Ericaceae (Kron et al. 2002). The Arbutoideae form a distinct type of mycorrhiza (termed an arbutoid mycorrhiza) that shares morphological characteristics with both ectomycorrhizal (formation of a well-developed hyphal sheath and Hartig net) and ericoid mycorrhizal (hyphal colonization of the epidermal cells) types (Brundrett 2004; Harley 1969; Molina and Trappe 1982; Setaro et al. 2006a,b). Harley (1969) hypothesized a relationship between arbutoid mycorrhizae and ectomycorrhizae based on shared morphological similarities and the restricted position of mycorrhizae on the short, fine roots. Molina and Trappe (1982) subsequently classified arbutoid mycorrhizae as a subtype of ectomycorrhizae based both on these morphological similarities and on in vitro synthesis experiments showing that arbutoid mycorrhizae are formed by mycobionts that form ectomycorrhizae with other plant hosts. While arbutoid mycorrhizae of related *Arctostaphylos* and *Arbutus* spp. have been studied in some depth, mycorrhizae of *C. arbutoides* have not to our knowledge been previously described in the literature. Given the unusual geographic distribution and ecological setting of *C. arbutoides*, a closer examination of its mycorrhizal host associations is warranted. In addition to its implications for improved understanding of páramo ecology, such an examination can shed further light on the biogeography and evolution of host specificity patterns in *Leccinum*, species of which appear to exhibit a high degree of host specificity (den Bakker et al. 2004b). To our knowledge, this is the first study conclusively documenting an arbutoid mycorrhizal association for *C. arbutoides* from within its native range.

Materials and methods

Specimen collection and description of root morphology

Soil specimens were collected by coring beneath *L. monticola* basidiomes to increase the probability of recovering *L. monticola* mycorrhizae (Guidot et al. 2001). Putative mycorrhizal root tips were removed from soil samples within 8 h of collection, sorted by morphotype, and stored in CTAB buffer for DNA analyses and in 90% EtOH or ddH2O for anatomical examination. Root external morphology of multiple root tips from five soil cores was examined under a dissecting microscope and described using terminology from Agerer (1987–2002).

Light microscopy

Root cross sections for examining cellular structures were made by mounting hand sections in Hoyer's solution (Anderson 1954), and were examined using an Olympus BHS-2 compound microscope with Nomarski interference

optics. Anatomical examinations were made from multiple root tips from the same five soil cores used for the macromorphological examinations.

Genetic characterization of mycorrhizal symbionts

Positive identifications of symbionts were made by sequencing the nrDNA internal transcribed spacer (ITS) region and comparing the plant and fungal sequences obtained from a single, representative root tip to those from leaf or basidiome tissue. Polymerase chain reaction (PCR) amplifications used the basidiomycete-specific primer pair ITS1F/ ITS4B (Gardes and Bruns 1993) for fungal material and an angiosperm-specific primer pair designed by Ken Wurdack, ITS5A (CCTTATCATTTA GAGGAAGGAG; Stanford et al. 2000) and 241r (CAGTGCCTCGTGGTGCGACA; Michelangeli et al. 2004), for plant material. Voucher material of *L. monticola* (*Halling 8449*) and *C. arbutoides* (*Osmundson s.n.*, deposited with the *L. monticola* voucher) was deposited in the herbarium of the New York Botanical Garden.

The ITS region of many *Leccinum* species is characterized by length heterogeneity due to a minisatellite region in ITS1 (Binder 1999; den Bakker et al. 2004a). To observe whether this is the case in *L. monticola*, ITS amplicons were cloned from basidiome and root tip material using the Topo TA cloning kit (Invitrogen, Carlsbad, CA, USA) and the size of PCR-amplified inserts from five colony picks was observed by agarose gel electrophoresis. The structure of the minisatellite region in ITS1 was characterized by direct observation of sequence data and with the Tandem Repeats Finder, version 3.21 (Benson 1999), using default settings.

