Fine-level mycorrhizal specificity in the Monotropoideae (Ericaceae): specificity for fungal species groups

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Abstract
The Monotropoideae (Ericaceae) are nonphotosynthetic angiosperms that obtain fixed carbon from basidiomycete ectomycorrhizal fungi. In previous work, we showed that each plant species is associated with a single genus or a set of closely related genera of ectomycorrhizal fungi. Here we show that the level of specificity is much higher. We used a molecular phylogenetic approach to contrast specificity patterns among eight plant lineages and three fungal genera. We relied on fungal nuclear internal transcribed spacer (nrITS) sequence data obtained from 161 basidiocarps and 85 monotropoid roots representing 286 sampled plants screened using restriction length polymorphisms. From the phylogenetic placement of fungal symbionts in fungal phylograms, we found that three basal (Sarcodes, Pterospora, Pleurotus) and one derived lineage (Allotropa) of plants target narrow clades of closely related species groups of fungi, and four derived lineages (Monotropa hypopithys species group, Pityopus) target more distant species groups. Within most plant lineages, geography and photobiont association constrain specificity. Specificity extended further in Pterospora andromedea, in which sequence haplotypes at the plastid trnL–F region of 73 plants were significantly associated with different fungal species groups even in sympatry. These results indicate that both the macro- and microevolution of the Monotropoideae are tightly coupled to their mycorrhizal symbionts.

Keywords: coevolution, Monotropa, mycorrhizae, specialization, Tricholoma

Introduction
Myco-heterotrophic plants acquire fixed carbon from their mycorrhizal fungi and they have arisen independently multiple times in angiosperm evolution (Leake 1994). Many of these plants are epiparasitic because they obtain fixed carbon indirectly from a neighbouring photosynthetic plant through a shared mycorrhizal fungus (Björkman 1960; Duddridge & Read 1982; Cullings et al. 1996; Taylor & Bruns 1997; McKendrick et al. 2000; Bidartondo & Bruns 2001). Thus, mycorrhizal epiparasites are exploiters of one of the most widespread and ancient interspecific mutualisms. All mutualisms are susceptible to exploiters or cheaters, species that can obtain rewards without providing service in return (Janzen 1975; Inouye 1983; ‘aprovechados’ sensu Soberon & Martinez 1985; Bronstein 1994, 2001). Much doubt has been cast on the legitimacy of the most classic example of cheating, nectar-robbing, because robbers can and do pollinate (Maloof & Inouye 2000). Other examples are legitimate, such as the seed-eating nonpollinating yucca moths and fig wasps, because the insects lack the morphological traits required for pollen manipulation (Fellmyr & Leebens-Mack 2000) or their contribution to pollination is negligible (Compton et al. 1991). Nonphotosynthetic mycorrhizal exploiters are legitimate exploiters because, unlike green plants, they are unable to provide fixed carbon to their mycorrhizal fungi. Indeed, many lack chlorophyll b and have only traces of chlorophyll a (Cummings & Welschmeyer 1998). Even though in symbiotic interactions the larger symbiont is conventionally termed ‘host’ and in mycorrhizal interactions the plant is always the ‘host’, here we use this term to refer to the mycorrhizal fungi to imply that the fungi are the ‘lineages being tracked’ (sensu Page & Charleston 1998) by epiparasitic plants.

The Monotropoideae (Ericaceae) is a diverse subfamily of 10 genera of nonphotosynthetic mycorrhizal epiparasites
that associate with five distant families of ectomycorrhizal basidiozymcute fungi (Bidartondo & Bruns 2001). The centre of diversity of the Monotropoidea is western North America (eight genera), but they occur in evergreen and deciduous ectomycorrhizal forests throughout the northern hemisphere (dominated by Pinaceae, Fagaceae, Salicaceae, Dipterocarpaceae) (Wallace 1975). In a previous study, we showed that each plant lineage specializes on a single fungal genus, or in the case of Monotropa uniflora on two closely related genera (Bidartondo & Bruns 2001). In this study, we examine the pattern of symbiont affiliaton at a finer phylogenetic scale for eight plant lineages, and show that specificity patterns are much more narrow than previously realized. We use this information to address basic questions about mycorrhizal cheating. How diverse is the range of mutualists with which cheaters interact (Bronstein 1994)? What is the pattern of specificity when sympatric related plants (e.g. sister taxa) target closely related fungi (e.g. within a single genus)? Is there exclusive symbiont fidelity or is there symbiont overlap? Is exploiter phylogeny congruent with fungal phylogeny at low phylogenetic levels? What biological features are associated with the patterns of cheater specificity and radiation (Pellmyr & Leebens-Mack 2000)?

