Mycorrhiza (2011) 21:431–441
DOI 10.1007/s00572-010-0349-8

ORIGINAL PAPER

# Molecular characterization of pezizalean ectomycorrhizas associated with pinyon pine during drought

**Galena J. Gordon · Catherine A. Gehring**

Received: 24 September 2010 / Accepted: 22 November 2010 / Published online: 30 December 2010


**Abstract** Recent studies using molecular analysis of ectomycorrhizas have revealed that ascomycete fungi, especially members of the order Pezizales, can be important members of ectomycorrhizal (EM) fungal communities. However, little is known about the ecology and taxonomy of many of these fungi. We used data collected during a wet and a dry period to test the hypothesis that pezizalean EM fungi associated with pinyon pine (*Pinus edulis*) responded positively to drought stress. We also assessed the phylogenetic relationships among six, unknown pezizalean EM fungi, common to our study sites, using rDNA sequences from the internal transcribed spacer and large subunit (LSU) regions of the ribosomal DNA. Sequences of these fungi were then compared to sequences from known taxa to allow finer-scale identification. Three major findings emerged. First, at two sites, pezizalean EM were 44–95% more abundant during a dry year than a wetter year, supporting the hypothesis that pezizalean EM fungi respond positively to dry conditions. Second, four of the six unknown pezizalean EM fungi associated with *P. edulis* separated from one another consistently regardless of site or year of collection, suggesting that they represented distinct taxa. Third, comparison with LSU sequences of known members of the Pezizales indicated that these four taxa grouped within the genus *Geopora* of the family Pyronemataceae. Our results provide further evidence of the importance of pezizalean fungi in the ectomycorrhizal symbiosis and demonstrate high local abundance of members of the genus *Geopora* in drought-stressed pinyon–juniper woodlands.

**Keywords** Ascomycete ectomycorrhiza · Drought · *Geopora* · Pezizales

G. J. Gordon
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA
e-mail: galena.gordon@gcmail.maricopa.edu

C. A. Gehring (✉)
Department of Biological Sciences and the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011-5640, USA
e-mail: Catherine.Gehring@nau.edu

*Present Address:*
G. J. Gordon
Glendale Community College North,
5727 W Happy Valley Rd,
Phoenix, AZ 85310, USA

## Introduction

Ectomycorrhizal (EM) fungi are a species rich group of root symbionts that promote host plant growth by enhancing plant acquisition of soil resources and improving host tolerance of abiotic and biotic environmental stresses (Smith and Read 1997). At least 6,000 species of fungi form ectomycorrhizal associations (Rinaldi et al. 2008), though that number is likely much higher (Tedersoo et al. 2010). There is ample evidence of variation among species in resource acquisition and utilization (e.g., Lilleskov et al. 2002), presumed carbon cost to host plants (e.g., Saikkonen et al. 1999), and response to disturbance (e.g., Stendell et al. 1999; Jones et al. 2003). Ectomycorrhizal fungi have evolved independently multiple times, principally within the phyla Ascomycota and Basidiomycota (Tedersoo et al. 2010). However, the association between functional attributes and phylogenetic relatedness is unclear.

Although previous research on ectomycorrhizal fungi has emphasized fungi in the Basidiomycota because of their abundant production of epigeous sporocarps, recent research indicates that members of the Ascomycota are

important in many habitats (e.g., Fujimura et al. 2005; Egger 2006; Tedersoo et al. 2006; Smith et al. 2007; Morris et al. 2008), including environmentally stressful sites such as drought-prone forests (Izzo et al. 2005). Many ascomycetous EMF belong to the Pezizales, the basal order of Euascomycetes (Lutzoni et al. 2004) that is comprised of ~1,125 species of variable trophic status (Kirk et al. 2001; Egger and Paden 1986). While early reports of ectomycorrhizal members of the Pezizales were based on fruiting habits and sporocarp identification (Maia et al. 1996), the recent apparent increase in pezizalean EM fungal diversity has resulted from molecular analysis of root tips collected in the field (Tedersoo et al. 2006). Pezizalean ectomycorrhizal root tips have been difficult to identify beyond the level of order or family because the morphological characteristics used, especially septal pore untrastructure (Berndt et al. 1990) provide low taxonomic resolution (Kimbrough 1994). Sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) (Horton and Bruns 2001) has facilitated identification of some pezizalean EM fungi, but phylogenetic analysis using the large subunit (LSU) of the rDNA was necessary for identification beyond family and order in some study systems (Ogura-Tsujita and Yukawa 2008; Perry et al. 2007; Perry and Pfister 2008; Tedersoo et al. 2006). A greater understanding of the distribution and phylogenetic relationships of pezizealean EM fungi is important given their abundance in stressful habitats. Smith et al. (2006) reported that EM within the order Pezizales were well adapted to harsh environments and regenerated quickly after disturbance. Dominance by pezizalean EM has been observed on plants growing in low moisture and nutrient soils (Gehring et al. 1998), and in association with biotic stresses such as below-ground competition (Haskins and Gehring 2004) and mistletoe parasitism (Mueller and Gehring 2006).