Results

Morphology of mycorrhizal roots

Roots obtained beneath *L. monticola* basidiomes contained a single mycorrhizal morphotype, exhibiting fine roots that were swollen with pinnate to irregularly pinnate ramification, a morphology consistent with that of previously described arbutoid mycorrhizal roots (Smith and Read 1997). The hyphal sheath was smooth, shiny, semitransparent, and pale yellowish-brown, with sparse white, cottony emanating hyphae, and was moderately to strongly agglutinated with mineral particles (Fig. 1a,b). Examination of root cross sections revealed a moderately thin hyphal sheath, thin paraepidermal Hartig net, and dense epidermal intracellular hyphal networks typical of arbutoid mycorrhizae (Fig. 1c,d).

Molecular characterization of symbionts

Comparison of an approximately 900-bp complete rDNA ITS sequence showed 100% identity between photobiont DNA from the *C. arbutoides* root tip sample and DNA

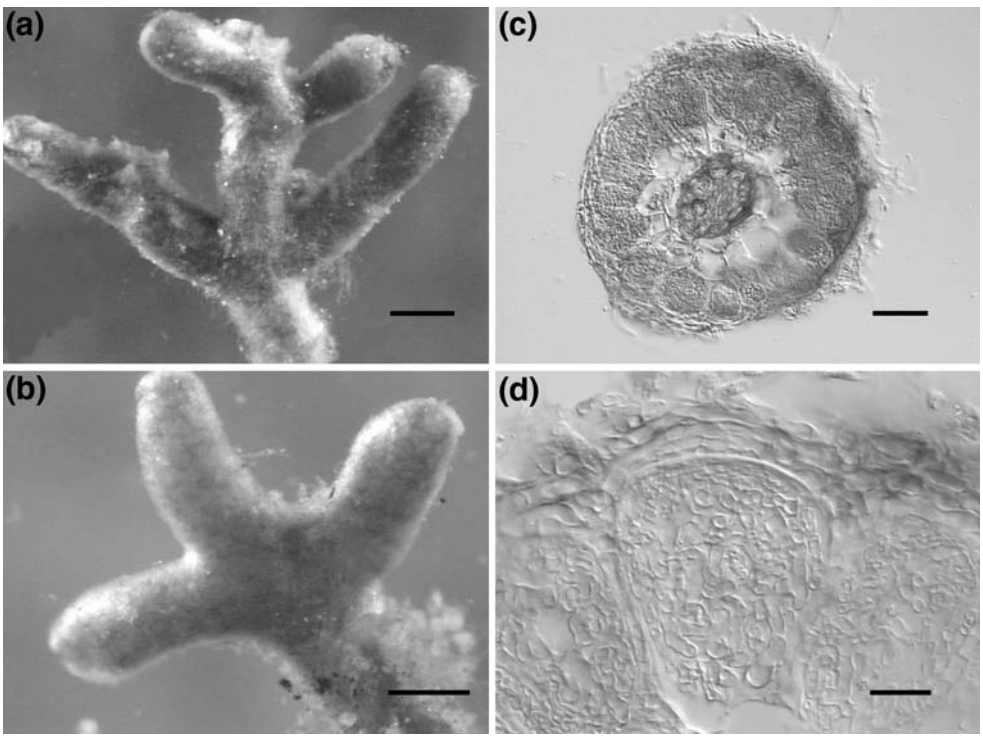

Fig. 1 Arbutoid mycorrhizae of *C. arbutoides*/*L. monticola*. **a**, **b** External morphology of branched arbutoid mycorrhizal roots. **c** Root cross-section showing the hyphal sheath and epidermal cells with intracellular hyphae. **d** Magnified view of root cross-section showing intracellular hyphae. Scale bars: 1 mm (**a**, **b**); 200 μm (**c**); 50 μm (**d**)

extracted from *C. arbutoides* leaf tissue. Comparison of an approximately 700-bp rDNA ITS1 sequence showed nearly complete identity between mycobiont DNA from the mycorrhizal root tip and DNA extracted from *L. monticola* basidiome tissue, with the exception of nucleotide transitions (C-T) in two sequence positions (i.e., >99.7% sequence identity).