Detailed knowledge of symbiont phylogenies is required to interpret the phylogenetic pattern of host association, and so phylogenetically explicit studies of the evolution of cheaters are still few (Pellmyr & Leebens-Mack 1999). The evolutionary relationships of the plants involved in the monotropoid mycorrhizal symbiosis have been re-examined recently (Bidartondo & Bruns 2001), but the evolutionary relationships of the fungi involved are poorly understood. To remove this problem, we built the evolutionary relationships of the fungi involved in the monotropoid mycorrhizal symbiosis have been re-examined recently (Bidartondo & Bruns 2001). A list of sampling localities follows. Monotropa hypopithys N. Am: Albany County (WY), Mary’s Pk., Benton Co. (OR), Chittenden Co. (VT), Perkins Cr., Silicoos Lk., Lane Co. (OR), Crescent Lk., Klamath Co. (OR), Umpqua N.F., Douglas Co. (OR); M. hypopithys Sweden: Hakeberga, Orckellunga, Janne, Skane Prov. (SE), Uppland Prov. (SE); M. hypopithys Eurasia: Derbyshire (UK), Anglesey (UK), Iwate Prefecture (JP), Lappeenranta (FI), Dalby, North Yorkshire (UK), Sylvaäkä, Skåne Prov. (SE); Pityopus californicus: Mary’s Pk., Benton Co. (OR), Umpqua N.F., Douglas Co. (OR), Perkins Cr., Lane Co. (OR), Whitaker, Tulare Co. (CA); Allstrope virgata: Siskiyou N.F., Curry Co. (OR), Umpqua N.F., Douglas Co. (OR), Perkins Cr., Waxyntyle, Lane Co. (OR), Stevensom S.P., Napa Co. (CA), Crescent Lk., Klamath Co. (OR); Pleurocortus fimbriolata: Umpqua N.F., Douglas Co. (OR), Gaddis Road, Stumpy Mds., Blodgett, Lk. Tahoe, El Dorado Co. (CA), Sierra N.F., Dinkey Cr., Fresno Co. (CA), Perkins Cr., Lane Co. (OR), Lk. Almanor, Plumas Co. (CA), Sequoia N.P., Tulare Co. (CA); Sarcodes sanguinea: Siskiyou N.F., Curry Co. (OR), Lk. Tahoe, El Dorado Co. (CA), Dinkey Cr., Sierra N.F., Fresno Co. (CA), Mt. Abel, Kern Co. (CA), Yuba Pass, Nevada Co. (CA), Quincy, Fredonry Summit, Plumas Co. (CA), San Bernardino Co. (CA), Mt. Pinos, Ventura Co. (CA); Hart, Sequoia N.P., Tulare Co.; Pterospora andromedea: Chuska Mts., Apache Co. (AZ), Blodgett, El Dorado Co. (CA), Sierra N.F., Fresno Co. (CA), Siskiyou N.F., Josephine Co. (OR), Crescent Lk., Klamath Co. (OR), Round Valley, Quincy, Plumas Co. (CA), Whitaker, Tulare Co. (CA), Quebec Prov. (Canada).

Identification of fungal lineages
We identified fungi using methods described by Gardes & Bruns (1996). In summary, we extracted genomic DNA from individual monotropoid root tips and amplified the ITS of the nuclear ribosomal repeat using a polymerase chain reaction (PCR) with the fungal-specific primer pair ITS1F/ITS4 (White et al. 1990; Gardes & Bruns 1993). We screened PCR products by restriction fragment length polymorphisms (RFLP) using the restriction endonucleases...
Low phylogenetic breadth in epiparasites

We selected 73 Pterospora andromedea plants from populations in the Sierra Nevada of California where some plants associated with the Rhizopogon salebrosus species group and others with the R. arctostaphyli species group. The plastid trnL intron and trnL–F intergenic spacer regions were amplified from root DNA extracts using primers c and f (Taberlet et al. 1991). These were then sequenced. We tested for haplotype–fungal symbiont association with a G2 likelihood ratio $\chi^2$ test using JMP (SAS Institute, Cary, NC, USA).