In this study, we examined ectomycorrhizas from pinyon pine (*Pinus edulis* Engelm.) collected over multiple sites and years to determine: (a) if there was an association between pezizalean EM and drought stress and (b) the phylogenetic relationships of a group of EM fungi frequently observed on *P. edulis*, but presently classified only to order (Pezizales). We focused our study on the EM fungi of *P. edulis* for two major reasons. First, *P. edulis* is a dominant tree of the southwestern US which has experienced more than 10 years of drought including 2 years of extremely dry conditions (1996 and 2002) that resulted in widespread mature *P. edulis* mortality (Breshears et al. 2005; Mueller et al. 2005). We have data on EM fungal community composition from the same sites before and during the drought, allowing us to determine if changes in the abundance of pezizalean EM fungi were associated with drought stress. Second, the EM fungal community of *P. edulis* can be dominated by pezizalean EM (Gehring et al. 1998; Mueller and Gehring 2006; McHugh and Gehring 2006; Hubert and Gehring 2008; Sthultz et al. 2009), including several putative species that were morphologically similar when observed as EM root tips but that had different restriction fragment length polymorphism patterns and ITS sequences. The pinyon study system thus provided us with the opportunity to examine the phylogenetic relationships of several unknown Pezizales of ecological importance. We tested two hypotheses: (1) the relative abundance of members of the order Pezizales will increase during drought. To test this hypothesis, we sampled ectomycorrhizal root tips associated with *P. edulis* from two study sites in northern Arizona at two times, once before the onset of long-term drought in the region in April 1995 and once during an ongoing drought in April 2004. (2) The six commonly observed members of the order Pezizales associated with *P. edulis* represent distinct taxa. We tested the latter hypothesis because morphologically similar ectomycorrhizas formed by widespread "species" such as *Pisolithus tinctorius* and *Cenococcum geophilum* can represent genetically and physiologically distinct phylogenetic lineages or cryptic species that co-occur (e.g., Martin et al. 2002; Douhan and Rizzo 2005). We also compared the DNA sequences generated in this study to those of other members of the Pezizales (Perry et al. 2007) to determine their phylogenetic relationships to described species.

## Materials and methods

### Hypothesis 1—abundance of Pezizales EM fungi increases during drought

To determine if the relative abundance of pezizalean EM fungi varied in association with drought ectomycorrhizal root tips associated with pinyon pine were sampled at two study sites in northern Arizona during both a relatively wet year (1995) and a relatively dry year (2004). The two study sites were located in northern Arizona, USA, approximately 20 km from one another on different soil types. Site S (35° 23′ 25″N, 111° 25′ 40.8″ W) with an elevation of ~2,050 m, was located near Sunset Crater National Monument and consisted of cinder soils classified within the US Department of Agriculture Soil Taxonomic Sub-Group of Typic Ustorthents (Miller et al. 1995). These soils were low in nutrients and water-holding capacity (Gehring et al. 1998). Site B was located 20 km northwest of site S (35° 32′ 55.5″N, 112° 50″ 49″W) on more fertile sandy–loam soils classified as Typic Haplustafs (Miller et al. 1995) and was at an elevation of ~1,975 m. Pinyon pine and one-seed juniper (*Juniperus monosperma*) dominated both sites. Shrubs such as *Fallugia paradoxa* and

*Rhus trilobata* were abundant at site S, while another shrub species (*Chrysothamnos nauseosus*) and grasses, especially *Bouteloua gracilis*, were the dominant understory vegetation at site B. Pinyon pine is the only host for ectomycorrhizal fungi in many pinyon–juniper woodlands, including these study sites (Haskins and Gehring 2005).

The 1995 samples were collected prior to the onset of a long-term drought in the region that began in the winter of 1995. The 2004 samples were collected during a dry year in the midst of an ongoing drought in the southwestern USA. The average Palmer Drought Severity Index (PDSI) for the region including our study sites (Arizona division 2) was 1.5 for 1995 and −3.2 for 2004 (www.noaa.gov). PDSI ranges from 6.0 to −6.0, with values between 1 and −1 indicating average conditions and more negative values indicating drought. One root tip was analyzed per tree for 50 mature trees per site as described in Gehring et al. (1998). This sampling scheme was used because previous work demonstrated that individual *P. edulis* trees tended to be dominated by one or two species of EM, so that replication within trees added little to the description of the EM community at a site (Gehring et al. 1998). The trees sampled at both sites during both years were similar in size (basal trunk diameter 25–30 cm) and covered an area of ~0.5 $km^2$. Several EM root tips were collected per tree, but one was selected at random for restriction fragment length polymorphism (RFLP) analysis.

To determine the EM fungal community composition of the trees at a site, DNA was extracted from one root tip per tree using either the mini-prep method of Gardes and Bruns (1993) for 1995 samples or a Qiagen DNEasy (Qiagen, Valencia, CA, USA) plant kit for the 2004 samples. The ITS region was amplified using polymerase chain reaction (PCR) with the forward ITS1F (5′-cttggtcatttagaggaagtaa-3′) and reverse ITS4 (5′-tcctccgcttattgatatgc-3′) primer pair (Gardes and Bruns 1993). RFLP data were obtained following the methods of Gehring et al. (1998) using restriction enzyme digestion with *Hinf*I and *Mbo*I. If the first root tip per tree failed to amplify after two attempts, an additional root tip from the same tree was selected at random and subjected to the same PCR–RFLP procedure. The amplification success of the first root tips attempted exceeded 90% in both years.

Our comparison of the dominance of ascomycete EM fungi before and during drought was focused on a common morphological type of ectomycorrhiza (reddish brown mantle, smooth surface, generally coralloid with sparse emanating brown hyphae and no rhizomorphs). Molecular analysis of this morphotype resulted in six distinct RFLP patterns (designated C, E, J, K, N, and Z) using the ITS region of the ribosomal DNA with ITS primers, ITS1F and ITS4, and restriction enzymes, *Hinf*I and *Mbo*1 (Table 1). When the RFLP patterns were sequenced and compared to the database in GenBank, they were 94–97% similarity to several GenBank sequences, identified only as members of the order Pezizales (Haskins and Gehring 2004; Mueller and Gehring 2006). The abundance of the combined total EM from these six RFLP types relative to all EM RFLP types sampled before and during the drought at the two sites were compared using a contingency table chi-square analysis. We predicted that the relative abundance of pezizalean EM would be higher during the dry year, 2004, than the relatively wet year, 1995.