Sequence analysis showed that the ITS1 region of *L. monticola* contains an insert composed largely of minisatellite repeat sequences similar to that previously observed in other *Leccinum* species (Binder 1999; den Bakker et al. 2004a). The minisatellite region in *L. monticola* has a length of 552 bp. Like previously described *Leccinum* minisatellite regions (den Bakker et al. 2004a), it is composed largely of two core sequences: "a," a 12-bp sequence consisting of (CTTATTGAAAAG) or derivatives, and "b," an 11-bp sequence consisting of (CTAATA GAAAG) or derivatives (Fig. 2). The analyzed *L. monticola* accession contains 30 core sequences in an a:b ratio of 15:15, with core sequences in the arrangement (ababababababaabbabaabbabaabbab). The number and order of core sequences in *L. monticola* is thereby identical to that observed in European accessions of *Leccinum piceinum* and *Leccinum duriusculum* (den Bakker et al. 2004a). Examination of five fungal ITS clones obtained from root tips showed no apparent length heterogeneity between clones and no apparent length difference when compared to a clone derived from basidiome material (data not shown).

Analysis using the Tandem Repeats Finder (Benson 1999) revealed tandemly repeated sequences of two repeats of 34 bp, present twice in the sample (the two occurrences differing only by the presence of an additional adenine residue at the 5′ end of the second occurrence), and a 2×17-bp repeat that partially overlaps the second 2×34-bp repeat (Table 1). The structure of tandem repeats in *L. monticola* is most similar to that of a *L. piceinum* accession (Austrian *L. piceinum* clone 1) analyzed by den Bakker et al. (2004a). In the *L. piceinum* accession, the 2×34 and 2×17 repeats occur within a 3×116-bp tandem repeat. Although a 3×116-bp repeat was not identified in *L. monticola* by the Tandem Repeats Finder, the insert size, order of core sequences, and position and characteristics of the smaller tandem repeats are nearly identical to those observed for *L. piceinum* clone 1. Furthermore, tandem repeats may fail to be identified by the Tandem Repeats Finder when the repeats differ significantly due to mutation (den Bakker et al. 2004a).

Table 1 Results of the Tandem Repeats Finder analysis of the minisatellite insert region of the internal transcribed spacer 1 sequence of *L. monticola* from Cerro de la Muerte, Costa Rica

Period size	Copy number	% Matches	Sequence position
34	2.1	86	271–342
34	2.2	90	387–460
17	1.9	100	444–476

```
ATTAATGAATTTGGAGGCTGTCGCTGGCTAGGTGATTCTAGCATGTGCAC   [50]
GTCTACACTTTTAAACACACTTGTGAACCCATTGTAGATCGAGTCCATCG   [100]
AACTACCTAATAGAGGGCTAATTGGCTTATCGAAAAGTAACCTAATAGAG   [150]
GCTTATCGAAAAGTAGTGTGAACTACCTAATAGAAAGCTTACCGAAAAGT   [200]
ACCTAATAGAGAGAGCTAGTTGGCTTATCGAAAAGTGGTGAGAAACTACC   [250]
TAATAGAGGGCTGAGTTGGCTCATTGAAACGTAGTTAATAGAGGGCTGGT   [300]
TGGCTTATCGAAAAGTATGAGGCTTATTGAAAAGTAGGTGTGAAACTACC   [350]
TAATAGAAAGCTTAGTACCTAATAGAGGGCTAGTTGGCTTATCGAAAAGT   [400]
ACCTAATAGAAGGTTGGTTGGCTTATTGAAAAGTATGAAGCTTATTGAAA   [450]
AGTAGTGTAAACTACCTAATAGAAAGCTCAAGTACCTAATAGAAGGCTAG   [500]
TTGACTTGTCGAAAAGTACCTAATAGAAGGTTAGTTGGCTTATTGAAAAG   [550]
TACGGCTTATTGAAAAGTAGTTTGAACTACCTAATAGAAAGCTTAGTACC   [600]
TAATAGAGAGCTAGTTGGCTTATCGACAGACTACCTAATAGAGGGACCCC   [650]
GGAAGATCTATGTT                                       [664]
```