DNA sequencing

Sequencing of both strands was performed with an ABI model 377 Sequencer (Applied Biosystems Co., Foster City, CA, USA) using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Co.) or a Thermo Sequenase™ Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). We then sequenced the ITS region revealed that specific epithets were not applied consistently to other specimens. We extracted DNA from 136 basidiocarps and sequenced their nrITS region. We did not rely on ITS–RFLP comparisons (Kårén et al. 1997; Taylor & Bruins 1999) because these alone can be insufficient to discriminate closely related species groups (Kretzer et al. 2000). A list of herbaria and specimen vouchers follows.

Low phylogenetic breadth in epiparasites

Alignments were produced with clustal x (Thompson et al. 1997), adjusted manually using a colour font, analysed by neighbour joining and parsimony, and bootstrapped (1000 replicates) using paup Version 4.0b8 (Swofford 2001). Neighbour joining was used with the Kimura 2-parameter model. In the Rhizopogon section Amylopogon and in the Allotropa–Pituyopsis–Monotropa hypopithys sequence alignments, we coded informative indels as in Kretzer et al. (2000). Because of computational difficulties associated with parsimony analysis of the large data sequence sets on a Power Macintosh G4 (Apple Computer Inc., Cupertino, CA, USA), we used a stepwise approach to remove taxa until the analyses were feasible. First, from sets of identical sequences only one was chosen arbitrarily and retained. Second, from sets of sequences differing by 1 or 2 bp only one was chosen arbitrarily and retained. These steps sufficed for Rhizopogon section Amylopogon and Gauteria. For Tricholoma, the largest data

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set, we used an additional third criterion: only one arbitrarily chosen sequence from each clade with > 95% distance bootstrap support (in the full data set) was retained for parsimony analysis. *Tricholoma* and *Rhizopogon* section *Amylopegon* were midpoint rooted. *Gautieria* was rooted with *Kamaria botrytis* based on results from nrLSU and mtSSU analyses (Humphert et al. 2001). The resulting phylogenograms were compared with the latest classification schemes such as those of Riva (1988), Noordeloos & Christensen (1999), and Shanks (1997) for *Tricholoma*, Smith & Zeller (1986) for *Rhizopogon* section *Amylopegon* and Stewart (1974) for *Gautieria*. Because we knew that there were multiple nrDNA lineages within *Monotropa hypopithys* and *Pityopus californicus* (Bidartondo & Bruns 2001), we tested congruency between plant ITS phylogeny and fungal ITS phylogeny with a partition homogeneity test (Farris et al. 1994) using 1000 random taxon addition sequences in PAUP excluding parsimony-uninformative characters. In this analysis we included *Allotropa virgata*, and excluded all *Tricholoma* species not associated with Monotropoideae lineages. We used 17 plant ITS sequences from Bidartondo & Bruns (2001) and 14 additional plant ITS sequences.

**Results**

All *Pterospora andromedea* symbions were restricted to the *Gautieria monticola* species group in the Sierra Nevada of California and the southern Cascade Range in Oregon (Fig. 1). The full data set for *Gautieria* had 55 fungal taxa and 649 characters. The parsimony data set had 27 fungal taxa and 108 parsimony-informative characters, which produced 16 most parsimonious trees. The systematics of taxa and 108 parsimony-informative characters, which produced 1890 most parsimonious trees. The strict consensus topology mirrors that of the parsimony trees are indicated on the neighbour joining phylogram in Fig. 2. *Rhizopogon* is also a taxonomically challenging genus, and our preliminary analyses of specimen collections revealed that specific epithets applied to specimens often did not correspond to the phylogenetic placement of holotype specimens. Even paratypes were often found to significantly differ from holotypes. For this reason, in Fig. 2 specific epithets were removed from all specimens except type specimens.