**Table 1** RFLP band sizes for two enzymes used to digest DNA from the ITS region of six pezizalean EMF commonly found in association with pinyon pine

| RFLP Type | Restriction enzyme | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Hinf*I | | | | | *Mbo*1 | |
| C | 122 | 146 | 294 | | | 300 | 322 |
| E | 167 | 205 | 295 | | | 165 | 193 |
| J | 131 | 169 | 246 | | | 294 | 308 |
| K | 91 | 112 | 148 | 159 | 184 | 296 | 347 |
| N | 120 | 158 | 172 | | | 284 | 307 |
| Z | 110 | 151 | 184 | 210 | | 298 | 302 |

## Hypothesis 2—pezizalean EM fungi represented distinct taxa

To test the hypothesis that the six pezizalean RFLP types represented distinct taxa, sequence analysis using two regions of the ribosomal DNA, the ITS and the LSU was conducted on EM samples collected from *P. edulis* at three sites in northern Arizona. Root samples were collected from *P. edulis* at these sites during various years (1999, 2001, 2002, 2004, and 2005) from trees of varying ages and condition (e.g., degree of attack by herbivores or parasites). The purpose of using such a diverse sample of trees was to determine if the six pezizalean RFLP types were distinct from one another despite variation in the timing or location of sampling and the characteristics of the host plant. Most of the DNA samples used were from EM root tips collected for other studies that are now published (Haskins and Gehring 2004; Mueller and Gehring 2006; McHugh and Gehring 2006; Sthultz et al. 2009). The LSU region was chosen in addition to the ITS because taxonomists frequently use the LSU region for phylogenetic analyses of ascomycete fungi (Taylor and Bruns 1999; Smith et al. 2006; Hansen and Pfister 2006), as it codes for proteins and is less variable than the ITS. Tedersoo et al. (2006) reported that phylogenetic analysis using the LSU data resolved the identity of most pezizalean EM fungal sequences to the genus or species level. Forward primer LROR (5′–acccgctgaacttaagc-3′) and reverse primer LR5 (5′-tcctgagg gaaacttcg-3′) were used to amplify the LSU region and the

ITS1F and ITS4 primers described above were used to amplify and sequence the ITS region.

The DNA used to test hypothesis two came from EM root tips that were collected from sites S and B described for hypothesis 1 above, as well as at a third site approximately 5 km from site S, called site H (35° 23′ 26.9″ N, 111° 23′ 23.3″ W). Use of the three sites (S, B, and H) allowed sequences to be compared among sites as well as among RFLP types. Site H has similar soils to site S, but occurred at lower elevation (~1,725 m) where *P. edulis* occurred at low density and juniper (*J. monosperma*) and shrubs, especially *F. paradoxa*, dominated the vegetation (McHugh and Gehring 2006). Pinyon pines at this latter site experienced significantly greater environmental stress than pinyons at the other two sites due to lower soil moisture and higher soil temperatures (Sthultz et al. 2007). Table 2 shows the number of ITS and LSU sequences per site used for subsequent sequence analyses. Replicate samples of a given RFLP type at a site were collected from different trees at least 50 m apart from one another. In total, sequences from 59 EM root tips were used for the ITS and sequences from 80 EM root tips were used for the LSU.

Both the ITS and LSU regions from the six dominant unknown RFLP taxa were purified using a 96-well Millipore PCR purification block on a Biomek FX robot and sequenced using the same primers described previously for PCR (forward and reverse) at the Genomic Analysis and Technology Core at the University of Arizona on an ABI 3730xl (Applied Biosystems) Genetic Analyzer. Editing and assembling of sequences were performed using BioEdit version 7.0.9 (Hall 2007) and SeqMan 8.0.2 software suite for sequence analysis (1988–2008, DNASTAR Lasergene). Sequences were exported to ClustalW multiple alignment in BioEdit software (Hall 2007) for final alignment and manual adjustment. Characters that were considered variable at any base pair or indel position were verified using their appropriate chromatogram. Ambiguous base pairs were excluded from all sequences. Before analyses, up to 200 total nucleotides were trimmed from the ends of the alignments due to poor sequence quality in some sequences. Bi-directional sequences of ≈500 bases within the ITS region and ≈700 bases within the LSU region were used for analyses. Sequences were combined to form two datasets of independently aligned regions, one for the LSU and one for the ITS. Phylogenetic analyses were conducted using *MEGA* version 4 1993–2008 (Tamura et al. 2007). Distance trees were achieved by neighbor-joining (NJ; Saitou and Nei 1987) under the maximum composite likelihood (MCL) method (Tamura et al. 2004). This method was chosen because it does not require the assumption of a constant rate of evolution. Gaps were treated as missing data and eliminated from the datasets. Pair-wise distance tests were performed among representative ITS and LSU sequences of each of five RFLP types (C, E, J, K, Z) to compare the level of divergence among taxa. Representative sequences were identical to at least three (range 3–14) other sequences of that RFLP type and deemed representative of that taxon and were deposited in GenBank. The relative strength of branches for clade stability was determined using the bootstrap (BS) method (Felsenstein 1985) with 1,000 replicates on all characters. Trees were then exported to Dendroscope version 2.2.2 2009 (Huson et al. 2007) for visualization and editing.