Fig. 2 *L. monticola* ITS1 sequence obtained from basidiome tissue. Minisatellite core sequences as identified by den Bakker et al. (2004a) are underlined

Discussion

Morphological and molecular data provide strong evidence supporting an arbutoid mycorrhizal association between *C. arbutoides* and *L. monticola* in the Costa Rican páramo. Beyond verifying this specific association, the results of this study suggest that arbutoid mycorrhizal associations may play an important role in maintaining populations of the dominant plant species in this ecosystem. A similar role has been suggested for ectomycorrhizal fungi in high-elevation temperate (i.e., alpine) habitats (e.g., Cripps and Eddington 2005; Gardes and Dahlberg 1996; Graf 1994; Senn-Irlet 1988). *L. monticola* was originally encountered in subpáramo habitats containing *Q. costaricensis*, and it was thought that this oak species was the mycorrhizal host (Halling and Mueller 2003). However, *L. monticola* was subsequently found in páramo habitats where *C. arbutoides* is the dominant cover plant and *Q. costaricensis* is lacking, and *C. arbutoides* was found to be sympatric with *Q. costaricensis* in the subpáramo habitats where *L. monticola* was originally found. These field data, combined with the results of the present study, strongly indicate that *L. monticola* forms mycorrhizae either solely with *C. arbutoides* or with both *C. arbutoides* and *Q. costaricensis*. Field data support the former scenario, as *L. monticola* has thus far not been reported with *Q. costaricensis* where *C. arbutoides* was lacking. However, further field studies of the type reported here, as well as in vitro mycorrhizal resynthesis experiments, are necessary before the host specificity limits of *L. monticola* can be accurately delimited. *L. monticola* populations have been observed to produce large numbers of basidiomes on a yearly basis and

over a significant portion of the rainy season (R.E. Halling & G.M. Mueller, unpublished data), suggesting a high energetic investment in reproduction and the possibility of requiring high levels of root colonization and/or high carbon sink strength to support such an investment.

The characterization of an arbutoid mycorrhizal relationship in a páramo ecosystem is significant both in terms of biogeographic patterns and in terms of ecological and conservation relevance. Members of the *L. manzanitae* Thiers species group, closely related to *L. monticola* (den Bakker et al. 2004b), have been reported in association with the arbutoid genera *Arbutus* and *Arctostaphylos* in the western US (Acsai and Largent 1983; Thiers 1975). Both of these genera have ranges that overlap with that of *Comarostaphylis* (which has its center of diversity in Mexico; Diggs 1995). The results of a previous phylogenetic study (den Bakker et al. 2004b) and the similarity of structure in the ITS1 minisatellite-like region (den Bakker et al. 2004a) suggest that a recent radiation of arbutoid-associated *Leccinum* species has occurred.

Previous research has suggested that *Arbutus* and *Arctostaphylos* exhibit low mycorrhizal specificity and may play an important role in forest succession by providing shared mycorrhizal networks that support the establishment of trees such as *Pseudotsuga menziesii* (Mirb.) Franco (Horton et al. 1999; Molina and Trappe 1982; Molina et al. 1992; Trappe and Fogel 1977). Currently, it is unknown whether *Comarostaphylis* plays a similar role in neotropical forests, supporting the establishment of trees such as *Q. costaricensis*. Examination of *Q. costaricensis* mycorrhizae from adjacent subpáramo forests could provide interesting insights into such a relationship. The further characterization of specific associations in the Arbutoideae will contribute to untangling the ecology and evolutionary history of mycorrhizal formation in the Ericaceae as a whole.