All *Allotropa virgata* symbions were restricted to the *Tricholoma magnivelare* species group. *Pityopus californicus* and *Monotropa hypopithys* are closely associated with various paratypes were often found to significantly differ from holotypes. For this reason, in Fig. 2 specific epithets were removed from all specimens except type specimens.

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Fig. 1. Phylogenetic placement of mycorrhizal associates of *Pleuricospora fimbriolata* within the basidiomycete fungal genus *Gautieria*. This fungal nuclear internal transcribed spacer (nrITS) phylogram is based on neighbour joining (full data set), bootstrap (full data set, values > 70 are near branches), and parsimony analysis (restricted data set) of 36 basidiocarp sequences, and 18 sequences from monotropoid roots representing 13 populations and 42 plants. Symbols for sequences from monotropoid roots are followed by a locality and population/plant number. Branches present in the strict parsimony consensus are in bold. Bootstrap values are near branches. *Ramaria botrytis* was used as the outgroup.
Fig. 2 Phylogenetic placement of mycorrhizal associates of *Sarcodes sanguinea* and *Pterospora andromedea* within the basidiomycete fungal genus *Rhizopogon* (the upper left small phylogram is from Grubisha et al. 2002), section *Amylopogon* (large phylogram). The large fungal nuclear internal transcribed spacer (nrITS) phylogram is based on neighbour joining (full data set), bootstrap (full data set, values > 70 are near branches), and parsimony analysis (restricted data set) of 51 new basidiocarp sequences, 15 basidiocarp sequences from Kretzer et al. (2000), 26 new sequences from monotropoid roots, seven monotropoid root sequences from Kretzer et al. (2000) and 1 sequence from a *Pinus muricata* root. Species names have only been retained for sequences from type specimens (HT, arrow: holotype; PT: paratype).
Fig. 3 Phylogenetic placement of mycorrhizal associates of *Monotropa hypopithys* N.Am., *M. hypopithys* Eurasia, *M. hypopithys* Sweden, *Pityopus californicus* and *Allotropa virgata* within the basidiomycete fungal genus *Tricholoma*. This fungal nuclear internal transcribed spacer (nrITS) phylogram is based on neighbour-joining (full data set), bootstrap (full data set, values > 70 are near branches) and parsimony analysis (restricted data set) of 10 basidiocarp sequences from GenBank, 49 new basidiocarp sequences, and 34 sequences from monotropoid roots representing 33 populations and 73 plants. Symbols for sequences from monotropoid roots are followed by locality and population/plant number(s). Branches present in the strict parsimony consensus are in bold. Selected putative photobiont associations and Riva’s (1988) sectional classification are to the right.
The contingency table for the *Pterospora andromedea* plastid *trn* L–F haplotypes and fungal symbiont identity were used for a G2 likelihood ratio \( \chi^2 \) test (Table 1). The results of the test show a significant departure from expectations of random assortment between plant haplotypes and fungal species groups (\( P < 0.0001 \), likelihood ratio = 35.035, \( df_{\text{error}} = 64 \)). Five distinct haplotypes were scored. Haplotypes 4 and 5 were characterized by conserved 14 and 7 bp insertions, respectively. Linked single indels and substitutions were also detected. Recombinant haplotypes were not observed. Every haplotype, except haplotype 5, was detected co-occurring with every other haplotype at least at one sampled site (data not shown). Haplotypes 2 and 5 were excluded from the statistical test because they were too rare. One DNA sequence per haplotype was submitted to GenBank.