### Relationship to other members of the Pezizales

The LSU sequences generated in this study were compared to sequences of 24 other members of the Pezizales (Perry et al. 2007) to determine their phylogenetic relationships to described species (Table 3). Of the target unidentified RFLP types from *P. edulis*, sequences of five types (C, E, J, K, Z) were combined with GenBank sequences to construct an original tree using the NJ method in *MEGA* 4. Based on the results of our test of hypothesis 2, RFLP type N was not included in this comparison. As described for the pair-wise distance tests for hypothesis 2, one representative sequence for each of the RFLP types was used for this analysis. The sequences selected were identical to at least six (range 6–20) other sequences of that RFLP type and were thus deemed representative of that taxon. Two of the sequences retrieved from GenBank were selected as outgroup taxa (*Rhizina undulata* DQ220410 and *Peziza varia* AF335151) based on the phylogeny of the Pyronemataceae, the largest family of the Pezizales (Perry et al. 2007). The final tree was rooted with these two outgroup taxa. Nodal support was assessed by bootstrap values as described above. A

**Table 2** Number of ITS and LSU sequences of each RFLP type used from each of three study sites

| RFLP name | C | | E | | J | | K | | N | | Z | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | ITS | LSU | ITS | LSU | ITS | LSU | ITS | LSU | ITS | LSU | ITS | LSU |
| B | 4 | 6 | 5 | 12 | 3 | 6 | 1 | 4 | 0 | 3 | 0 | 0 |
| S | 6 | 4 | 9 | 12 | 3 | 4 | 4 | 3 | 5 | 4 | 9 | 11 |
| H | 0 | 0 | 5 | 6 | 0 | 0 | 1 | 1 | 0 | 0 | 4 | 4 |
| TOTAL | 10 | 10 | 19 | 30 | 6 | 10 | 6 | 8 | 5 | 7 | 13 | 15 |

**Table 3** LSU sequences from GenBank used to assess phylogenetic relationships

| GenBank accession no. | Species | Geographic origin | Year and collector |
|---|---|---|---|
| DQ220336 | *Geopora arenicola* (1) | Denmark | 1994, K. Hansen, S.K. Sandal |
| DQ220337 | *Geopora arenicola* (2) | Denmark | 1994, K. Hansen, S.K. Sandal |
| DQ220344 | *Geopora cf. cervina* | Norway | 2003, K. Hansen, C. Lange |
| DQ220339 | *Geopora clausa* | California, USA | 1980, J. Trappe |
| DQ220340 | *Geopora cooperi* (1) | California, USA | 1981, R. Trial |
| DQ220341 | *Geopora cooperi* (2) | Wyoming, USA | 1989, J. Ammarati |
| DQ220342 | *Geopora cooperi f. gilkeyae* | California, USA | 1996, E. Cázares |
| DQ220343 | *Geopora pellita* | Michigan, USA | 1969, D.H. Pfister |
| DQ220338 | *Geopora sp. A* | Denmark | 2001, S.A. Elborne |
| DQ220345 | *Geopora sp. B* | Norway | 2003, K. Hansen |
| AF335151 | *P. varia* | New Hampshire, USA | 1999, Z.-L. Yang |
| AF266707 | *Pezizales* sp. B | California, USA | M. Bidartondo |
| DQ220396 | *Pustularia patavina* | Norway | 2003, K. Hansen, C. Lange |
| DQ247805 | *Pyronema domesticum* | Netherlands | 1988, H.A. van der Aa |
| DQ220397 | *Pyronema omphalodes* (1) | Carchi, Ecuador | 2004, K. Hansen et al. |
| DQ220398 | *Pyronema omphalodes* (2) | California, USA | 2003, B.A. Perry, M. Wood |
| DQ220399 | *Pyronemataceae sp. B* | New Mexico, USA | 2004, N. Weber, K. Hansen, B.A. Perry |
| DQ220410 | *R. undulata* | Ostfold, Norway | 2002, D.H. Pfister, B.A. Perry, K. Hansen |
| DQ220442 | *Tricharina gilva* (1) | Norway | 1981, H. Dissing |
| DQ220443 | *Tricharina gilva* (2) | California, USA | 2002, D.E. Desjardin |
| DQ220444 | *Tricharina gilva* (3) | California, USA | 2002, B.A. Perry |
| DQ220445 | *Tricharina ochroleuca* | Greenland | 1983, H. Dissing |
| DQ220447 | *Tricharina sp. A* | Ecuador | 2003, J. Salazar, T. Laessoe |
| DQ220446 | *Tricharina sp. B* | Massachusetts, USA | 1971, M. E. Barr |

pair-wise distance test was calculated to assess divergence between sequences of each RFLP type in *MEGA* 4.

Using the data described above, a consensus tree was also produced using maximum parsimony (MP) in *MEGA* 4. When looking for the most parsimonious tree, gaps were treated as missing data and all nucleotide changes were weighted equally. The initial trees for branch swapping algorithm were performed via close neighbor interchange by random addition of 10 replications. Nodal support was assessed by bootstrap values analyzed as described above. Branch lengths were calculated using the average pathway method (Nei and Kumar 2000). By default, branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed.