Molecular characterization of the ITS1 spacer in *L. monticola* revealed two interesting characteristics, namely, a difference in two nucleotide positions (both T-C transitions) between basidiome and mycorrhizal root sequences, and the presence of a minisatellite-containing insert. The high similarity (>99.7%) between the basidiome and mycorrhizal sequences is strongly suggestive of conspecificity, though the lack of complete sequence identity suggests intraspecific genetic variation within a very small area. This result is concordant with previous studies that have identified the presence of multiple fungal genets within a single root system in ericoid (Allen et al. 2003) and ectomycorrhizal (Gryta et al. 1997) plants. The identification of a minisatellite-containing insert in the ITS1 region of *L. monticola* further confirms the widespread occurrence of such inserts in species of *Leccinum*. The minisatellite region in *L. monticola* is extremely similar to that observed in an accession of *L. piceinum*, a similarity consistent with the close phylogenetic relationship between the two species indicated by a molecular phylogenetic analysis of glyceraldehyde 3-phosphate dehydrogenase sequences (den Bakker et al. 2004b). The similarity of the ITS1 insert between these species indicates that there may be some conservation of insert structure within clades and that insert structure may therefore be useful in some cases for diagnosis above the species level. However, den Bakker et al. (2004a) caution that homoplasy in insert structure at the generic level makes the region unsuitable for use in phylogenetic reconstruction.

The present study, while suggesting the importance of arbutoid mycorrhizae in páramo ecology, represents only a first step toward understanding the structure, function, and dynamics of páramo mycorrhizal communities. Important steps such as demonstrating carbon transfer between the photobiont and mycobiont organisms, examining the level of host specificity of *L. monticola*, and fully characterizing the composition of the mycorrhizal community associated with *C. arbutoides*, have yet to be completed. Additional mycorrhizal genera (e.g., *Tricholoma*, *Cortinarius*, *Russula*, and *Laccaria*; unpublished data) collected in the study area are other likely associates of *C. arbutoides*. Further characterization of these arbutoid mycorrhizal communities is important in fostering a better understanding of the evolution of mycorrhizal associations in the Arbutoideae and the ecology and conservation of páramo habitats.

Acknowledgements We wish to thank Maria Alice Neves for field assistance, and Lisa Campbell, Susan Pell, and Lawrence Kelly for assistance with laboratory methods. Funding support was provided by the National Science Foundation (DEB-9972018 and DEB-9972027), National Geographic Society Committee for Research and Exploration, and the Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies (NYBG). Further information on macrofungi of the Costa Rican páramo is available from the Macrofungi of Costa Rica project web site (http://www.nybg.org/bsci/res/hall/index.html). Photographs of páramo habitat, *C. arbutoides*, and *L. monticola* are available on the project web site and in Halling and Mueller (2006).

References

Acsai J, Largent D (1983) Fungi associated with *Arbutus menziesii, Arctostaphylos manzanita*, and *Arctostaphylos uva-ursi* in central and northern California. Mycologia 75:544–547

Agerer R (1987–2002) Colour atlas of Ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmünd, Munchen, Germany

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

Anderson L (1954) Hoyer's solution as a rapid permanent mounting medium for bryophytes. The Bryologist 57:242–244

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Bidartondo MI, Bruns TD (2001) Extreme specificity in epiparasitic Monotropoideae (Ericaceae): widespread phylogenetic and geographical structure. Mol Ecol 10:2285–2295

Binder M (1999) Zur molekularen Systematik der Boletales: Boletineae und Sclerodermatineae subordo nov. Dissertation, University of Regensburg, Germany

Brundrett M (2004) Diversity and classification of mycorrhizal associations. Biol Rev 79:473–495