the northern hemisphere, shifting among fungal genus (plants are sister taxa and they target the same section of one M. hypopithys sticky mass, but are wind-dispersed and Rhizopogon both taxa have a similar phylogenetic breadth of associated sticky mass and are more distant fungal species groups within a single genus. This is even narrower specificity than previously shown (Bidartondo & Bruns 2001) and indicates that recent evolutionary radiations have been tightly constrained to host-shifts within fungal genera. This is impressive because Monotropoideae species are massively prolific producers of dust-like seeds (each inflorescence of M. hypopithys produces in excess of 2×10^6 seeds < 0.5 mm in diameter; Bakshi 1959), many are wind-dispersed (Wallace 1975), and their habitats are characterized by high mycorrhizal fungal diversity (Bidartondo et al. 2000). All of these factors should provide opportunities for frequent radical jumps (sensu Roy 2001). Instead, in the Monotropoideae the genetic basis of specificity is a stronger determinant of diversification than ecological opportunity. Furthermore, adaptations for dispersal are decoupled from fine-level mycorrhizal phylogenetic breadth: sister taxa with very different adaptations have similar breadth and sister taxa with very similar adaptations differ. Sarcodes species that are not found associated with mature plants (Bruns & Read 2000). This suggests that the first constraint on jumps may be host chemistry, which is probably shared among related hosts. The second constraint must involve physiological interactions between the germinating seed and the potential fungal host. Positive interactions between the plant and the fungus recognize the plant as a parasite, lead to rejection. The former fails if the genetic match is not adequate. The latter, essentially a co-evolutionary arms race (Thompson & Burdon 1992), fails if the fungus has been selected to recognize the plant. Either of these mechanisms might lead to shifts among related host taxa that were initially recognized by a positive germination cue. However, the arms

Table 1 Contingency table for Pterospora andromedas plastid trn L–F haplotypes and fungal symbiont identity (Rhizopogon salebrosus or R. arctostaphylli). There is a significant association between plastid haplotype and mycorrhizal fungal associate (P < 0.0001). Every haplotype, except haplotype 5, was detected co-occurring with every other haplotype at least at one sampling site (data not shown).

<table>
<thead>
<tr>
<th>Fungal symbiont</th>
<th>Haplotype 1</th>
<th>Haplotype 2</th>
<th>Haplotype 3</th>
<th>Haplotype 4</th>
<th>Haplotype 5</th>
</tr>
</thead>
<tbody>
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<td>Rhizopogon salebrosus</td>
<td>4</td>
<td>3</td>
<td>16</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Rhizopogon arctostaphylli</td>
<td>30</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Mycorrhizal specificity

Our findings indicate that each lineage of the Monotropoideae exhibits specificity for select species groups within single fungal genera (Figs 1–3). We found that three basal (Sarcodes, Pterospora, Pleuricospora) and one derived plant lineage (Allotropa) target single or very closely related species groups of fungi, and four derived sister lineages (Monotropa hypopithys group, Pitheopus) target more distant fungal species groups within a single genus. This is even narrower specificity than previously shown (Bidartondo & Bruns 2001) and indicates that recent evolutionary radiations have been tightly constrained to host-shifts within fungal genera. This is impressive because Monotropoideae species are massively prolific producers of dust-like seeds (each inflorescence of P. andromedas produces in excess of 2×10^6 seeds < 0.5 mm in diameter; Bakshi 1959), many are wind-dispersed (Wallace 1975), and their habitats are characterized by high mycorrhizal fungal diversity (Bidartondo et al. 2000). All of these factors should provide opportunities for frequent radical jumps (sensu Roy 2001). Instead, in the Monotropoideae the genetic basis of specificity is a stronger determinant of diversification than ecological opportunity. Furthermore, adaptations for dispersal are decoupled from fine-level mycorrhizal phylogenetic breadth: sister taxa with very different adaptations have similar breadth and sister taxa with very similar adaptations differ. Sarcodes species that are not found associated with mature plants (Bruns & Read 2000). This suggests that the first constraint on jumps may be host chemistry, which is probably shared among related hosts. The second constraint must involve physiological interactions between the germinating seed and the potential fungal host. Positive interactions between the plant and the fungus recognize the plant as a parasite, lead to rejection. The former fails if the genetic match is not adequate. The latter, essentially a co-evolutionary arms race (Thompson & Burdon 1992), fails if the fungus has been selected to recognize the plant. Either of these mechanisms might lead to shifts among related host taxa that were initially recognized by a positive germination cue. However, the arms
race model provides a mechanism for partitioning of fungal associates between Monotropoideae species when all partners occur in sympatry, as there would be strong selection for recognition. Nonetheless, the selective pressure to resist an exploiter is likely lower than that against a costly pathogen (Levin & Lenski 1985; Bronstein 2001). The arms race model also provides selection for races of the plants that are specialized on particular hosts, as may be the case in *Pterospora*. It predicts that host shifts might be more likely when seeds encounter naive fungal populations, e.g. after rare dispersal events.