## Results

### Hypothesis 1—abundance of pezizalean EM increases with drought

Ectomycorrhizal fungi in the order Pezizales were more abundant in the drought year of 2004 than the more mesic year of 1995 at both sites ($X^2$=9.72, $p$<0.01 for site S and $X^2$=28.57, $p$<0.001 for site B; Fig. 1). The difference between years was more dramatic at site B as pezizalean EM were rare during the mesic year, but dominated during the drought year. Pezizalean EM were dominant during both years at site S, but made up nearly 90% of all ectomycorrhizas during the drought year.

### Hypothesis 2—pezizalean EM fungi represent distinct taxa

Neighbor joining trees produced using both ITS and LSU sequence data partially supported our second hypothesis that four of the six members of the order Pezizales observed as pinyon EM represented distinct taxa. Sequences of RFLP types C, E, J, and K generally formed distinct clades with moderate to strong bootstrap support (Figs. 2 and 3). In contrast, RFLP type N was observed at varying locations in both the ITS and LSU neighbor-joining trees, indicating that it was not a well defined taxon. RFLP type Z was nested within representatives of RFLP type E in the LSU tree and there was low bootstrap support for the separation of E and Z in the ITS tree.

Specifically, for the LSU, 79 sequences with formed a tree with clades that represented four of the six RFLP types

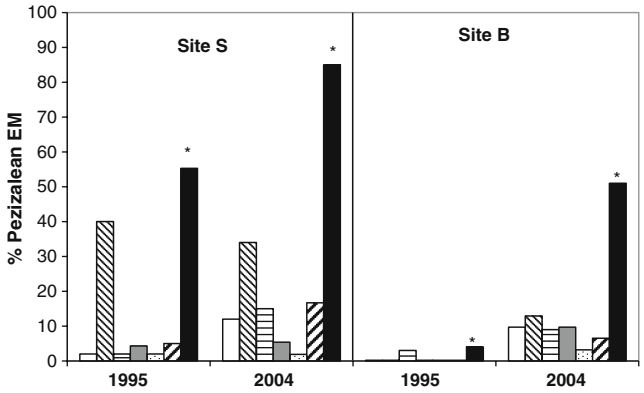


**Fig. 1** The relative abundance of six RFLP patterns of EM within the order Pezizales associated with pinyon pine at two study sites in northern Arizona was higher in a drought year (2004) than in a year of higher precipitation (1995). The six EM RFLP types are indicated by different patterns and are shown in the following order: C, E, J, K, N, and Z. *The solid bar denoted with an asterisk* represents the combined abundance of the six EM RFLP types at each time–site combination

examined (Fig. 2). Representatives of RFLP C formed a clade with strong support (BS 99%). RFLP J produced a clade with 92% support and K produced a separate group with lower support. RFLP Z was nested with E with moderate support (BS 82%). J and E appeared as sister groups with low resolve. The pair-wise distance calculations supported these results as C differed the most from the other RFLP types (~3.0% difference) while Z and E were the most similar (<1% difference; Table 4). GenBank accession numbers for LSU consensus sequences of RFLP types C, E, J, K, and Z are HQ630381, HQ630382, HQ630383, HQ630384, and HQ630385, respectively.

Within the ITS tree, clades E and Z nested together although the relationships were not well supported (Fig. 3). Clades J and K were well-supported distinct clades, 100% and 99%, respectively. RFLP type C had low support as a distinct clade and identified with K as a moderately supported sister group. Analysis of the phylogenetic distance among five representative sequences of each RFLP type revealed that K and J showing the highest difference at 13.0%, while E and Z showed the closest identity with only 2.5% divergence (Table 4). GenBank accession numbers for ITS consensus sequences of RFLP types C, E, J, K, and Z are HQ630376, HQ630377, HQ630378, HQ630379, and HQ630380, respectively.

### Relationship to other members of the Pezizales

The topology of an original tree generated using the NJ method (Fig. 4) and a consensus tree (of 1,000 replicates) constructed with MP (tree not shown) in the LSU were similar, as were the bootstrap values. Comparison of LSU sequences of the five *P. edulis* RFLP types with pezizalean samples from GenBank indicated a close association between these samples and the genus *Geopora*. The *Geopora* formed a strongly supported group (BS 91%), that included two members of the genus *Tricharina*, as well as all five of the pinyon pine EM RFLPs. RFLP types E, J, K, and Z grouped with *Geopora cf. cervina* and *G. clausa* in a separate clade from RFLP type C, which was most closely associated with *Geopora* sp. B and with the well supported *Geopora cooperi* clade (BS 99%). Other members of the Pezizales were more distant with well supported lineages that were generally in agreement with Perry et al. (2007).

## Discussion

The higher relative abundance of pezizalean EM at a site with young cinder soils and at both sites during drought supported our first hypothesis and was consistent with previous research. Members of the Pezizales were observed in early successional environments e.g., glacial forefronts (Trowbridge and Jumpponen 2004) and forest edges (Dickie and Reich 2005) and areas that had been exposed to disturbances such as clear cutting (Mah et al. 2001) and wildfire (e.g., Vrålstad et al. 1998; Baar et al. 1999; Fujimura et al. 2005). In *P. edulis*, pezizalean EM fungi, including those described in this study, were more abundant when their hosts were exposed to poorer soils (Gehring et al. 1998), greater interspecific competition (Haskins and Gehring 2004), and higher mistletoe parasitism (Mueller and Gehring 2006) than in the absence of these stressors. However, our conclusion that pezizalean ectomycorrhizas increase with stresses such as drought must be tested more rigorously as our study was limited to a comparison of 2 years with markedly different precipitation rather than a series of years that varied in precipitation.