Cripps C, Eddington L (2005) Distribution of mycorrhizal types among alpine vascular plant families on the Beartooth Plateau, Rocky Mountains, USA, in reference to large-scale patterns in Arctic-alpine habitats. Arct Antarct Alp Res 37:177–188

den Bakker HC, Gravendeel B, Kuyper TW (2004a) An ITS phylogeny of *Leccinum* and an analysis of the evolution of minisatellite-like sequences within ITS1. Mycologia 96:102–118

den Bakker HC, Zuccarello GC, Kuyper TW, Noordeloos ME (2004b) Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. New Phytol 163:201–215

Diggs GM (1995) *Comarostaphylis*. In: Luteyn JL (ed) Ericaceae, Part II. The superior-ovaried genera. New York Botanical Garden Press, Bronx, NY, pp 146–193

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, Dahlberg A (1996) Mycorrhizal diversity in arctic and alpine tundra: an open question. New Phytol 133:147–157

Graf F (1994) Ecology and sociology of macromycetes in snow-beds with *Salix herbacea* L. in the alpine Valley of Radont (Grisons, Switzerland). J. Cramer, Berlin–Stuttgart

Gryta H, Debaud J-C, Effosse A, Gay G, Marmeisse R (1997) Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. Mol Ecol 6:353–364

Guidot A, Debaud J-C, Marmeisse R (2001) Correspondence between genet diversity and spatial distribution of above- and below-ground populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Mol Ecol 10:1121–1131

Halling RE, Mueller GM (2003) *Leccinum* (Boletaceae) in Costa Rica. Mycologia 95:488–499

Halling RE, Mueller GM (2006) Common mushrooms of the Talamanca mountains, Costa Rica. New York Botanical Garden Press, Bronx, NY

Harley JL (1969) Biology of mycorrhiza, 2nd edn., Leonard Hill, London

Horton TR, Bruns TD, Parker VT (1999) Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Can J Bot 77:93–102

Kappelle M, van Vuuren MMI, Baas P (1999) Effects of climate change on biodiversity: a review and identification of key research issues. Biodivers Conserv 8:1383–1397

Kron KA, Judd WS, Stevens PF, Crayn DM, Anderberg AA, Gadek PA, Quinn CJ, Luteyn JL (2002) Phylogenetic classification of Ericaceae: molecular and morphological evidence. Bot Rev 68:335–423

Luteyn JL (1999) Páramos: a checklist of plant diversity, geographical distribution, and botanical literature. New York Botanical Garden Press, Bronx, NY

Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skean JD (2004) A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. Taxon 53:279

Molina R, Trappe JM (1982) Lack of mycorrhizal specificity by the ericaceous hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi*. New Phytol 90:495–509

Molina R, Massicotte H, Trappe J (1992) Specificity phenomena in mycorrhizal symbioses: community–ecological consequences and practical implications. In: Allen M (ed) Mycorrhizal functioning: an integrative plant–fungal process. Chapman and Hall, New York, pp 357–423

Senn-Irlet B (1988) Macromycetes in alpine snow-bed communities—mycocoenological investigations. Acta Bot Neerl 37:251–263

Setaro S, Kottke I, Oberwinkler F (2006a) Anatomy and ultrastructure of mycorrhizal associations of neotropical Ericaceae. Mycol Prog 5:243–254

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

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, San Diego, CA

Stanford AM, Harden R, Parks CR (2000) Phylogeny and biogeography of *Juglans* (Juglandaceae) based on matK and ITS sequence data. Am J Bot 87:872–882

Thiers HD (1975) California mushrooms: a field guide to the boletes. Hafner Press, New York

Trappe JM, Fogel RD (1977) Ecosystematic functions of mycorrhizae. In: Marshall JK (ed) The belowground ecosystem: a synthesis of plant-associated processes. Colorado State University Press, Fort Collins, CO, pp 205–214