The Monotropoideae as a whole, and the *M. hypopitys* complex in particular, show that obligate exploiters of obligate mutualisms are not necessarily species-poor lineages, as documented in seed-eating nonpollinator (i.e. cheating) fig wasps and yucca moths; there is one described obligate cheat associated species in the wasps that pollinates 750–800 species of figs (Compton et al. 1991), and two narrow lineages of cheaters among the over 15 species of moths that pollinate yuccas (Pellmyr & Leebens-Mack 2000). To elucidate how common adaptive radiations of obligate mycorrhizal exploiters have been, it is necessary to examine the other nine vascular plant families in which nonphotosynthetic mycorrhizal lineages have evolved (Leake 1994) and also determine: (i) whether the plants target mycorrhizal fungi associated with green plants; (ii) whether they are derived from the breakdown of mutualism; (iii) what are the evolutionary relationships of the plants; and (iv) idem for the fungi. In return, these mycorrhizal systems can contribute to developing a general theory for the evolution of parasites amid mutualists. For instance, the obligate need for the coexistence of multiple species has been emphasized as a critical barrier to the stability of cheating (Compton et al. 1991; Pellmyr & Leebens-Mack 2000), but this barrier may be lowered for mycorrhizal cheaters because the mycorrhizal mutualism is diffuse (i.e. a single fungal interact simultaneously with several green plants and single green plants interact simultaneously with several fungi), which leads to highly diverse mycorrhizal communities even at local spatial scales (e.g. 130–350 species/hectare; Bruns 1995).

The observation that mycorrhizal epiparasitic plants in the Ericaceae (Cullings et al. 1996; Kretzer et al. 2000; Bidartondo & Bruns 2001; this study) and Orchidaceae (Taylor & Bruns 1997, 1999) display high levels of mycorrhizal specificity is notable on several grounds: (i) regardless of how much functional redundancy there may be within mycorrhizal communities, from an epiparasitic plant’s perspective, mycorrhizal fungi are unique, which is striking because mycorrhizal fungal communities are impressively diverse; (ii) there are no known morphological or ecological traits shared by the fungi associated with the Monotropoideae, other than their obligate ectomycorrhizal habit; and (iii) epiparasites are one of the few known systems involving extreme specialization toward fungi, which have been considered to have exceptionally few specialized parasites (Jaenike 1978; Lacy 1984; Hanski 1989).

In addition, the *Pterospora andromedea* results show that additional specificity may exist at the plant genotype level. A similar observation comes from the nonphotosynthetic orchid *Corallorhiza maculata* in which individual plant genotypes only form mycorrhizae with narrow clades of the ectomycorrhizal basidiomycete family Russulaceae and their specificity is maintained regardless of co-occurrence with other genotypes (D.L. Taylor, personal communication). The latter suggests that crossing is limited. This raises the question of what factors select for the evolution and maintenance of mycorrhizal specificity in lineages of epi-parasites, as it is difficult to envision how mycorrhizal interactions could lead directly to genetic isolation. Note that no nuclear sequence variation was detected among plants with different plastid trn L–F haplotypes in the nrITS from six *P. andromedea* plants (Bidartondo & Bruns 2001; Bidartondo, unpublished data), or in an intron-containing 444 bp region of the nuclear Calmodulin gene from 16 *P. andromedea* plants (Bidartondo, unpublished data).

The need for obligate manipulation of the host may select for extreme specificity in parasites and host manipulation may be explained by the need of the parasite to complete development on one host individual (Thompson 1994). There are two sources of evidence indicating that mycorrhizal exploiters are highly manipulative of their symbionts. First, the cell–to–cell interactions in the mycorrhizal roots of the Monotropoideae are tightly orchestrated and involve the development of unique fungal ‘transfer’ structures not observed in photobiont roots (Lutz & Spjeldn 1973; Duddridge & Read 1982; Robertson & Robertson 1982). Second, *Sarcodes sanguinea* appears to locally stimulate both the mycorrhizal fungus and the photobiont roots (Bidartondo et al. 2000). Unlike other parasites, mycorrhizal exploiters do not colonize their symbionts or transmit vertically to them, instead mycorrhizal exploiters are actively colonized by the hyphae of their associated fungi. In addition, the evolution and maintenance of the biochemical mechanisms that allow mycorrhizal exploiters to actively sequester fungal carbon (whose direction of flow is reversed relative to green plant mycorrhizae) at the cellular level should also provide a source of selective pressures.
toward specialization. If these mechanisms are homologous to those leading to cheating among green plants connected to a common mycorrhizal network (Simard et al. 1997), then nonphotosynthetic mycorrhizal exploiters are the plant parasitic extreme of the mycorrhizal symbiotic continuum.