Our results indicating that four of the six undescribed pezizalean EM fungi observed to increase with drought represented distinct taxa based on ITS and LSU sequence data provided partial support for our second hypothesis. Sequence divergence among the four distinct RFLP taxa in the ITS were consistent with species level differentiation based on the 97% cut off for species identification frequently used in molecular phylogenetic analysis of EM fungi (e.g., Izzo et al. 2005; Smith et al. 2007). Based on phylogenetic analysis of the LSU, the pezizalean EM fungal taxa dominant on *P. edulis* grouped most closely with members of the genus *Geopora*. Members of this genus have been observed as EM in several other study systems including a ponderosa pine forest after low intensity fire (Fujimura et al. 2005), later successional boreal coniferous and deciduous woodlands (Tedersoo et al. 2006) and in association with a woody member of the Rosaceae (*Cercocarpus ledifolius*) growing on semi-arid rocky outcrops (McDonald et al. 2010). Very recently, a

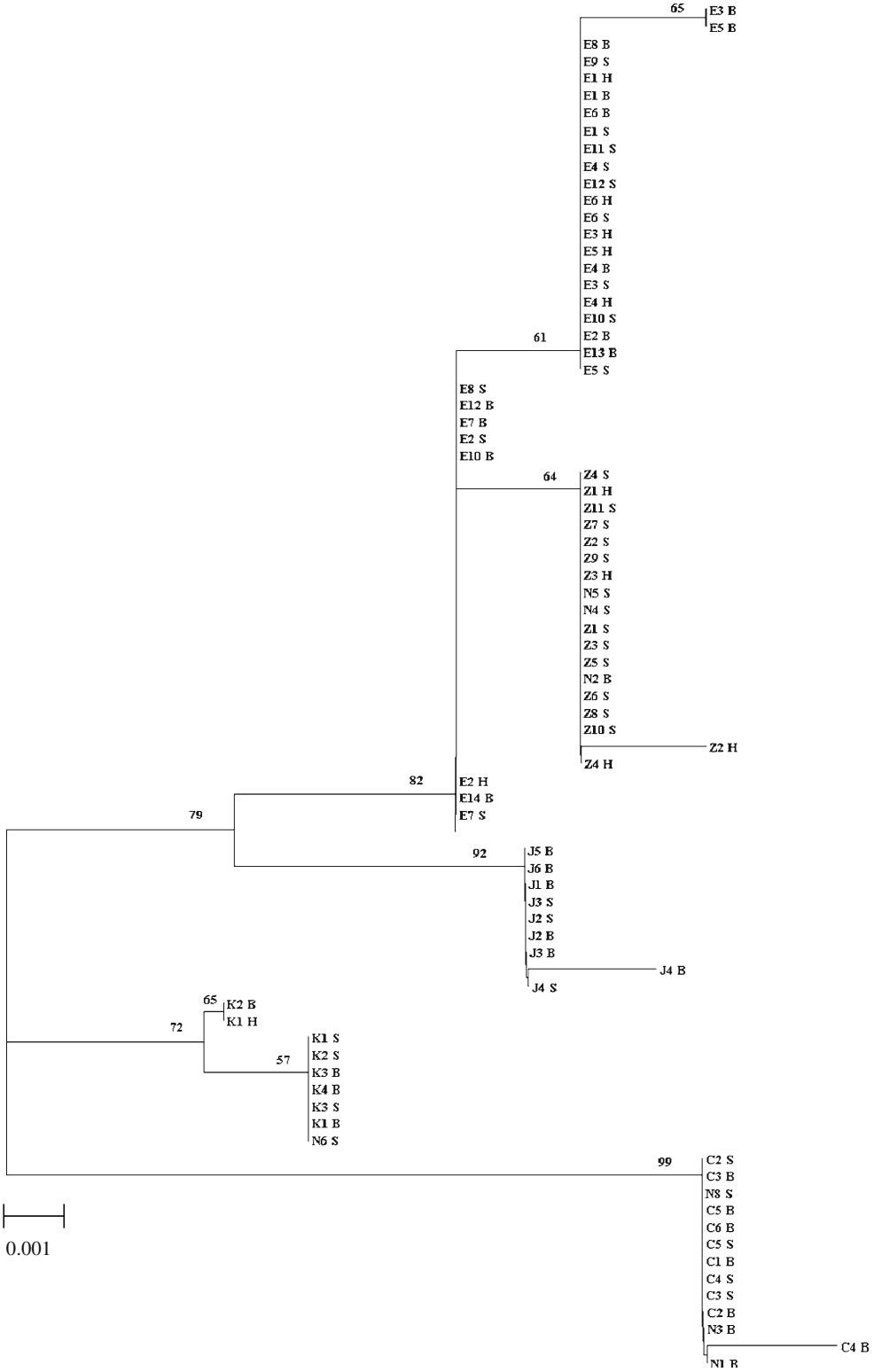


**Fig. 2** Evolutionary relationships of 80 sequences of six different EM RFLP types of *P. edulis* using the LSU region of the rDNA. The inferred optimal NJ tree with the sum of branch length=0.05938394 is shown. Bootstrap support is shown *above the branches* (1,000 replicates). Evolutionary distances were computed using the MCL method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. *Bar* represents 1 change per 1,000 characters

species of *Geopora* was found in fly ash (the principal byproduct of coal-fired power stations) where it formed ectomycorrhizas with willow clones that had been inoculated with *Laccaria spp* (Hrynkiewicz et al. 2009), suggesting that *Geopora* propagules colonized the area independently and could be useful in restoration. In our study system, we previously observed *G. cooperi* sporocarps and ectomycorrhizas at site S and locations near site B (Gehring et al. 1998; Mueller and Gehring 2006). The RFLP patterns of *G. cooperi* at this site were distinct from the other pezizalean ectomycorrhizas in this study and matched GenBank sequences with high affinity (99%; Mueller and Gehring 2006).

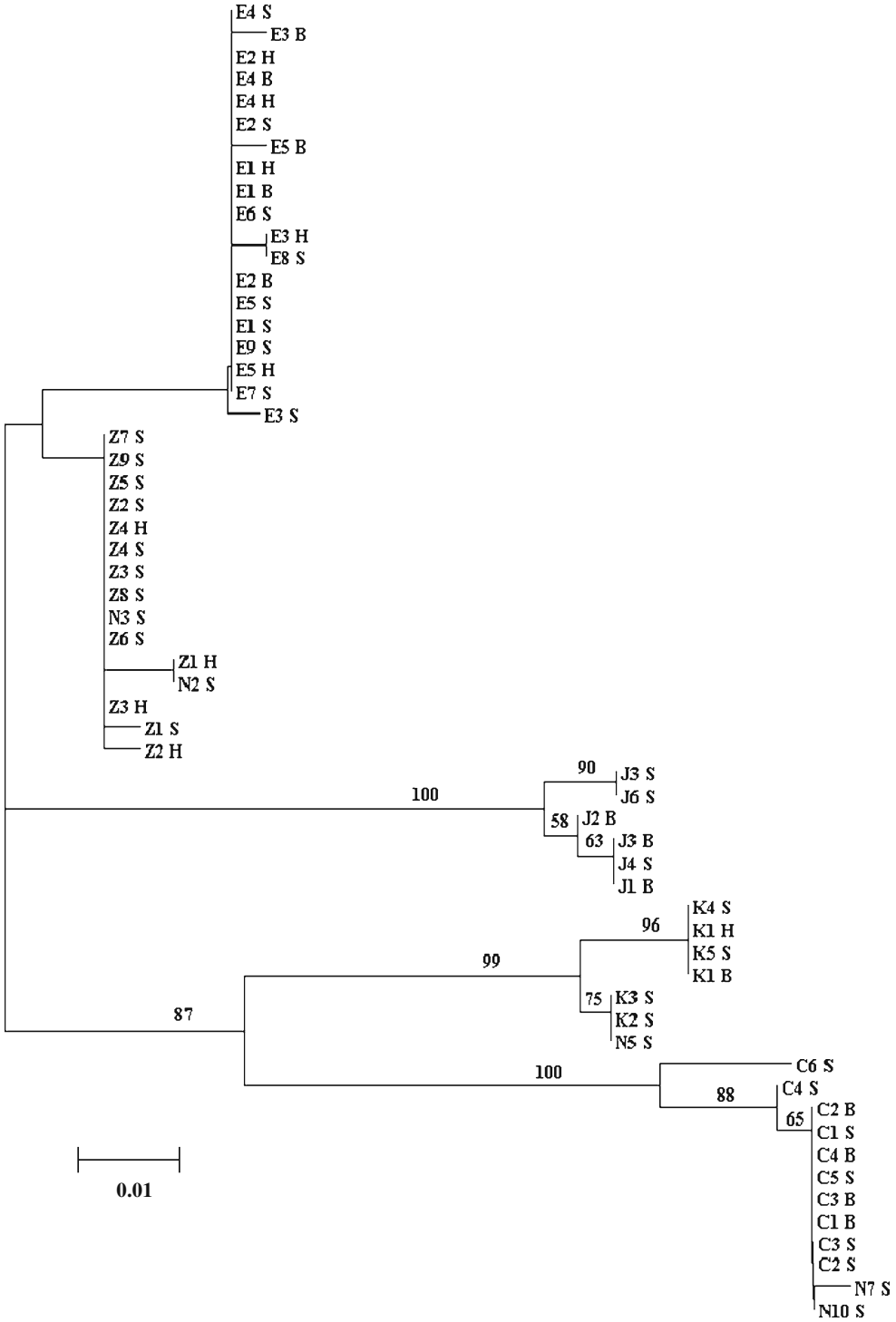


**Fig. 3** Evolutionary relationships of 59 sequences of six different EM RFLP types from *P. edulis* using the ITS region of the rDNA. The optimal NJ tree with the sum of branch length= 0.22809593 is shown. Bootstrap support is shown *above the branches* (1,000 replicates). The evolutionary distances were computed using the MCL method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. *Bar* represents 1 change per 100 characters

The genus *Geopora* has been difficult to characterize phylogenetically and ecologically. Some species are known only from ascocarps while other putative species are known only from molecular analysis of ectomycorrhizas, similar to the taxa described in this study. Although members of the genus have been observed to fruit away from putative plant hosts, they are generally considered to be ectomycorrhizal and can form associations with diverse plant taxa including conifers, poplars, and orchids (Tamm et al. 2010). A recent phylogenetic study comparing morphological traits and ITS sequence data indicated low correspondence between the two types of measurements, with molecular analysis resulting in twice as many lineages as described using morphology (Tamm et al. 2010).