**Fungal phylogenies**

Even though the objective of this study was to examine patterns of mycorrhizal specificity in the monotropoid symbionts, new phylogenetic results emerged from our analyses of sequence data from basidiocarps of taxa related to the mycorrhizal symbionts of the Monotropoideae. The *Rhizopogon* section *Amylopogon* phylogram with type specimens from every species in the section indicates that: (i) Smith & Zeller’s (1966) stripes are polythetic; (ii) paratypes are often different to holotypes of the same species, so that para- and holotype specimens of those species are polythetic (as found for other species in *Rhizopogon* section *Villosuli*, A. Kretzer personal communication); (iii) there is no ITS variation within several sets of species, which may reflect lack of resolution in the ITS or description of developmental stages of a single species as different species (as inferred in the *K. villosulus* species group by Martin et al. 1998); and (iv) *R. radus* does not belong within section *Amylopogon* but in section *Villosuli* (data not shown). Assuming that different ITS sequences represent different species (there is no evidence of paralogous or xenologous copies in *Rhizopogon*), we conclude that conflicts are due to the reduced morphology of *Rhizopogon* coupled to its high diversity in western North America (over 100 described species); these two factors led Smith, Zeller and subsequent taxonomists to admittedly have difficulties in distinguishing many *Rhizopogon* morphospecies. However, a single locus (e.g. ITS) may not distinguish closely related species (Avise & Wollenberg 1997; Maddison 1997; O’Donnell et al. 1998); therefore, some of the species groups we detected could be composed of more than one evolutionary lineage.

Similar taxonomic problems were encountered in *Gautieria*, another diverse false-truffle genus exhibiting reduced morphology. Many of the conflicts in the *Gautieria* phylogram probably stem from the inappropriate usage of specific epithets from one continent in another. For instance, *G. chilensis* from Mexico are polythetic and not closely related to *G. chilensis* from Chile. A small-spored clade (*G. monticola* species group) and a large-spored clade are strongly supported as reciprocally monophyletic. *G. monticola* has the smallest spores in the genus (Zeller & Dodge 1918; Stewart 1974; J. States, personal communication), but this character is not reflected in existing sectional divisions.

Various *Tricholoma* clades comprising several species groups were strongly supported and many of these are largely congruent with morphologically defined sections: *Tricholoma* and *Terra* (Noordeloos & Christensen 1999), *Genuina* (Shanks 1997), *Eiseeria* and *Atrosquamosa* (Riva 1988). Nonetheless, sectional monophyly cannot be systematically evaluated here as many described *Tricholoma* species remain unsampled in this diverse genus (>70 described species in Europe alone).

**Acknowledgements**

We thank the following individuals for specimens: Jun–ichi Abe, Arne Anderberg, Michael Castellano, Morten Christensen, Frédéric Cousoul, Dennis Dejauring, Hans Ek, Robert Fogel, Gro Gudlen, Fred Huber, Thomas Horton, Janne Johansson, Ralph Kingsbury; Annette Kretzer, Steven Miller, Cathy Paris, David Read, Daniela Roth, Perti Salo, Béatrice Sen-Irlet, Jack States, James Trappe, Else Vellinga, Håkan Wallander, Molly Widmer and Akiyoshi Yamada. Timothy Szaro provided computer assistance. Michael Mågroom and two anonymous reviewers offered thoughtful comments on the manuscript. The following organizations provided funding: Berkeley Vice-Chancellor for Research Fund, East Bay Chapter of the California Native Plant Society, Berkeley Chapter of the Sigma Xi Society, Mycological Society of San Francisco (to MB), National Science Foundation (grant DEB9628852 to TDB).

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The authors share an interest in the evolution and ecology of ectomycorrhizal symbioses and epiparasitic mycorrhizal plants.