Our study showing extreme dominance of closely related species of *Geopora* EMF in drought-affected pinyon–

**Table 4** Pair-wise distances between consensus sequences of the ITS and LSU regions for five *P. edulis* EMF RFLP types

| RFLP | ITS | | | | | LSU | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | E | J | K | Z | C | E | J | K | Z |
| C | – | | | | | – | | | | |
| E | 0.111 | – | | | | 0.030 | – | | | |
| J | 0.127 | 0.094 | – | | | 0.029 | 0.018 | – | | |
| K | 0.098 | 0.077 | 0.1301 | – | | 0.034 | 0.019 | 0.022 | – | |
| Z | 0.108 | 0.025 | 0.0828 | 0.0768 | – | 0.029 | 0.005 | 0.015 | 0.014 | – |

juniper woodlands suggests that members of this genus may be well suited to stressful environments. Many species of multiple lineages within the Pezizales, including some *Geopora*, produce hypogeous fruiting bodies that reduce the risk of desiccation (Thiers 1984). Although ectomycorrhizas of the genus *Geopora* have not been widely described morphologically (but see Tedersoo et al. 2006), they may share the thin mantle and few emanating hyphae characteristic of other pezizalean EMF (Tedersoo et al. 2006) that have been hypothesized to reduce the carbon

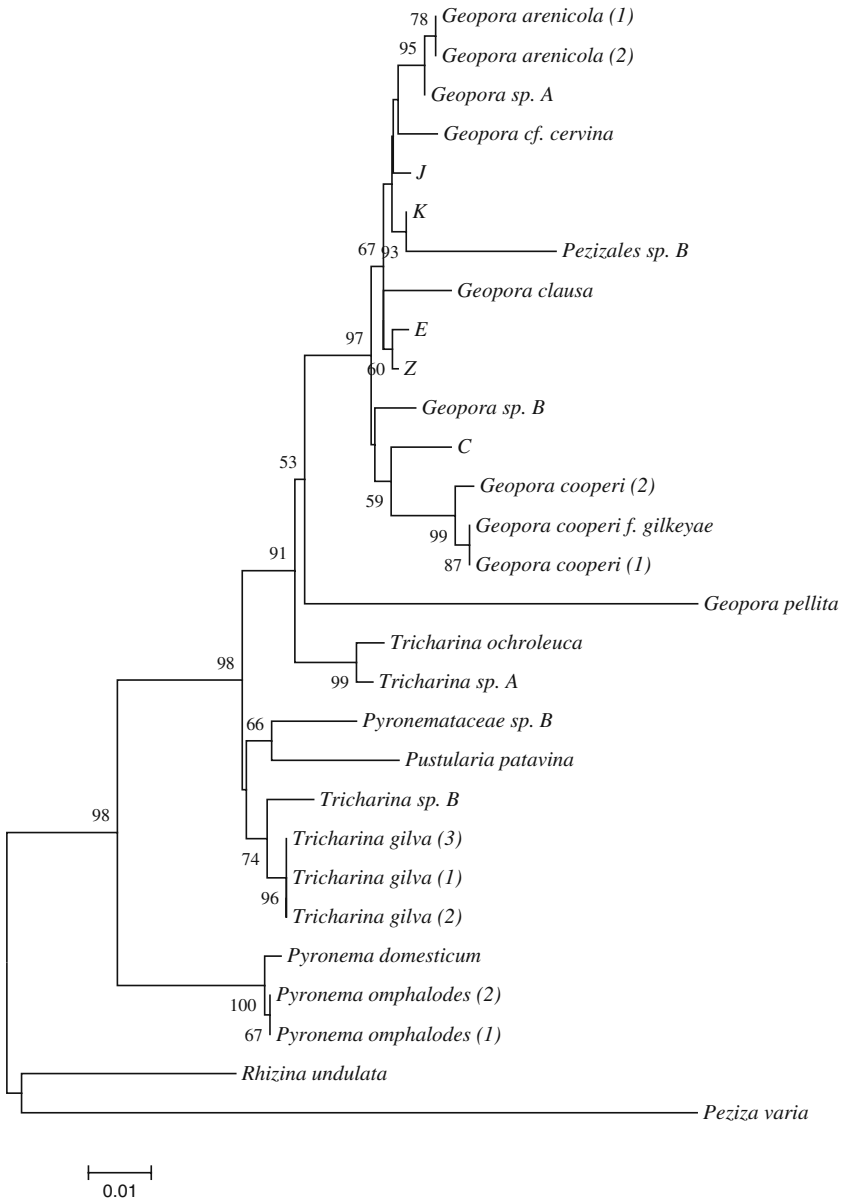


**Fig. 4** Evolutionary relationships of 29 sequences of the LSU (5 RFLP types from *P. edulis*—C, E, J, K, and Z) and 24 identified members of the order Pezizales described in Table 2. Bootstrap support is shown *above the branches* (1,000 replicates). The evolutionary distances were computed using the MCL method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. There were 579 positions in the final dataset. *Bar* represents 1 change per 100 characters

cost of these symbionts for host plants. Likewise, some pezizalean EMF have significant saprotrophic capabilities (Tedersoo et al. 2010), which could allow reduced reliance on host plant carbon during times when photosynthesis might be limited, such as during drought. Further studies are needed to understand the functional significance of the dramatic shifts towards *Geopora* EMF that we observed, particularly given that the arid conditions that appear to favor this genus are predicted for the duration of this century in the southwestern USA (Seager et al. 2007).

**Acknowledgments** We thank the US Forest Service and National Park Service for access to study sites, K. Haskins, T. McHugh, R. Mueller, T. Rubow, and C. Sthultz for EMF DNA, G. Allan for assistance with phylogenetic analysis, and Nancy Johnson, members of the Gehring, Allan and Johnson lab groups and two anonymous reviewers for helpful comments. Financial support was provided by the Minority Student Development Program at NAU, National Science Foundation grants DEB-0236204, DEB-0816675 and Science Foundation Arizona grant 0236-08.

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